

Ivan A. Ross

Plant-Based Therapeutics, Volume 1

Cannabis sativa



Springer

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Ocala, FL, USA

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Contents

1	The History of <i>Cannabis</i>	1
	<i>Cannabis sativa</i> L., fam. Cannabinaceae	1
	Botanical Description	1
	Origin and Distribution	5
2	The Bioactive Components of Plants	11
	Flavonoids	12
	Proteins and Peptides	20
	Glycosides	21
	Alkaloids	23
	Lignans	25
	Resins	27
	Saponins	28
	References	29
3	Chemical Constituents of <i>Cannabis</i>	39
	Chemical Constituents	39
	References	52
4	The Bioactive Components of <i>Cannabis</i>	59
	The Endocannabinoid System	60
	The Entourage Effect of the Phytochemicals	62
	Biosynthetic Pathways	63
	Cannabinoid Receptors	64
	Endocannabinoids in the Circulation	65
	Endocannabinoids and Food	66
	Endocannabinoids and Body Mass Index	68
	Endocannabinoid and Circadian Rhythm	70
	Endocannabinoids and Exercise	71
	Endocannabinoids and Inflammation	73
	The Endocannabinoids and Stress	74
	Endocannabinoids and Chronic SNS or HPA Axis Activation	76

Endocannabinoids in Reward, Anxiety, and Cognition	77
Cannabinoids and Cognitive Functions	79
The Endocannabinoids and Pain.....	80
Terpenes	81
Phenols	83
Trichomes	84
Adverse Effects of Cannabinoids	85
References.....	85
5 Neurological Effects of <i>Cannabis</i>	101
Factors Affecting the Impact of <i>Cannabis</i> Use on the Brain	103
Duration of Abstinence.....	103
Age of Onset of <i>Cannabis</i> Use.....	104
Frequency and Magnitude of <i>Cannabis</i> Use.....	104
Errors in Monitoring Awareness, Reward Processing, and Affective Processing.....	104
Functional MRI Studies on <i>Cannabis</i>	106
Endocannabinoid-Mediated LTP in Learning.....	108
The Homosynaptic and Heterosynaptic eCB-Mediated Potentiation...	109
Memory and Cognitive Impairments	112
<i>Cannabis</i> on Attention and Working Memory	115
<i>Cannabis</i> and Spatial Working Memory.....	121
<i>Cannabis</i> and Executive Function	123
Neuropsychological Functioning in First-Episode Psychosis.....	125
<i>Cannabis</i> and Olfactory Neuroepithelium Cells.....	126
<i>Cannabis</i> and Audio/Visual/Verbal Memories	127
<i>Cannabis</i> and Motor Skills	128
<i>Cannabis</i> and the Mismatch Negativity	129
<i>Cannabis</i> and Traumatic Brain Injury.....	130
The Cognitive and Emotional Impairments Induced by Carotid Artery Occlusion.....	131
Relapse/Reinstatement of Drug-Seeking Behavior.....	132
Dysregulatory Risk Factor	133
<i>Cannabis</i> and Driving.....	134
<i>Cannabis</i> and Microglia Function	134
Endocannabinoids and Astrocytes	135
<i>Cannabis</i> and $\alpha 2A$ -Adrenoceptor.....	135
<i>Cannabis</i> and Catechol-O-Methyl Transferase.....	136
<i>Cannabis</i> and Cyclooxygenase-2	137
Neurobiological Alterations During Pregnancy and Breastfeeding	138
<i>Cannabis</i> in Adolescence	140
<i>Cannabis</i> and Schizophrenia.....	145
<i>Cannabis</i> and Bipolar Disorder	150
<i>Cannabis</i> in Alzheimer's and Parkinson's Diseases	154
<i>Cannabis</i> and Alcohol Consumption	155

<i>Cannabis</i> and Tobacco Coadministration	157
Medical vs. Recreational <i>Cannabis</i> Use	158
<i>Cannabis</i> Flowers vs. Concentrate	162
Acute Administration of Delta-9-THC vs. CBD	163
Delta-9-THC vs. Delta-9-Tetrahydrocannabivarin	165
Delta-8-THC vs. Delta-9-THC	166
Drug Addiction	167
Cannabinoids and Pain	167
<i>Cannabis</i> and Gender Differences	167
<i>Cannabis</i> and HIV	169
<i>Cannabis</i> on Heart Rate and EEG	169
The Residual Impact of <i>Cannabis</i>	170
Therapeutic Perspectives.	171
References.	172
6 Psychotropic Effects of <i>Cannabis</i>.	185
Structural and Functional Effects of <i>Cannabis</i> on the Brain	186
Cannabinoids on Physical and Mental Health	189
Cannabinoids in the Pathophysiology of Psychosis	190
The Endocannabinoid System and HPA Axis Regulation	196
<i>Cannabis</i> and Auditory Evoked Mismatch Negativity	197
<i>Cannabis</i> and Cognitive Performance	198
Delta-9-Tetrahydrocannabinol and Psychosis.	201
The Antipsychotic Properties of Cannabidiol.	202
First Episode Psychosis.	203
Cannabidiol on Anxiety, Depression, and Psychosis	205
Bipolar Disorder	206
Addictive and Psychiatric Comorbidity	207
Motor and Cognitive Impairments	210
<i>Schizotypal</i> Personality Disorder	211
Implicit and Explicit Psychotic Disorders	212
Delta-9-THC and CDB on Cognitive Functions.	212
Psychosis and Schizophrenia	214
Dependence and Behavioral Disturbances	224
<i>Cannabis</i> and Pregnancy.	225
<i>Cannabis</i> and the Vulnerable Adolescent Group	228
Posttraumatic Stress Disorder.	235
Schizophrenia-Like Psychoses	236
<i>Cannabis</i> and Psychosocial Stress	237
Synthetic Cannabinoids	237
Cannabidiol and Parkinson's Patients.	238
Metabolic Dysregulation.	239
Creatine Kinase Elevation.	240
The Cardiac Electrophysiological Effects of Cannabidiol	241
References.	241

7	The Analgesic Effects of <i>Cannabis</i>	249
	Routes of Administration	251
	Modulation of Chronic Neuropathic Pain	255
	Pathological or Injury-Related Persistent Pain	261
	The Antinociceptive Effects of Delta-9-THC	261
	Terpenes in <i>Cannabis</i> -Mediated Analgesia	262
	Coadministration of <i>Cannabis</i> and Opioid	263
	Cannabidiol and the Serotonin (5-HT) _{1A} Receptor	270
	TRPV1 as a Target of the Therapeutic Potential of Myrcene	270
	Cannabinoids and the Glycine Receptors	271
	Gender Differences in Cannabinoid-Mediated Antinociceptive Effects	272
	Cannabidiol and Osteoarthritis	273
	Rheumatologic Pain	274
	<i>Cannabis</i> in Acute Pain and Hyperalgesia	275
	The Endocannabinoid System and Integumentary Wounds	276
	The Endocannabinoid System in Female Reproduction	278
	Labor Pain	278
	Endometriosis	279
	The <i>Cannabis</i> Flower in Pain Management	280
	<i>Cannabis</i> in Capsaicin-Induced Pain	280
	<i>Cannabis</i> Use Preoperatively	281
	<i>Cannabis</i> and Postoperative Pain	281
	The Tetrad Effects – Analgesia, Hypothermia, Catalepsy, and Suppressed Locomotion	285
	Stress-Induced Ulcerogenesis	288
	<i>Cannabis</i> and the Psychotropic Side Effects in Migraine Pain	289
	Capsaicin in Cannabinoid Hyperemesis	290
	The Analgesic Effects of MAGL and FAAH Inhibitors	291
	The Endocannabinoid System and Glutamate N-Methyl-D-Aspartate	292
	Synthetic Cannabinoids on the Visual, Auditory, and Tactile Responses	293
	Spasticity in Multiple Sclerosis	294
	<i>Cannabis</i> in Amyotrophic Lateral Sclerosis	294
	<i>Cannabis</i> in Takotsubo Cardiomyopathy	295
	References	296
8	<i>Cannabis</i> and Perinatal Development	303
	The Health Effects of <i>Cannabis</i> and Cannabinoids	304
	Physiological and Neurodevelopmental Outcomes of <i>Cannabis</i> Exposure	305
	Behavioral Consequences	310
	Genetic Involvement in <i>Cannabis</i> Exposure	312
	Genetic Mutations	316
	Cannabinoids and the Immune System	317

<i>Cannabis</i> Use and the Perception of the User During Pregnancy	318
Maternal Age and <i>Cannabis</i> Use	318
Pregnancy Risk Assessment	319
<i>Cannabis</i> in Nausea and Vomiting During Pregnancy	320
Parental <i>Cannabis</i> Use on Child Development.	320
Prenatal Tobacco and Co-exposure to Tobacco and <i>Cannabis</i>	322
Prenatal Exposure to Alcohol and Delta-9-THC	325
Prenatal Tobacco, Alcohol, and <i>Cannabis</i> Exposures.	326
Prenatal Exposures to Adult Electronic Cigarette.	327
Prenatal <i>Cannabis</i> Exposure on Emotional, Social, and Cognitive Endpoints.	328
<i>Cannabis</i> and Prenatal Choline on Fetal Brain Development	329
<i>In Utero</i> Exposure to <i>Cannabis</i> , Tobacco, and Cocaine.	329
<i>Cannabis</i> Use Late in Pregnancy	330
Infant Neurobehavioral Development.	330
Tobacco and <i>Cannabis</i> Exposure on Child Behavior Problems	332
The Hypothalamic/Pituitary/Adrenal Axis	332
Somatometric Alterations and Risk Factors	333
Child Maltreatment and Its Association with <i>Cannabis</i> Use	334
Prenatal <i>Cannabis</i> Exposure and Transport-Related Injuries	335
Postpartum Exposure to <i>Cannabis</i> and Tobacco on Immune Function.	335
Eye Disorders in Infants Prenatally Exposed to Opioids, Cocaine, and <i>Cannabis</i>	338
Retinal Development.	338
Adverse Neonatal Outcomes.	339
Birth Weight	340
Neonatal Withdrawal Syndrome	342
<i>Cannabis</i> During Lactation.	342
Adult Working Memory	343
Cannabinoid Hyperemesis Syndrome.	343
<i>Cannabis</i> , Tobacco, Alcohol, and Cocaine	344
Modes of <i>Cannabis</i> Administration	345
Stressful Life Events and <i>Cannabis</i> Use.	346
The Demographics of <i>Cannabis</i> Consumption.	347
The Association of Prenatal Substance Use with Prenatal and Postpartum Care.	348
Patterns of Maternal <i>Cannabis</i> Use Predicted Offspring Substance Use.	349
Encephalograms in Prenatal Exposure to <i>Cannabis</i>	349
<i>Cannabis</i> and Children's Dental Health	350
<i>Cannabis</i> and Maternal Choline	350
<i>Cannabis</i> and Tobacco Use.	351
Maternal and Paternal Tobacco and <i>Cannabis</i> Use.	352
<i>Cannabis</i> Use and Fetal Birth Weight.	352

<i>Cannabis</i> and Tobacco Use	353
Prenatal <i>Cannabis</i> Exposure	353
Development and Infancy Study of Prenatal Drug Use	354
The Genetics of <i>Cannabis</i> Use Behavior	355
The Impact of <i>Cannabis</i> Laws on <i>Cannabis</i> Use	356
References	357
9 <i>Cannabis</i> and Multiple Sclerosis	363
The Endocannabinoid System and MS	364
Cannabigerol in Multiple Sclerosis	365
<i>Cannabis</i> for Spasticity Symptoms and Neuropathic Pain	369
<i>Cannabis</i> -Based Treatments for Tremors	381
<i>Cannabis</i> and Cognitive Impairment	382
<i>Cannabis</i> on Processing Speed, Memory, and Executive Functioning	386
Acute Effects of <i>Cannabis</i> Use	393
Chronic Effects of <i>Cannabis</i> Use	393
Structural and Functional Abnormalities in Young <i>Cannabis</i> Users	394
The Effects of <i>Cannabis</i> on Serum Cytokines	395
<i>Cannabis</i> Consumption and Urinary Functions	396
Glucose Uptake and Cognitive Functions in Multiple Sclerosis	397
Physical and Psychological Impact <i>Cannabis</i>	398
<i>Cannabis</i> Use in Depression	404
Pain Management in MS	405
Immunomodulatory Effects of Cannabinoids	406
<i>Cannabis</i> and Cytochrome P450	414
Anti-inflammatory Effects of Cannabidiol	415
Risk Factors of Multiple Sclerosis	416
References	418
10 <i>Cannabis</i> and Cancer	427
Endocannabinoids and Cancer	428
Antitumorigenic Effects	429
Genome and Epigenome	429
Endocannabinoids in Male Reproductive Functions	429
Cannabinoids	430
Delta-9-THC	434
Cannabigerol	435
Tetrahydrocannabivarin	437
Cannabinol	437
Cannabidivarin	437
Cannabichromene	438
Cannabidiol	438
Cell-Cycle Progression	452
Autophagy and Apoptosis	453
Angiogenesis	455

Cytotoxicity	456
Pancreatic Cancer	457
Glioblastoma, Glioma, and Neuroblastoma	464
Lung Cancer	470
Urogenital Cancer	473
Hodgkin's Lymphoma	475
Prostate Cancer	475
Myeloma	477
Oral Cancer	479
Colorectal Cancer	479
Melanoma	483
Hepatocellular Carcinoma	484
Endometrial and Cervical Cancers	487
Breast Cancer	489
Bone Cancer	495
Leukemia	496
<i>Cannabis</i> and Tobacco	499
<i>Cannabis</i> in Immunotherapy	501
<i>Cannabis</i> and Radiation Therapy	502
References	503
11 <i>Cannabis</i> and Parkinson's Disease	513
The Endocannabinoid System and Parkinson's Disease	517
Cannabinoids and Parkinson's Disease	519
Cannabidiol and Delta-9-THC	520
<i>Cannabis</i> Use and Dopaminergic Gene Polymorphism	545
Parkinson's Disease and Complementary Therapies	549
Synthetic Cannabinoids and Parkinson's Disease	551
Opiates and Parkinson's Disease	552
Morphine and Parkinson's Disease	554
Cocaine and Parkinson's Disease	554
Amphetamines and Parkinson's Disease	555
Innovative Therapies in Parkinson's Disease	559
References	560
12 <i>Cannabis</i> and the Skin	569
Keratinocyte Proliferation	570
Melanocytes	575
Kaposi's Sarcoma	578
Psoriasis	582
Acne Vulgaris and Seborrhea	587
Alopecia and Hair Growth Disorders	590
Pruritus	591
Atopic Dermatitis	597
Allergic Contact Dermatitis	602
Systemic Sclerosis	606

Cutaneous Melanoma	610
Dermatomyositis	613
Cutaneous Lupus Erythematosus	614
Epidermolysis Bullosa	616
Wound Healing and Skin Aging	619
References	621
13 Antiviral Activities of <i>Cannabis</i>	629
Immune Response	631
Hepatitis Virus	631
Herpes Simplex Virus	633
Human Immunodeficiency Virus	633
Influenza Virus	634
Coronavirus	634
References	637
14 Alkaline Phosphatase Stimulation	641
CDB and Methylsulfonylmethane	641
<i>Cannabis</i> and Alcohol Co-administration	642
<i>Cannabis</i> and β -Carotene	642
<i>Cannabis</i> and Dill Seeds on Gut Health	643
<i>Cannabis</i> and Physical Health	643
Cannabidiol for Enhancing Osteoblastic Differentiation	645
<i>Cannabis</i> and Spermatogenesis	646
Cannabidiol and Liver Tests	646
Cannabidiol and Sublesional Bone Loss	648
Delta-9-THC and the Anticancer Drug Irinotecan	648
References	649
Index	651

Abbreviations

17-AAG	17-allylamino-17-demethoxygeldanamycin
2-AG	2-arachidonoylglycerol
2-OG	2-oleoylglycerol
5-HT	5-hydroxytryptamine
5-HT1A	5-hydroxytryptamine receptor 1A
5-HT2AR	5-HT2A receptors
6-OHDA	6-hydroxydopamine
AAE	acyl-activating enzyme
ABC	ATP-binding cassette
ABHD6	α/β -hydrolase domain-containing 6
ABPP	activity-based protein profiling
ACD	allergic contact dermatitis
ACE2	angiotensin-converting enzyme 2
AD	Alzheimer's disease
ADHD	attention-deficit disorder with impulsivity or hyperactivity
AEA	arachidonylethanolamide
Aer	aerial parts
AEs	adverse events
ALK	anaplastic lymphoma kinase
AM	associative memory
AMPAR	α -amino-3-hydroxy-5-methyl-isoxazole propionic acid receptors
AMPK	AMP-activated protein kinase
An	anther
ANT	attention network task
AOM	azoxymethane
APDs	antipsychotic drugs
APS	attenuated psychotic symptoms
AR	androgen receptor
As	ash
ASCs	adipose-derived stem cells
ASL	arterial spin labeling

ATF6	activating transcription factor 6
BCCSS	β -carotene, degraded by <i>Cannabis</i> smoke
BCP	beta-caryophyllene
BCRP	breast cancer resistance protein
Bd	bud
BDNF	brain-derived neurotrophic factor
bFGF	basic fibroblast growth factor
BIR	baculoviral IAP repeat
Bk	bark
BKCa	calcium-activated potassium channel
BPRS	Brief Psychiatric Rating Scale
BRNB	Brief Repeatable Battery of Neuropsychological Tests
Bu	bulb
CA1	cornu ammonis 1
Call tiss	callus tissue
CANTAB	Cambridge Neuropsychological Test Automated Battery
CAPE	Community Assessment of Psychic Experiences
CB1R	cannabinoid 1 receptors
CB2R	cannabinoid 2 receptors
<i>CBC</i>	cannabichromene
<i>CBCA</i>	cannabichromenic acid
<i>CBCAS</i>	cannabichromenic acid synthase
CBCL	Achenbach Child Behavior Checklist
<i>CBD</i>	cannabidiol
<i>CBD A</i>	cannabidiolic acid
CBDAS	cannabidiolic acid synthase
CBGA	cannabigerolic acid
CBGV	cannabigerovarin
CBM	<i>Cannabis</i> -based medicine
CBME	<i>Cannabis</i> -based medicinal extract
CBNA	cannabinolic acid
CBNDA	cannabinodiolic acid
CC	chronic cancer
CCI	controlled cortical impact
CEQ	<i>Cannabis</i> Experience Questionnaire
CEVs	<i>Cutibacterium acnes</i> -derived extracellular vesicles
CFP	cingulo-fronto-parietal
CGI-I	clinical global impression-improvement
CHO	Chinese hamster ovary
CHS	contact hypersensitivity
CIDI	Composite International Diagnostic Interview
CIF	<i>Cannabis</i> influence factor
CIP	<i>Cannabis</i> -induced psychosis
<i>CMK</i>	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase
CnC	chronic non-cancer

Co-IP	co-immunoprecipitation
COMT	catechol-O-methyl transferase
COX2	cyclooxygenase 2
CPP	cell-penetrating sequence
CPT	cold pressor test
Cr	crown
CRC	colorectal cancer
CRCE	cannabidiol-rich <i>Cannabis</i> extract
CRD	secretory immunoglobulin A (SIgA) <i>Cannabis</i> -related diagnosis
CREAE	chronic-relapsing experimental autoimmune encephalomyelitis
CREATE	chronic-relapsing experimental allergic encephalomyelitis
CREB	cAMP response element-binding protein
CRS	Category Rating Scale
Ct	coat
CTAB	cetyltrimethylammonium bromide
CTMMP	Connecticut Medical Marijuana Program
CUD	<i>Cannabis</i> use disorder
CVLT	California Verbal Learning Test
CVLT-II	California Verbal Learning Test, Second Edition
CVS	cyclic vomiting syndrome
Cx	calyx
Cy	cotyledon
DA	dopamine
DB	digit backwards
DDS	Descriptor Differential Scale
Delta-9-THCP	(-)-trans-delta-9-tetrahydrocannabinophorol
DHA	docosahexaenoic acid
DiOHTHC	8,11-dihydroxy-THC
DIS	Diagnostic Interview Schedule
DISC1	disrupted-in-schizophrenia-1
DLPFC	dorsolateral prefrontal cortex
DMAPP	dimethylallyl diphosphate
DMN	default-mode network
DMT	disease-modifying therapy
DNFD	2,4-dinitrofluorobenzene
DNMT	DNA methyltransferase
<i>Drd2</i>	dopamine D2 receptor gene
DRM	Deese/Roediger-McDermott
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
<i>DXR</i>	1-deoxy-D-xylulose 5-phosphate reductoisomerase
<i>DXS</i>	1-deoxyxylulose-5-phosphate synthase
EAE	experimental autoimmune encephalomyelitis
EB	epidermolysis bullosa
ECs	endothelial cells
EDSS	expanded disability status scale

EIH	exercise-induced hypoalgesia
Em	embryo
EMT	epithelial-to-mesenchymal transition
EMV	exosomes and microvesicles
ENDS	electronic nicotine delivery devices
EO	essential oil
Ep	epidermis
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
<i>EST</i>	expressed sequence tag
FAAH	fatty acid amide hydrolase
FEP	first-episode psychosis
<i>FIGE</i>	field inversion gel electrophoresis
<i>FISH</i>	fluorescence in situ hybridization
FITC	fluorescein isothiocyanate
Fl	flower
fMRI	functional magnetic resonance imaging
FOXP3	forkhead box P3
FPP	farnesyl diphosphate
FPS	farnesyl diphosphate synthase
Fr	fruit
GABA	gamma-aminobutyric acid
<i>Gb</i>	giga base pair
GBM	glioblastoma multiforme
G-CSF	granulocyte colony-stimulating factor
GDNF	glial-derived neurotrophic factor
Gel	jell
GFP	green fluorescent protein
GM-CSF	granulocyte macrophage-colony stimulating factor
GMM	growth mixture modeling
<i>GPP</i>	geranyl diphosphate
GPR55	G protein receptor fifty-five
GRO- α	growth-regulated protein α
GRP94	glucose regulated protein 94
GRs	glucocorticoid receptors
GSCs	glioma stem cells
GWAS	genome-wide association studies
GWEIS	genome-wide environment-interaction study
HADS	Hospital Anxiety and Depression Scale
HAM-D-21	Hamilton Depression Rating Scale
HAQ-DI	Health Assessment Questionnaire
HCC	hepatocellular carcinoma
HCT	hematopoietic stem cell transplantation
HCV	hepatitis C virus
HDFs	human dermal fibroblasts

<i>HDR</i>	4-hydroxy-3-methylbut-2-enyl diphosphate reductase
<i>HDS</i>	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
HEK	human embryonic kidney
HER2	human epidermal growth factor receptor 2
HMOXI	heme oxygenase 1
HMVECs	human dermal microvascular endothelial cells
HPA	hypothalamic-pituitary-adrenal
HPG	hypothalamic-pituitary-gonadal
<i>HPL</i>	hydroperoxide lyase
HRQoL	health-related quality of life
HSP	heat shock proteins
Hsp90	heat shock protein 90
HSV	herpes simplex virus
Hu	hull
IAA	intra-abdominal adipose
IAP	inhibitor of apoptosis
IASP	International Association for the Study of Pain
IAT	implicit associate task
ICRP	intractable cholestatic-related pruritus
IECs	intestinal epithelial cells
IGT	Iowa Gambling Task
IMDs	immune-mediated disorders
iNOS	inducible nitric oxide synthase
IPP	isopentenyl diphosphate
ISGA	Investigator's Static Global Assessment
IVA/CPT	Integrated Visual and Auditory Continuous Performance Test
Ju	juice
KCs	Epidermal keratinocytes
KSHV	Kaposi's sarcoma-associated herpesvirus
Lf	leaf
L-NAME	N ω -Nitro-L-arginine methyl ester hydrochloride
L-NOARG	L-nitro-N-arginine
<i>LOX</i>	lipxygenase
<i>LRK2</i>	leucine-rich repeat kinase 2
LST	Lesion Segmentation Toolbox
LTD	long-term depression
LTP	long-term potentiation
LUTS	lower urinary tract symptoms
Lx	latex
MADRS	Montgomery-Asberg Depression Rating Scale
MAGL	monoacylglycerol lipase
MAO-B	monoamine oxidase type B
MAST	Michigan Alcoholism Screening Test
MAT	micropipette aspiration technique
MCD	methyl-beta cyclodextrin

M-CIDI	Munich version of the composite international diagnostic interview
MCP-2	monocyte chemotactic protein-2
MCT	medium chain triglyceride
MDD	major depressive disorder
<i>MDS</i>	2-C-methyl-D-erythritol 2:4-cyclodiphosphate synthase
MDSCs	Myeloid-derived suppressor cells
MEFs	mouse embryonic fibroblasts
MEK	mitogen-activated protein kinase
<i>MEP</i>	2-C-methyl-D-erythritol 4-phosphate
MHPCD	Maternal Health Practices and Child Development Study
MI	Motivational Intervention
MMN	mismatch negativity
MMP	mitochondrial membrane potential
MMSE	Mini-Mental State Examination
MORs	mu-opioid receptors
mPFC	medial prefrontal cortex
MPID1	mean pain intensity difference at the first postoperative hour
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MSIS-29	Multiple Sclerosis Impact Scale
MSIT	multi-source interference task
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of the rapamycin complex 1
MVA	mevalonic acid
NAAA	<i>N</i> -acylethanolamine-hydrolyzing acid amidase
NAc	nucleus accumbens
NAEs	<i>N</i> -acyl ethanolamines
NAPE	<i>N</i> -arachidonoyl-phosphatidylethanolamine
NAPLAN	National Assessment Program-Literacy and Numeracy
NARCOMS	North American Research Committee on Multiple sclerosis
NCE	non-cocaine/polydrug
NDO	neurogenic detrusor overactivity
NDUFA9	NADH dehydrogenase ubiquinone 1 α subcomplex subunit 9
NET	neutrophil extracellular trap
NHEKs	normal human epidermal keratinocytes
NMDA	<i>N</i> -methyl-D-aspartate
NMDARs	<i>N</i> -methyl-D-aspartate receptors
NMO	neuromyelitis optica
NOR	novel object recognition
NOS	nitric oxide synthase
NPCU	non-psychotic <i>Cannabis</i> users
Nrf2	nuclear factor erythroid 2-related factor 2
NSAIDs	non-steroidal anti-inflammatory drugs

NSCLC	non-small cell lung cancer
NSPCs	neural stem progenitor cells
NUC	non-uremic calciphylaxis
OAC	olivetolic acid cyclase
OEA	oleoylethanolamide
OLA	olivetolic acid
OLAC	olivetolic acid cyclase
OLS	olivetol synthase
OPPS	Ottawa Prenatal Prospective Study
<i>ORF</i>	open reading frame
OSC	oxidosqualene cyclases
PANAS	Positive and Negative Affect Schedule
PANSS	Positive and Negative Syndrome Scale
PANSS-EC	Excited Component of the Positive and Negative Syndrome Scale
PASAT	paced auditory serial addition test
PBMCs	peripheral blood mononuclear cells
Pc	pericarp
PCBs	phytocannabinoids
PCC	prostate carcinoma cell
PCE	prenatal cocaine/polydrug exposure
PCP	phencyclidine
PCS	Pain Catastrophizing Scale
PDDS	Patient-Determined Disease Steps
PE	phosphatidylethanolamine
PEA	palmitoylethanolamide
PERK	phospho-PKR-like ER-resistant kinase
PFC	prefrontal cortex
PFS	progression-free survival
PGIC	Patient Global Impression of Change
P-gp	P-glycoprotein
PI-NRS	11-point pain intensity numeric rating scale
<i>PK</i>	Purple Kush
PKM2	pyruvate kinase isoforms M2
PKS	polyketide synthase
Pl	plant
PLA2	phospholipase A2
PLC	phospholipase C
PLEs	psychotic-like experiences
pMD	pooled mean difference
Pn	panicle
PNS	peripheral nerve stimulation
PPAR	peroxisome proliferator-activated receptor
PPC	posterior parietal cortex
PPQ	Parkinson's Psychosis Questionnaire
PR	progesterone receptor

PRAMS	Pregnancy Risk Assessment Monitoring System
PROMIS-10	Patient Reported Outcome Measure Information System
PSA	prostate-specific antigen
PSQI	Pittsburgh Sleep Quality Index
Pt	part
<i>PT</i>	prenyltransferase
PTEN	phosphatase and tensin homolog
PTSD	post-traumatic stress disorder
Pu	pulp
PWI	perfusion-weighted imaging
pwMS	patients with MS
RAPTOR	regulatory-associated protein of the mammalian target of rapamycin
RD	risk difference
Rh	rhizome
RICTOR	regulatory-associated companion of mammalian target of rapamycin
RMI	Rivermead Mobility Index
RN	red nucleus
<i>RPKM</i>	reads per kb per million reads
RR	relapsing-remitting
RRMS	relapsing-remitting MS
Rt	root
rTC	Takotsubo cardiomyopathy
SAPS	Scale for the Assessment of Positive Symptoms
SCC	squamous cell carcinoma
SC-IAT	Single-Category Implicit Association Tests
SCID-I	Structured Clinical Interview for the DSM-IV Axis I Disorders
SCR	skin conductance response
Sd	seed
SDMT	Symbol Digit Modalities Test
SGOT	serum glutamic oxaloacetic transaminase
Sh	shoot
SIV	simian immunodeficiency virus
SLE	systemic lupus erythematosus
SMD	standardized mean difference
SNARE	soluble N-ethylmaleimide-sensitive factor attachment receptor
SNPs	single nucleotide polymorphisms
<i>SNV</i>	single nucleotide variants
SPECT	single photon emission computerized tomographic
SPID1	sum of pain intensity differences
SQS	squalene synthase
SRD	substance-related diagnoses
SRT	Selective Reminding Test
St	stem

STAT3	signal transducer and activator of transcription factors
STW	Spot-the-word test
SWM	spatial working memory
SZ	schizophrenia
TCBM	Topical <i>Cannabis</i> -based medicines
TDL	total dendrite length
Tensor-ICA	tensor-independent component analysis
TEWL	transepidermal water loss
<i>THC</i>	<i>delta</i> -9-tetrahydrocannabinol
<i>THCA</i>	<i>delta</i> -9-tetrahydrocannabinolic acid
<i>THCAS</i>	<i>delta</i> -9-tetrahydrocannabinolic acid synthase
THCCOOH	11-nor-carboxy-THC
THCV	delta-9-tetrahydrocannabivarin
TM	transmembrane
TM6	transmembrane helix 6
TMPRSS2	transmembrane serine protease 2
TMRE	tetramethylrhodamine, ethyl ester, and perchlorate
TMZ	temozolomide
Tr	trunk
TRAILS	TRacking Adolescents' Individual Lives Survey
TRP	transient receptor potential
TRPV1	transient receptor potential vanilloid type 1
TSLP	thymic stromal lymphopoietin
TSST	Trier Social Stress Test
Tu	tuber
TUNEL	TdT-mediated dUTP nick-end labeling
Tw	twig
UDS	urine drug screens
UPS	ubiquitin-proteasome system
VAS	Visual Analogue Scale
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VEGF-C	vascular endothelial growth factor C
VEGFR-3	vascular endothelial growth factor receptor 3
vGPCR	viral G protein-coupled receptor
VHipp	ventral hippocampus
VR1	vanilloid receptor 1
vSub	ventral subiculum
WCST	Wisconsin Card Sorting Test
WLG	word list generation
WM	working memory
WMT	working memory training
XIAP	X-linked inhibitor of apoptosis
YMRS	Young Mania Rating Scale
α -MSH	alpha-melanocyte-stimulating hormone

Chapter 1

The History of *Cannabis*



Cannabis sativa L., fam. Cannabinaceae

Botanical Description

Cannabis sativa is an annual herb of the Cannabinaceae family of the order Urticales. Cannabinaceae genera of importance are the *Cannabis* and *Humulus* (hop). The plants include trees, shrubs, and herbaceous plants, either annual and upright as in *Cannabis*, or perennial and voluble as in *Humulus*. They are sometimes grouped in a raceme of cymes (*Cannabis*), sometimes in cones or catkins (female hop). The ovule is pendant and campylotropous; the albumen is fleshy, and the embryo is curved.

Cannabinaceae is a family of apetalous plants, the flowers of which are generally unisexual, with a regular perianth which is sometimes different in the male and female flowers. The androecium is isostemonous in the male flower, whereas in the female flower, the ovary is unicarpellary or bicarpellary but uniocular and uniovular. The fruit is an indehiscent drupe of achene, the albumen small or nonexistent. The leaves are generally opposite, with persistent stipules; the flowers are dioecious and pentamerous at the calyx and the androecium, and the males spread out with straight stamens, the females sessile with bifid style and biflorous or uniflorous bracts.

The genus *Cannabis* consists of a single species, *Cannabis sativa* (L). *Cannabis indica* is a variety. The changes which *Cannabis* undergoes in order to adapt to the varying conditions it has been introduced led to the mistaken belief that different species existed. Attempts were made to differentiate between the varieties known as *C. erratica* (Silv.), *C. Foetens* (Gilibert), *C. lupulus* (Scop.), *C. macrosperma* (Stock), *C. gigantea* (Lapin), *C. excelsa*, *C. compressa* (Husson), and *C. sinensis* (Delille). After investigations by several botanists, it was concluded that there is only a single species, *Cannabis sativa* L., including two or three varieties. In light

of the taxonomic uncertainty, *C. sativa* is used to describe the plant described in this compilation.

Cannabis has a diploid genome ($2n = 20$) with a karyotype composed of nine autosomes and a pair of sex chromosomes (X and Y). Female plants are homogametic (XX) and males heterogametic (XY) with sex determination controlled by an X-autosome balance system. The estimated size of the haploid genome is 818 Mb for female plants and 843 Mb for male plants, owing to the larger size of the Y chromosome. The genomic resources available for *Cannabis* are mainly confined to transcriptome information: National Center for Biotechnology Information (NCBI) contains 12,907 ESTs and 23 unassembled RNA-Seq datasets of Illumina reads. Neither a physical nor a genetic map of the *Cannabis* genome is available.

Cannabis sativa grows to 5 meters tall, usually erect; stems variable, with resinous pubescence, angular, sometimes hollow, especially above the first pairs of true leaves; basal leaves opposite, the upper leaves alternate, stipulate, long petiolate, palmate, with three to eleven rarely single lanceolate, serrate, acuminate leaflets up to 10 cm long, 1.5 cm broad. Flowers are monoecious or dioecious, the male in axillary and terminal panicles, apetalous, with five yellowish petals and five poricidal stamens; the female flowers germinate in the axils and terminally with one ovulate ovary. The fruit is a brown, shining achene, variously marked or plain, tightly embracing the seed with its fleshy endosperm and curved embryo that matures in the late summer to early fall and year-round in the tropics.

The *indica* variety is an annual suffrutescent plant with five, seven, nine, or sometimes eleven folioles; female inflorescences are very thick, condensed, and contracted; achene small, the tegument with black spots and discs on a yellowish ground; very high stalk, up to approximately 4 meters in plants grown in the plains or hot, damp climates, but not over 1.50 meters in wild plants or those grown in high cold districts or hot but dry regions.

In morphological differences, wide varieties of *Cannabis* could easily be distinguished. However, it should be remembered that the characteristics of *Cannabis* are very readily modified in cultivation or by removal to different surroundings. Research has shown that plants grown from seeds brought from India are completely similar to indigenous plants after two or three generations. The converse reveals the same from studies by Husson in Cairo and Bouquet in Tunisia.

Both the *sativa* and *indica* varieties have textile fibers and oleaginous seeds and can provide inebriant resin if the circumstances of habitat and climate provide the necessary conditions. *Cannabis* is an annual herbaceous plant reaching a height of approximately 2 meters in the temperate regions of Europe. The stem is upright, rectilinear, fluted, and rough. The branches are fragile and short, the leaves palmate, generally with five, seven, or nine folioles (rarely eleven), very unequal, lanceolate, and regularly dentate. Most of the leaves are opposite, but in the neighborhood of the inflorescence and toward the top of the stalk, they are frequently alternate, simple, or trifoliate. On either side of a long petiole is a straight pointed stipule.

The male plants are smaller and more slender and wither and dry up fairly rapidly after flowering. The greater frailty formerly caused them to be referred to as female *Cannabis*. This confusion was found in Lémery (*Traité universel des*

Drogués Simples, 1733) and in many earlier writers Matthiöle, Dodoens, Tragus, and the Bauhins, who gave the name *Cannabis foemina* to *C. sterilis*, the male *Cannabis*. In German-speaking countries, the male *Cannabis*, which is shorter, is popularly known as Fimmel (from the Latin *foemina*, female), and the female *Cannabis*, which is thicker in appearance and grows to a greater height, as maschel (from the Latin *masculus*).

The male flowers are grouped in loose panicles. They comprise five greenish sepals and five episepal stamens with introrse anthers. In the center of the androe-cium, a rudimentary ovary is sometimes present. The floriferous branches of the female plant bear leaves and secondary branches, and the inflorescences form contracted racemes of cymes intermingled with foliaceous bracts that give the female plant a robust, bushy, compact appearance.

The female flower is topped by two long stigmas which are sometimes pink in color; the calyx is urceolate; the ovary sessile and containing a campylotropous ovule that on maturity develops into a smooth globular achene, decorated with webbing of varying fineness and color; it is surrounded by a bract, which is very rich in resin-secreting glands and is thought to be formed by the connection of two stipules. The seed is almost completely lacking in albumen; the fleshy, curved embryo, with an incumbent radicle folded back on the cotyledons, contains oil and aleurone.

When the female flower is fully developed, the organs which are reminiscent of the glandulose hairs of the Labiates will have a globular head formed by four cells with voluminous kernels with abundant protoplasm. They do not contain any chlorophyll pigments but secrete an oleoresin which, seen through the microscope, is of a light amber color. The base is constituted of two cubical cells, which likewise contain no chlorophyll and are supported by two wedge-shaped epidermic cells.

The hairs grow and vary in appearance, for, at a more advanced stage, their heads include eight and sometimes as many as twelve to sixteen cells arranged radially. They also vary in shape according to their position on the plant; for instance, on the outer surface of the bracts of the female inflorescence and the axes of the inflorescences, the hairs attain considerable development and have a distinctive appearance; they take the form of an elongated stem of homogeneous parenchyma, surmounted like a mushroom by an almost hemispherical top, consisting of eight to sixteen cells full of resin. Sometimes, on the other hand, especially on the lower surface of the leaves at the top of the plant, the stem does not develop, and the gland is reduced to a flat round head of eight to ten cells, which appears to be stuck on to the surface of the epidermis.

All parts of the plant, when rubbed between the fingers, give off a characteristic minty odor, which is particularly strong in the case of the female tops at the time of flowering. They are slightly sticky to the touch, and this stickiness is very marked in the highly-resinous *Cannabis* of hot, dry regions.

When the fresh plant is examined under the microscope, it can readily be observed that the content of the secretive hairs is a liquid. In dry samples, there is no liquid but a solid resinous mass. The pluricellular glandulose hairs are the external secretive organs that perform the function of preparing the resin. The oleoresin secreted is stored between the cuticle, which it dilates, and the central part of the

cell. It is easy to understand that if the temperature is very high or the secretion is very abundant, turgescence increases, the cuticle tears, and the oleoresin spreads over the surface of the bract, leaf, or branch, where it dries. That explains the method of harvesting still in use today in certain parts of Asia, where the cultivators, dressed in leather, go about in the plantations at the hottest time of the day in order that the resin may stick to their clothes or to the leather thongs which they draw over the tops of the plants.

Besides the glands of the glandulose resin-secretive hairs, the *Cannabis* plant also possesses other secretive organs, such as the laticifers in the fiber of the terminal portion of the adult stalks. It was revealed that laticifers are present in the plant's vegetative parts, on both sides of the cambium at the level of the inflorescences and lower down in the pericyclic region of the stalks, and also in the neighborhood of the veins of the leaves.

The inarticulate laticifers have a homogeneous content of the same appearance and color as the content of the glandulose hairs, and it is, therefore, possible that they assist in the preparation of the resinous element of *Cannabis*. However, it has been shown that the laticifers secrete only an extremely small proportion of the resin compared to the glandulose hairs.

The resin production is most intense between the times when the flowers begin to appear and when the seeds are completely mature; it ceases when the seeds are ripe. The secretion of resin does not vary in different varieties of *Cannabis* but depends largely on climatic conditions; it becomes considerable in the same variety of *Cannabis* when grown in hot, dry climates. It has been observed that *Cannabis* is grown where there the humidity and barometric pressure are low, and excessive secretion of resin is produced. It may be the content of the laticifers which is responsible for the fact that very young male or female plants sometimes produce resin even when they are still too young to have developed glandulose resin-secretive hairs on their surface.

Resin production is primarily a defensive plant reaction against high temperatures and lack of moisture in the atmosphere. When the female flowers are blossoming, and at the time when the ripened and enlarged ovaries are about to provide shelter for the formation and development of the seeds, it is vital for the plant that the particular part in which those processes are going to take place should be protected from the danger of desiccation. Therefore, it is at this period that the activity of the resin glands becomes intense; owing to the exudation of resin, the female tops are, as it were, smeared over with a protective coating, under which the life-giving sap of the plant can bring to the fertilized ovules those elements which are essential to their development; an insulating protective varnish is necessary, and the plant secretes it.

Where the drought is not severe, the plant does not need to devote its activity to producing resin. The vegetative apparatus (leaves) and maintenance apparatus (fibers and stalks) develop to an increased extent; the plants are thicker and higher but produce less resin. *Cannabis* plants from the same lot of seeds were grown, some of them on a dry plateau, and some in a hollow where the subsoil had retained a certain amount of moisture. The climatic conditions were, of course, the same, as

the two crops were only a few hundred yards apart. However, the plants sown on the plateau did not grow higher than 1.50 meters; the others grew to 2 meters.

Drug-producing selections grow better and produce more drugs in the tropics; oil and fiber-producing plants thrive better in temperate and subtropical areas. The form of the plant and the yield of fiber from it vary according to climate and particular variety. Varieties cultivated for their fibers have long stalks and branches very little and yield only small quantities of seed. Oil seed varieties are small, mature early, and produce large quantities of seed. Varieties grown for medicinal purposes are small, much-branched with smaller dark-green leaves. Between the three main types of plants are numerous varieties that differ from the main one in height, the extent of branching, and other characteristics.

The unique pharmacological properties of *Cannabis* are due to the presence of cannabinoids, a group of more than 100 natural products that mainly accumulate in female flowers. Delta-9-tetrahydrocannabinol is the principle psychoactive cannabinoid and the compound responsible for the analgesic, antiemetic and appetite-stimulating effects of *Cannabis*. Nonpsychoactive cannabinoids such as cannabidiol, cannabichromene, and delta-9-tetrahydrocannabivarin, which possess diverse pharmacological activities, are also present in some varieties or strains. Cannabinoids are synthesized as carboxylic acids and upon heating or smoking decarboxylate to their neutral forms; for example, delta-9-tetrahydrocannabinolic acid is converted to delta-9-THC.

Cannabinoid content and composition is highly variable among *Cannabis* plants. There are large differences in the minor cannabinoid constituents within the basic chemotypes. Breeding of *Cannabis* for use as a drug and medicine, as well as improved cultivation practices, has led to increased potency with median levels of delta-9-THC in dried female flowers of ca. 11% by dry weight; levels in some plants exceed 23%. This breeding effort, largely a covert activity by *Cannabis* growers, has produced hundreds of strains that differ in cannabinoid and terpenoid composition, as well as appearance and growth characteristics.

Origin and Distribution

Cannabis has been used as a fiber, food, and drug plant. It is among the oldest economic plants. Varieties have evolved through natural selection within the diverse environment into which it has been introduced. There are three population types of *Cannabis* plants based on their natural origins and their association with humans: wild, cultivated, and those that grow spontaneously in areas associated with humans, either derived from truly wild populations or feral escapes from cultivation.

From its site of domestication in Central Asia, the cultivation of *Cannabis* spread in ancient times throughout Asia and Europe and is now one of the most widely distributed cultivated plants. Fibers were used for textile production in China more than 6000 years ago. Archaeological evidence for the medicinal use of *Cannabis* has been found in a 2700-year-old tomb in north-western China and a Judean tomb

from 1700 years ago. *Cannabis* use is increasingly recognized in the treatment of a range of diseases such as multiple sclerosis and conditions with chronic pain.

The Southern Himalayan foothills from Kashmir through Nepal and Bhutan to Burma and northeastern India have been identified as the region of spontaneous growth where it has been extensively utilized. *Cannabis* has a long and rich history in that part of the world and can still grow wild in many areas, particularly in regions at the foothills of the Himalayas.

The Sanskrit word for *Cannabis* – “bhang” or “bhanga” – is believed to have been the source for the Arabic word “benj” and Persian “beng.” *Cannabis* in ancient Indian societies comes largely from ancient Hindu scriptures known as the Vedas. The sacred texts were recorded between 3500 and 2500 years ago. Before that, it is believed that the teachings of the Vedas were passed from master to student for generations and memorialized to keep the original message intact. In the Vedas, *Cannabis* is listed as one of the five sacred crops alongside barley and soma (an unidentified plant of which the juice was a ritual drink). It is believed that the plant has been used as part of Ayurveda (traditional medicine) since the Vedic Period, and it is largely associated with the god Shiva.

The two dominant species are *Cannabis sativa* and *Cannabis indica*. It is assumed that an area with the greatest diversity within a species is also the area in which the natural origin of the species occurred, rather than recognizing that such variation may be derivative instead of ancestral. If *Cannabis* was introduced to the southern slopes of the Himalayas Mountain range and intensely cultivated, the evolution of wide varieties through artificial selection and hybridization by humans and ecological variation may have occurred subsequent to its introduction. Although *Cannabis* grows spontaneously throughout Eurasia, not necessarily as a native plant, it seems unlikely that the genus originated north and south of the mountain ranges.

Human migrations spread *Cannabis* throughout the Himalayan and Hindu Kush Mountains early in prehistory, probably sometime after the beginning of the Holocene or earlier as modern humans first began their advance across Eurasia. In China, the oldest document relating to *Cannabis* is a Chinese treatise, the *Rh-ya*, of the fifteenth century B.C.E. It describes two varieties, one producing seed and the other only flowers. There is also evidence of *Cannabis* being used by Assyrians in the eighth century B.C.E. to produce intoxication. They called it Quonoubou Qunnapu (cf. the Hebrew Qanneb, the Arabic Qannob and Qinnab, the Persian Quonnab, the Celtic Quannab, and the Greek Kannabas).

The use of *Cannabis* spread to the Tigris and Euphrates regions, Iran, and Arabia. *Cannabis* appears to have been unknown to the Egyptians and the Hebrews, as there is no mention of it either in hieroglyphic texts or in the scriptures and no *Cannabis* has been found in ancient tombs. Later, *Cannabis* spread to the Ionian Archipelago and Hellas. It has been claimed that *Cannabis* could be identified with the Nepenthes of Homer (Odyssey, Book IV, 219–232). During Herodotus (Historiae, IV, 75), *Cannabis* appears in Greek literature. Herodotus says that the plant is cultivated in Scythia and Thrace and that the inhabitants of those countries make clothing from it

and intoxicates them by breathing the vapors given off from the seeds when roasted on white-hot stones. That is a clear allusion to the inebriating properties of *Cannabis*.

Diodorus Siculus relates that the Egyptian women of Thebes dispel anger and sorrow with the help of a potion based on *Cannabis*. The Greek physicians, however, were ignorant of the properties of the plant, for Dioscorides (IV-5) speaks only of the emollient qualities of its seeds. *Galen* and his contemporaries were a little more informed.

Among Latin writers, Pliny (Hist. Nat. XIX., 2, 9, and XX, 23), *Columella* (De re rustica, II), Lucilius, and Celsius, mentioned *Cannabis*. In the East, on the other hand, it was already known for its intoxicating qualities. The Sanskrit work Zend Avesta, composed in Northern Iran about six centuries before the Christian era, mentioned *Cannabis* (Cadaneh) and its inebriating resin. The Fourth Book of the Vedas refers to it sometimes under the name of Vijahia (source of happiness) and sometimes under that of Ananda (laughter-provoker). It was not, therefore, for its textile properties that *Cannabis* was used in India; at the beginning of the Christian era, the use of its fiber was still unknown there. It is solely to its inebriating properties that *Cannabis* owes the signal honor of being sung in the Vedas, and it was probably the peoples of Northern Iran who discovered those properties, for they were already using the leaves (Cheng) and the resin (Cers) as inebriants and narcotics before the Hindus. Thus, through the Iranian tribes, the priestly class in India – the only educated class at that time – learned of the properties of *Cannabis*.

Some documents contain information about the part played by *Cannabis* in certain religious ceremonies in India. Kaempfer, a doctor in the service of the East India Company, said:

In Malabar, at the time of the sacrifices in honor of Vishnu, virgins pleasant to behold and richly adorned were brought from the temple of the Brahmins. They came out in public to appease the god who rules over plenty and fine weather. To impress the spectators, the young women were previously given a preparation with a basis of hemp and datura. When the priest saw, by certain symptoms, that the action of the drugs was about to show itself, he began his invocations. The Devadassy (servants of the gods) then danced, leapt about yelling, contorted their limbs, and, foaming at the mouth, their eyes ecstatic, committed all sorts of eccentricities. Finally the priests carried the exhausted virgins into the sanctuary, gave them a potion to destroy the effect of the previous one, and then showed them again to the people in their right mind so that the crowd of spectators might believe that the demons had fled and the idol was appeased.

It was also through religious sects that *Cannabis* became known in Asia Minor and Syria. While it is easy to show that the priestly classes knew of the properties of *Cannabis* and used it, it is difficult to establish where and how the common people learned about its use as an inebriant.

In Hindustan, when the secret of the priests was revealed, *Cannabis* was used solely for the preparation of potions. The Brahmins appear to have attempted to control its use. They authorized it only on the occasion of certain important religious celebrations (Kali festivals, Druga-Puja). It is not known whether the people readily accepted the restriction of their consumption of *Cannabis* potions to the permitted dates; nor if it was precisely in order to gratify the passion for the

intoxicating drug while at the same time respecting the law promulgated by the ministers of the divinity, that the custom of smoking *Cannabis* arose. The practice, more attractive, quicker in its effects, and less dangerous, spread with great rapidity, and smokers form the great majority of the hashish users of India.

Persia, Asia Minor, Egypt, and other countries, which became acquainted with the use of *Cannabis*, learned simultaneously to ingest it and smoke it. The great impetuous wave from Asia, which, beginning in the seventh century, flooded Syria and then Northern Africa with believers, brought the use of hashish in its wake. Doctors of the period frequently used it as a remedy. Iraq became acquainted with it in the reign of the Caliph Mustansir Billah in the thirteenth century. The Arab invasions probably introduced it into Northern Africa and made it known in Spain, where its use was not established. In the Muslim world, despite the repeated prohibitions of the imams and the edicts and penalties of the emirs and sultans, hashish triumphed, Meunier said:

Every Oriental is at once an artist and a metaphysician; he has the gift of conceiving forms and unity, the abstract in the concrete; but he has a great disdain for works and a lazy disinclination to act; he is an artist only for himself, whence his taste for intoxicants and especially for hashish, which conjures up visions of dazzling forms, of which each possesses an intense emotive coefficient.

In Europe, *Cannabis* was known only for its textile properties. Occasionally its seeds were used as medicine. In the sixteenth century, more was learned about *Cannabis* in Europe; it was the age of colonialism. Indian *Cannabis* has then reached Europe. The plant was drawn by H. van Rheede (*Hortus Malabaricus*, 1678–1692), who states that the natives of Malabar intoxicate themselves by smoking its leaves. More information was given in the *Herbarium Am-boynense*, published at Hanau about 1690 by Rumphius, Councilor to the Dutch East India Company at Amboyna. He described the differences between Indian *Cannabis* and Europe; appearance, size, resin production, etc. He notes that *Cannabis* is smoked with tobacco and dispels melancholy and produces pleasant dreams, but its effects vary with the individual and occasionally provoke a kind of madness.

In 1692, Kaempfer tried the effects of *Cannabis* preparations on himself and his companions during a cruise in the Persian Gulf. Chardin, in his *Voyage en Perse et autres lieux de l'Orient*, written about 1711, stated that various compounds (banghi, buengh) with a basis of Indian *Cannabis*, poppy, and *Nux vomica* are used in those regions. Linnaeus, in 1762, in *Amaenitates Academiae*, described the effects of hashish. He regards *Cannabis* as a narcotic, whereas Murray (*Apparatus Medicamentorum*, 1787) stated it is a stimulant and aphrodisiac.

After Bonaparte's campaign in Egypt, attention was drawn to the special properties of *Cannabis*, owing to the works and observations of the scientists who accompanied the expedition. Silvestre de Sacy, Rouyer, and Desgenettes studied hashish on the spot. In contrast, others, like Virey, examined substances brought back from Egypt, and Lamarck studied samples brought from India by the botanist Sonnerat. As a result, various memoranda were published in France, where Indian *Cannabis* was studied more thoroughly than ever before.

Attention has thus been drawn to the plant; it was discovered that it was used in several countries. Von Martius (1825) is the first to mention its use in America by the people of Brazil. The first experiments in the therapeutic use of *Cannabis* were made in 1838, in Calcutta, by O'Shaughnessy; at the same time, Raleigh, Esdale, and O'Birest attempted to carry out a chemical study of the resin extracted from the plant.

In 1840, Moreau de Tours produced a remarkable work on the physiological action of hashish. Leaving the pharmacology of *Cannabis* aside, he studied only its action on the mental faculties, comparing hashish intoxication with a condition of artificially-induced madness (*Du hachich et de l'aliénation mentale*, Paris, 1845).

Improvements in laboratory equipment and progress in research techniques soon made it possible for scientists to direct their activities along less uncertain paths. Many chemists set to work with great keenness, anxious to isolate the active elements in *Cannabis*. At the same time, physiologists and physicians determined to study the effects of *Cannabis* on man and the addiction that it produces.

Cannabis is now widespread in tropical and temperate regions. It has been cultivated for over 4000 years for different purposes, such as fiber, oil, or narcotics. The oldest use of *Cannabis* seems to be for fiber, and later the seeds began to be used for culinary purposes. Plants yielding the drug seem to have been discovered in India, where it was cultivated for medicinal purposes as early as 3000 years ago. In medieval times, it was transplanted to North Africa, where it has been cultivated exclusively for hashish.

If *Cannabis* is to be cultivated for resin production, it should be grown in a hot, dry environment where there is no rain at the time of flowering and fruiting. The fiber produced is practically useless industrially, being hard, short and brittle. A hot, damp environment must be chosen to obtain textile fiber with a high industrial value. In temperate climates, valuable fiber is also obtained, but the crops are always better in hot, rainy years. *Cannabis* cultivated in the plains gradually loses the property of supplying active resin; a certain altitude is required for resin production.

It is essential, particularly in the case of *Cannabis* grown in a hot, damp environment, if the crop is for textile fiber, the crop should be cut when the male plants start flowering. At the time of flowering, *Cannabis* devotes its whole energy to the perpetuation of the species. The fibers do not develop anymore, and the yield of textile fiber will not increase. It is therefore better to harvest at the period when the production of resin – if it takes place – is still slight. The method has the disadvantage of sacrificing the seed, the market value of which is far from negligible. One solution is allowing cultivation to obtain seeds for industrial purposes in temperate districts, where very little resin is produced.

Chapter 2

The Bioactive Components of Plants



Humans, in most cultures, have been using natural substances as a primary source of medicine until the discovery of modern medicine in the nineteenth century. With developments in chemistry, it became possible to closely examine plant materials and identify biologically active constituents to understand their mechanisms of action. In 1804, Sertürner purified morphine from *Papaver somniferum* and found that it produced the opium's analgesic and sedative effects. He called the isolated alkaloid "morphism" after the Greek god of dreams, Morpheus. His success led others to seek the active principles of plants. A few years later, quinine was purified from *Cinchona* and cocaine from *Erythroxylum coca*. Those have been the most fruitful approach for validating ancient cultures' use of plants for medicine.

With the development of microbial resistance to antibiotics and the emergence of other viruses and bacteria, there has been a revival of the quest for natural therapeutics. The synthesis of salvarsan as an arsenic-based drug for syphilis in 1910, the development of the sulfa drug prontosil in 1935, and penicillin purification and production in the 1940s set up the paradigms for the exploration of new drugs. Fleming's identification of the antibacterial properties of penicillin in 1929 revolutionized the treatment of bacterial infections by natural products. It led to the extensive screening of microbes, particularly soil actinomycetes and fungi, to identify other antibiotics. Large numbers of the original agents were discovered and, together with chemical derivatives, were adequate for treating infectious diseases.

It has been recognized that plants contain either harmful or toxic substances. The Paracelsian dictum "*sola dosis fecit venenum*" has been appreciated since ancient times. Many highly toxic materials used for lethal purposes have also found applications in modern medicine throughout the ages. However, it is wrong to think that plant toxins are likely to adversely affect any and every form of life. A common feature of most toxins is that they are also known for their curative properties, and although they may cure an individual's disease at one dose, they may cause the death of the same individual at another dose. Poisons are widespread in plants, and

humans have tried to eliminate them or convert them to their advantage. By their very nature, poisons are biodynamic substances that affect the functioning of the body. It means that they have been, and continue to be, essential sources of medicine.

The bioactive compounds that plants produce have pharmacological and toxicological effects. Although nutrients in plants also elicit effects, they are generally not categorized as bioactive compounds; the typical bioactive compounds in plants function as secondary metabolites. Secondary metabolites are compounds that are not required for the growth or reproduction of the plant but are produced to confer a selective advantage to the plant. Secondary metabolites may inhibit the growth of organisms with which the plant competes. As such, they often inhibit critical biological processes. Fungal secondary metabolites constitute a significant source of medically essential compounds, from antibiotics such as penicillin to the anticholesterol compound lovastatin.

The sequencing of the genomes of *A. nidulans* and other *Aspergillus* species revealed that they have many genes predicted, based on sequence, to be involved in secondary metabolism [157]. The genes are clustered, and the individual clusters generally encode the genes for a single secondary metabolite biosynthetic pathway. Most of the clusters are cryptic; they are not expressed under normal laboratory growth conditions. The secondary metabolite biosynthetic clusters are usually a significant source of valuable compounds.

Flavonoids

Plants produce flavonoids that have a range of biological activities, such as antioxidant, anti-inflammatory, anticancer, antibacterial, antifungal, and antiviral activities. Natural flavones include apigenin, baicalein, chrysin, luteolin, scutellarein, tangeritin, wogonin, and 6-hydroxyflavone. The antiviral activity of flavones was revealed when the simultaneous application of apigenin with acyclovir resulted in an enhanced antiviral effect on herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) in cell culture [1].

More than twenty flavonoids have been identified in *C. sativa*, most of which are flavone (apigenin and luteolin) and flavonol (kaempferol and quercetin) aglycones and glycosides. Three prenylated/geranylated flavones, cannflavin A, B, and C, were isolated in *C. sativa*. Although cannflavins are often referred to as flavonoids unique to *C. sativa*, cannflavin A has also been identified in *Mimulus bigelovii*, a plant in the *Phrymaceae* family.

Apigenin (4',5,7-trihydroxyflavone) is a flavone under the category of natural flavonoid that is abundantly present in common fruits, vegetables, nuts, onions, oranges, and tea. Apigenin has various beneficial health effects such as antioxidant, anti-inflammatory, and chemoprevention. Apigenin can downregulate the expression of IL-1 β and TNF- α in LPS-stimulated mouse macrophages and human monocytes. It has anti-inflammatory activity through the inhibition of COX-2 and iNOS

expression in LPS-stimulated mouse macrophages. It can suppress inflammatory responses by inactivating NF-kappa B. Apigenin can attenuate the cellular-mediated inflammatory response by reducing neutrophil and lymphocyte adhesion to endothelial cells. Adhesion of monocytes to human umbilical vein endothelial cells (HUVECs) also occurs by regulating the expression of ICAM and VCAM. In addition, apigenin also inhibits allergen-induced airway inflammation. The studies suggest that apigenin-mediated anti-inflammatory effects are associated with several intracellular signaling pathways, such as NF- κ B, MAPK/ERK, and JNK [133].

Apigenin is an aglycone of several naturally occurring glycosides. It is abundant in the ligulate flowers of the chamomile plant and is found in low concentrations in other sources, such as celery, parsley, and grapefruit. Concentrations of apigenin in rats reached 1.2 μ M after daily intraperitoneal administration of 20 mg/kg apigenin potassium. The compound is reported to cross the blood–brain barrier, and even at high doses, no severe toxicity was observed.

Apigenin inhibits IL-6 in mouse macrophages, suppresses the CD40 marker, and lowers TFN- α and IL-6 production by downshifting IFN gamma-induced phosphorylation of STAT-1 in murine microglia [141]. In human monocytes and mouse macrophages, apigenin has been shown to attenuate the release of inflammatory cytokines by inactivating nuclear factor- κ B (NF- κ B) [140].

Apigenin is commonly isolated from the family Asteraceae. The organic and aqueous extracts of Asteraceae plants with apigenin were active against HSV-1, poliovirus type 2, and hepatitis C virus (HCV) [2, 3]. Apigenin isolated from *Ocimum basilicum* produced a potent antiviral activity against adenoviruses (ADV) and hepatitis B virus in vitro [4].

Apart from the DNA viruses, apigenin was found to exert an antiviral effect against the African swine fever virus (ASFV) by suppressing the viral protein synthesis and reducing the ASFV yield by 3 log [5]. Apigenin is also active against RNA viruses. For picornaviruses, it has been shown that apigenin inhibits viral protein synthesis by suppressing viral internal ribosomal entry sites (IRES) activity [6, 7]. Apigenin affects enterovirus-71 (EV71) translation by disrupting viral RNA association with trans-acting factors regulating EV71 translation [8, 9]. It was demonstrated that apigenin has an antiviral effect on HCV by reducing mature microRNA122, a liver-specific microRNA that positively regulates HCV replication.

The in vitro inhibitory activity of methyl alcohol extract derived from leaves and resin of *Cannabis* against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) revealed a binding the affinity of selected phytochemicals of *Cannabis* to AChE and BChE. Phytochemicals of *Cannabis* have a binding affinity toward AChE ranging from -6.4 kcal/mol (beta-pinene) to -11.4 kcal/mol (campesterol) and BChE is ranging from -5.5 kcal/mol (alpha-pinene) to -9.8 kcal/mol (cannabioxepane). All cannabinoids, apigenin, terpenes, and phytosterols of *Cannabis* are dual inhibitors due to their hydrogen bonding and hydrophobic interaction with both catalytic triad and peripheral anionic site (PAS) of AChE and BChE. Delta-9-tetrahydrocannabinolic acid has been docked with AChE and BChE.

Baicalein and Luteolin Baicalein has been reported to have various pharmacological activities, such as anticancer, antioxidation, antiallergy, antiviral, and anti-inflammatory activities. Baicalein is also one of the active ingredients of a Japanese herbal supplement believed to be able to improve the liver health. It is highly insoluble in acidic medium and soluble in alkali medium but unstable. Poor solubility in the gastric fluid and instability in the intestinal fluid greatly limit its oral bioavailability and clinical efficacy.

Studies evaluating the antibreast cancer and estrogen receptor- α inhibitory activity of *Cannabis* have been reported to be effective in inhibiting breast cancer cell proliferation. The phytoconstituents of *Cannabis*: naringenin, dihydroresveratrol, baicalein, apigenin, and cannabitol have relatively better inhibitory activity than tamoxifen.

The main flavonoids found in *Cannabis* are luteolin-7-O-glucuronide and apigenin glucuronide. Baicalein significantly reduced the levels of human cytomegalovirus (HCMV) early and late proteins and viral DNA synthesis, although it did not affect viral polymerase activity [10, 11]. Baicalein impaired avian influenza H5N1 virus replication in human lung epithelial cells and monocyte-derived macrophages by interfering with neuraminidase activity [12].

Oral administration of baicalein to BALB/c mice infected with influenza H1N1 virus decreased the lung virus titer and increased the meantime to death [13]. Similar effects were recorded on mice infected with the Sendai virus [14]. The inhibitory effects in vivo were mediated by serum baicalin, a metabolite of baicalein with a glucose residue [15]. Baicalin exerts anti-influenza activity by modulating the function of NS1 protein, which downregulates IFN induction [16]. Baicalin directly induces IFN- γ production in human CD4⁺ T cells and CD8⁺ T cells and acts as a potent inducer of IFN- γ during influenza virus infection [17]. Novel baicalein analogs with B-rings substituted with bromine atoms demonstrated potent activity against influenza H1N1 Tamiflu-resistant virus, indicating that baicalein and its analogs can be favorable alternatives in managing Tamiflu-resistant viruses [18].

In vitro replication of HIV-1 was suppressed by baicalin when infected cells were treated early in the virus replication cycle [19]. HIV-1 envelope protein was the target site of baicalin's antiviral action by interfering with interactions between the virus structural protein and specific host immune cells [20].

Baicalein and baicalin were also investigated against the dengue virus (DENV). They exerted a significant virucidal effect on extracellular viral particles and interfered with different steps of DENV-2 replication [21, 22]. In silico studies revealed that baicalein has a strong binding affinity with DENV NS3/NS2B protein (−7.5 kcal/mol), and baicalin may interact closely with the virus NS5 protein at a binding affinity of −8.6 kcal/mol [24]. For baicalin, computational studies also showed a high binding affinity (−9.8 kcal/mol) against the chikungunya virus (CHIKV) nsP3 protein, suggesting that baicalin can potentially interfere with CHIKV infection [25].

Luteolin Luteolin has an antiviral effect on HIV-1 reactivation by blocking both clade B- and C-Tat-driven LTR transactivation [26]. Luteolin also indicated

significant inhibition of Epstein–Barr virus (EBV) reactivation in cells; it suppressed the activities of the immediate-early genes Zta and Rta by deregulating transcription factor Sp1 binding [27, 28]. Tests were conducted on 400 highly purified natural compounds to inhibit EV71 and coxsackievirus A16 infections. It was found that luteolin exhibited the most potent inhibition through disruption of viral RNA replication. Besides those antiviral activities, luteolin or luteolin-rich fractions showed antiviral effects against severe acute respiratory syndrome coronavirus (SARS-CoV), rhesus rotavirus, CHIKV, and Japanese encephalitis virus (JEV) [29–32].

Quercetin Quercetin-type flavonols are widely distributed in plants. They are found in various foods, including apples, berries, Brassica vegetables, capers, grapes, onions, shallots, tea, and tomatoes, as well as many seeds, nuts, flowers, bark, and leaves. One study found that organically grown tomatoes had 79% more quercetin than chemically grown fruit quercetin in various kinds of honey from different plant sources. In the determined food, the highest concentration is 234 mg/100 g of edible portion in capers (raw). The lowest concentration is 2 mg/100 g of edible portion in black or green tea (*Camellia sinensis*). The estimated flavonoid intake ranges from 50 to 800 mg/day (quercetin accounts for 75%), mainly depending on fruits, vegetables, and tea intake.

Quercetin displayed minimal competition with [³H]CP55,940 at CB1R but surprisingly produced a concentration-dependent and potent antagonistic inhibition of CB1R- and delta-9-THC-mediated inhibition of cAMP. Quercetin, have all been shown to have widespread and nonspecific antioxidant, lipid raft, and transcription factor modulatory effects that interfere with G protein-coupled receptors (GPCR) dependent signaling and trafficking. When exploring a subset of the phytomolecules in vivo, curcumin's and quercetin's effects are congruent with in vitro observations where both phytomolecules inhibited delta-9-THC-dependent catalepsy, hypothermia, and antinociception.

Quercetin exerts its anti-HSV activity by inhibiting the infection of HSV-1, HSV-2, and acyclovir-resistant HSV-1, mainly by blocking viral binding and penetration to the host cell. In vivo studies indicated that oral treatment with quercetin protected mice from the lethal Mengo virus [33]. Enhanced protection was observed when quercetin was administered in combination with murine type I interferon (IFN) [34]. Quercetin demonstrated a dose-dependent antiviral activity against poliovirus type 1, HSV-1, HSV-2, and respiratory syncytial virus (RSV) in cell cultures [35, 36]. *Epimedium koreanum* Nakai, which contains quercetin, has been shown to induce secretion of type I IFN, reducing the replication of HSV, Newcastle disease virus (NDV), vesicular stomatitis virus (VSV) in vitro, as well as influenza A subtypes (H1N1, H5N2, H7N3, and H9N2) in vivo [37, 38].

Quercetin suppresses NF-κB activation, which is essential for HSV gene expression. Quercetin interacts with influenza hemagglutinin protein, thereby inhibiting viral-cell fusion [39]. In silico analysis revealed that quercetin might be a potent inhibitor of the neuraminidase of influenza A H1N1 and H7N9 viruses [40, 41].

Molecular docking analysis found that quercetin may interact with HCV NS3 helicase, NS5B polymerase, and p7 proteins [42, 43]. The results correlate with studies indicating the anti-HCV activity of quercetin by inhibiting NS3 helicase and heat shock proteins [44, 45]. The inhibitory activity of quercetin and its derivatives have been indicated for ADVs, arthropod-borne Mayaro virus, porcine reproductive and respiratory syndrome virus, canine distemper virus, JEV, DENV-2, porcine epidemic diarrhea virus, and equid herpesvirus [22, 46–52]. Quercetin also possesses antirhinoviral effects by inhibiting endocytosis, transcription of the viral genome, and viral protein synthesis. In mice infected with rhinovirus, quercetin treatment decreased viral replication and attenuated virus-induced airway cholinergic hyper-responsiveness [53].

Kaempferol Produced by the *Kaempferia* species, kaempferol is used widely in folk medicine, including *K. parviflora*, *K. pulchra*, and *K. galangal*. Traditional medicines derived from *K. parviflora* rhizomes are reported to treat inflammation, hypertension, erectile dysfunction, and abdominal ailments and improve vitality and blood flow. Japanese use the extract of *K. parviflora* as a food supplement for treating metabolic disorders. *K. pulchra* is used extensively as a carminative, diuretic, deodorant, and euglycemic for treating urinary tract infections, fevers, and coughs. The rhizomes of *K. galanga* are used as an antitussive, expectorant, antipyretic, diuretic, anabolic, and carminative and for curing gastrointestinal ailments, asthma, wounds, rheumatism, epilepsy, and skin diseases.

Kaempferol and its derivatives bearing acyl substituents have shown inhibitory activity against HCMV [54]. Kaempferol derivatives isolated from *Ficus benjamina* leaves were more effective against HSV-1 and HSV-2 than their aglycon form [55]. Kaempferol derivatives with rhamnose residue are potent inhibitors of the 3a channel of coronavirus, which is involved in the mechanism of virus release [56]. One of the kaempferol derivatives, kaempferol 3-O- α -L-rhamnopyranoside, obtained from *Zanthoxylum piperitum*, significantly inhibited the replication of influenza A virus in vitro [57]. Kaempferol and kaempferol-7-O-glucoside have strong HIV-1 reverse transcriptase inhibitory activity [58]. The compounds exerted their effects, at a concentration of 100 μ g/mL, on the early stage of HIV replication in target cells.

Kaempferol-3,7-bisrhamnoside isolated from *Taxillus sutchuenensis* has potent in vitro activity on HCV NS3 protease function [59]. Mechanistic and structural studies indicated that the compound acts on the virus neuraminidase protein, and specific functional groups are responsible for kaempferol's efficacy [60]. The antiviral activity of kaempferol on the influenza viruses H1N1 and H9N2 was supported in a study conducted by researchers in South Korea.

A study comparing the antiviral activities of kaempferol and the isoflavone daidzein indicated that kaempferol exerted more potent inhibitory activities on JEV replication and protein expression than daidzein. JEV's frameshift site RNA (fsRNA) has been proposed as the target site for kaempferol's inhibitory activity against this flavivirus [61]. A study compared the potency of different classes of flavonoids against murine norovirus and feline calicivirus. The findings demonstrated that

among the flavonoids tested, kaempferol exhibited the most potent inhibitory activity against the viruses [62].

Rutin, Myricetin, and Fisetin Sulfated rutin, modified from glycoside rutin, demonstrated significant activity against different HIV-1 isolates [63]. The compound inhibited HIV-1 infection by blocking viral entry and virus–cell fusion, likely by interacting with HIV-1 envelope glycoproteins. Rutin at 200 μ M concentration inhibited EV71 infection by suppressing the activation of the MEK1-ERK signal pathway, which is required for EV71 replication [64]. Rutin and fisetin also inhibited the replication of EV-A71 by affecting the enzymatic activity of the 3C protease [65]. Fisetin treatment produced a dose-dependent decrease in CHIKV nonstructural proteins and inhibition of viral infection [66]. One study showed that the DENV-2 RNA copy number was significantly reduced after adding fisetin to infected cells [23]. It was also found that myricetin is a chemical inhibitor of SARS-coronavirus because it affects the ATPase activity of the viral helicase [67].

Catechin Catechin represents the chemical family name of the compounds derived from catechin. Catechin is derived from catechu of the extract of *Acacia catechu* L. (3,3',4',5,7-pentahydroxyflavone). Catechins are distributed in various foods, including tea, apples, persimmons, cacaos, grapes, and berries. Tea from the leaves and buds of *Camellia sinensis*, is one of the richest catechin sources and contains, as the major catechin, – epigallocatechin-3-gallate (EGCG), which has many beneficial properties for human health, such as anticancer, antiobesity, antidiabetic, antiscavenger, anti-infectious, hepatoprotective, and neuroprotective effects.

Several human epidemiological and clinical studies on tea have provided evidence of its anticancer benefits. In addition, detailed molecular mechanisms have been proposed for the action mechanism of EGCG and other catechins. One of the most attractive mechanisms is the one in which reactive oxygen species (ROS) are involved. EGCG is known to have dual actions, with ROS as an antioxidant and a pro-oxidant. Several lines of evidence have indicated that EGCG can eliminate ROS by scavenging and enhancing ROS production.

The antiviral activity of catechin and its derivatives epicatechin, epicatechin gallate, epigallocatechin (EGC), and epigallocatechin gallate (EGCG) found in the tea has been widely investigated [68]. Among different viruses studied as potential targets, the influenza virus has received the most attention after a report indicating that tea catechins, particularly EGCG, can bind to the hemagglutinin of the influenza virus, preventing its adsorption to Madin-Darby canine kidney cells [69]. EGCG may also interfere with the viral envelope's physical properties, inhibiting hemifusion events between the influenza virus and the cellular membrane [19]. EGCG competes with sialic acid for binding to the influenza A virus, thereby blocking the primary low-affinity attachment to cells [70].

Another tea catechin, EGC, exerted the inhibitory effect on the acidification of endosomes and lysosomes, thereby reducing viral entry via clathrin-mediated endocytosis [71]. Structure–function relationship analysis of tea catechins revealed the vital role of the 3-galloyl group of the catechin skeleton in its antiviral activity [72].

The results also indicated that modification of the 3-hydroxyl position significantly affected the antiviral activity. Catechin derivatives containing carbon chains at the 3-hydroxyl position demonstrated potent anti-influenza activity *in vitro* and *in ovo* [73].

It has been demonstrated that tea catechins have an antiviral effect against HIV infection. EGCG is the most effective in its antiviral effect throughout several steps of the HIV-1 life cycle among tea catechins. It directly binds to CD4 molecules with consequent inhibition of gp120 binding, an envelope protein of HIV-1 [74, 75]. Trp69, Arg59, and Phe43 of CD4 are the potential sites for interaction with the gallate moiety of EGCG. The identical residues are involved in interaction with viral gp120 [76]. Studies indicated that EGCG and ECG effectively inhibited HIV-1 reverse transcriptase *in vitro* [77, 78]. The molecular structure of EGCG was modified to determine the minimum structural characteristics necessary for HIV-1 reverse transcriptase inhibition [79]. In that study, the gallate ester moiety was involved in the inhibition. The EGCG also can reduce viral production in chronically infected monocytoid cells [80]. The inhibitory effect was increased by approximately 25% when EGCG was modified with liposomes.

Tea catechins are effective against herpes viruses. EGCG has been shown to block EBV lytic cycle by inhibiting the expression of viral genes, including Rta, Zta, and EA-D [81]. Studies indicated that one of the mechanisms by which EGCG may inhibit the EBV lytic cycle involves the suppression of MEK/ERK1/2 and PI3-K/Akt signaling pathways involved in the EBV lytic cycle cascade [82]. Isaacs et al. found that EGCG can inactivate HSV virions by binding to the envelope glycoproteins gB and gD, which are essential for HSV infectivity [83]. The EGCG digallate dimers theasinensin A, P2, and theaflavin-3,3'-digallate inactivated HSV-1 and HSV-2 more effectively than monomeric EGCG [84]. The dimers are stable at vaginal pH, indicating their potential to be antiviral agents against HSV infections.

The inhibitory effect of green tea extracts against hepatitis B virus (HBV) has been confirmed [85]. In HepG2 cells, EGCG inhibited HBV replication by impairing HBV replicative intermediates of DNA synthesis, thereby reducing the production of HBV covalently closed circular DNA [86]. In contrast, it was found that EGCG decreased HBV entry into immortalized human primary hepatocytes by more than 80% but had no effect on HBV genome replication [87]. EGCG can also enhance lysosomal acidification, an unfavorable condition for HBV replication [88].

EGCG has been found to exert antiviral activity against HCV by preventing the attachment of the virus to the cell surface and suppressing RNA replication steps [89, 90]. One study demonstrated the inhibitory activity of EGCG against another flavivirus, the Zika virus (ZIKV). In that study, foci forming unit reduction assays were performed to evaluate the antiviral activity of EGCG on ZIKV at different stages of virus replication. Foci observed indicated more than 90% inhibition when the cells were treated with EGCG during virus entry [91]. Similarly, EGCG can block CHIKV attachment to target cells but does not affect other stages of infection [92].

Naringenin and Hesperetin Naringenin, belonging to the flavanones class, has been shown to reduce the replication of a neurovirulent strain of the Sindbis virus in vitro [93]. It reduced Sindbis virus- and Semliki Forest virus-induced cytopathic effect in virus yield experiments [94]. Naringin, the glycoside form of naringenin, did not have anti-Sindbis virus activity, indicating that the rutinose moiety of this flavanone blocks its antiviral effect. Naringenin can block the assembly of intracellular HCV particles, and long-term treatment leads to a 1.4 log reduction in HCV [95, 96]. The alphavirus CHIKV was effectively inhibited when infected Vero cells were treated with naringenin at the postentry stage. In that study, hesperetin, another flavanone found in citrus fruits, exerts the most potent anti-CHIKV effect during the virus intracellular replication, with an IC_{50} of 8.5 μ M [97]. Molecular docking and molecular dynamics studies revealed strong and stable interactions between hesperetin and CHIKV nonstructural protein 2 (nsP2) and nonstructural protein 3 (nsP3), suggesting that the proteins may be the target of hesperetin's anti-CHIKV activity [98].

Genistein A tyrosine kinase inhibitor that is an isoflavonoid found in many plants, including soybeans and fava beans. Genistein exerts its antiviral effects on the replication of HSV-1, HSV-2, and avian leucosis virus subgroup J, by inhibiting virus transcription [104, 105]. Genistein reduced bovine herpes virus type 1 and New World arenavirus Pichinde replication by preventing the phosphorylation of viral proteins [99, 100]. Kinase inhibitor cocktails containing genistein displayed a broad-spectrum antiviral activity against arenaviruses and filoviruses [101]. Genistein inhibited HIV infection of resting CD4 T cells and macrophages through interference with HIV-mediated actin dynamics [102]. It acted against the HIV ion channel since it can block the viral Vpu protein, which forms a cation-permeable ion channel in infected cells [103].

Despite the availability and wide range of biological health benefits that flavonoids possess, the challenge is for the intake of flavonoids to be successfully absorbed and utilized [106]. Factors such as molecular sizes, glycosylation, esterification, lipophilicity, interactions with the enteric microorganisms, pKa, and other metabolic conjugations along the digestive process, affect the absorption and bioavailability of flavonoids in humans [27, 107–112]. Efforts to enhance the bioavailability of flavonoids upon human intake are vitally necessary to develop them into potential antiviral drugs.

While different methodologies can increase the bioavailability of flavonoids, it is vital that their biological efficacies are not affected but maintained or enhanced. Phosphorylated icariin has been found to inhibit duck hepatitis virus A more effectively than the parent compound [113]. Isorhamnetin is a methylated flavonol derived from the structure of quercetin. Investigating the antiviral potency of isorhamnetin against influenza A H1N1 virus revealed that the methyl group on the B ring enhances its antiviral activity compared with the other tested flavonoids. The efficacy of isorhamnetin against the influenza virus was also shown when in vivo and in ovo models [114].

Proteins and Peptides

Plant proteins are an essential source of food. Amino acids are absorbed from the intestinal tract and then built into proteins that exert their specific actions on the body. The Euphorbiaceae, *Ricinus communis*, produces ricin in its seeds. The potent protein (lectin), ricin inhibits protein synthesis and induces systemic effects in humans. Far less potent lectins are also present in seeds of several species of Fabaceae, the bean family. Colic and other gastrointestinal symptoms may occur if seeds are eaten without sufficient cooking, which inactivates many lectins.

A *Cannabis* seed protein isolate, prepared from defatted *Cannabis* seed meals by alkaline solubilization/acid precipitation, was subjected to extensive chemical hydrolysis under acid conditions (6 M HCl). Semipreparative RP-HPLC fractionated the resulting hydrolysate, and the purified fractions were tested as inhibitors of angiotensin-converting enzyme. Mono- and bidimensional NMR experiments and LC-MS analyses led to the identification of four potentially bioactive peptides, i.e., GVLV, IEE, LGV, and RVR. They were prepared by solid-phase synthesis and tested for ACE-inhibitory activity. The IC₅₀ values were GVLV 16 ± 1.5 μM, LGV 145 ± 13 μM, and RVR 526 ± 33 μM, confirming that *Cannabis* seed may be a valuable source of hypotensive peptides.

Cationic peptides possess antiviral activity against HIV [115], avian influenza H5N1 virus, herpes simplex virus [117], and dengue virus [118]. Since the cationic peptides possess antibacterial activity mediated by permeabilization of bacterial membranes, it could be hypothesized that the lipid membrane of enveloped viruses such as the dengue virus may also be a potential target of antimicrobial peptides [119].

The peptides block viral infection at the entry step by binding to heparan sulfate, blocking viral attachment [120, 121]. Some of the cationic peptides also indicated potential inhibition against viral proteases [122].

Plectasin Plectasin is an antimicrobial cationic peptide produced by the fungus *Pseudoplectania nigella*. It is a fungus-derived defensin with therapeutic potential, especially against gram-positive bacteria [127]. The peptide has secondary structures similar to defensins found in other organisms [124]. It consists of 40 amino acids (NH₂-MGFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY-COOH) with three intramolecular disulfide bridges between C1 and C5, C2 and C4, and C3 and C6, which stabilize its alpha helix-beta motif tertiary structure [124, 128].

Plectasin could be considered a candidate for antimicrobial drug development for clinical application due to its low toxicity, immunogenicity, and high stability. It has been shown that plectasin has a low ability to induce cytokine production, such as interleukin 8 (IL-8), and low cytotoxicity against mammalian cells [116]. Low in vivo toxicity was also observed in mice treated with peptides [123]. Immunization studies were used to assess the immunogenic properties of plectasin in mice. After five days of daily subcutaneous injections of plectasin, no antibody response, signs

of hypersensitivity, or injection site reactions were observed [125]. Surprisingly, the antibacterial activity of plectasin was stable over a wide pH range (2.0–10.0). Plectasin also showed high thermal stability (stable at 100 °C for one hour) and remarkable resistance to papain and pepsin [126].

One of the main limitations of using cationic peptides as antiviral drugs is the cost of production by chemical synthesis. As an alternative, producing recombinant peptides in *Escherichia coli* may represent one of the most attractive approaches toward large-scale production. However, *E. coli* almost always produce short peptides insoluble form that requires further purification, which is an obstacle to large-scale production [120, 129]. Five plectasin peptides were designed to be expressed in tandem in *E. coli* as inclusion bodies to solve that problem. They were purified by minimal washing, followed by solubilization, refolding, and cleaving to produce single plectasin peptide units. Subsequent assays on the recombinant plectasin peptide indicated inhibitory activities against dengue NS2B-NS3pro and viral replication in Vero cells.

Glycosides

The glycosides consist of secondary metabolites bound to a mono- or oligosaccharide or uronic acid. The saccharide or uronic acid part is called glycone, and the other is the aglycone. Following ingestion, the glycosides usually hydrolyze in the colon, and the more hydrophobic aglycone is absorbed. The main groups of glycosides are cardiac glycosides, cyanogenic glycosides, glucosinolates, saponins, and anthraquinonoid glycosides. Furthermore, flavonoids frequently occur as glycosides.

Cannabinoids, as well as other valuable bioactive compounds such as glycosidic flavones, may be recovered from the residues of the essential oil extraction, representing medically interesting substances. A study assessed the variables affecting the microwave irradiation extraction process for recovering essential oil, polyphenols, and phytocannabinoids from *Cannabis*. The products obtained using the optimized conditions were characterized by yield, chemical profile, and antioxidant potential. The by-products were further analyzed in terms of their biological activity using enzymatic and nonenzymatic assays. The aqueous residue demonstrated a powerful α -glucosidase inhibitory activity in terms of superoxide radical scavenging activity, a modest efficacy in inhibition of advanced glycation end-product formation, and no activity in terms of lipase inhibition. The residual deterpenated biomass did not possess significant biological activity.

Cardiac Glycosides Have a long history of treating cardiac diseases. However, recent reports have suggested that cardiac glycosides also possess antiviral activities. Cardiac glycosides have developed as potential antiviral agents by targeting cell host proteins to reduce resistance to antiviral therapies, making them a promising approach against human viral infections. Cardiac glycosides were reported effective against several DNA and RNA viral species such as influenza, human

cytomegalovirus, herpes simplex virus, coronavirus, tick-borne encephalitis virus, and Ebola virus. Cardiac glycosides were reported to suppress the HIV-1 gene expression and viral protein translation and alter viral pre-mRNA splicing to inhibit viral replication.

Cardiac glycosides have aglycones with a steroidal structure. Their effect is the inhibition of Na^+/K^+ -ATPase-pumps in the cell membranes. The pumps are critical for the functioning of the cardiac cells, and the effects of the compounds are very pronounced in the heart, resulting in increased contractility and reduced rate. The cardiac glycosides are present in plants of Scrophulariaceae, particularly *Digitalis purpurea*, and in Convallariaceae with *Convallaria majalis*.

Cyanogenic glycosides have aglycones derived from amino acids. Several of the compounds can interfere with iodine utilization and result in hypothyroidism. The other significant effect is their release of hydrogen cyanide, which is very toxic and lethal at high dosages. Cyanogenic glycosides are present in species of Rosaceae, such as in the leaves of *Anacolosia pervilleana*. In one study, extracts of the leaves were studied, and their inhibitory potencies were evaluated on CHIKV, DENV RdRp, and West-Nile polymerase virus (WNV RdRp). The extracts showed moderate activity against CHIKV (EC_{50} 77 μM and 86 μM , respectively), and the acetylenic acids produced IC_{50} values around three μM in the DENV RdRp assay [149].

Glucosinolates Sulfur-containing, aromatic amino acid-derived aglycones that show a complex set of effects on cytochrome P450 isoforms in various cells and decrease hepatic bioactivation of environmental procarcinogens. The glucosinolates can irritate the skin and also induce hypothyroidism and goiter. Brassicaceae is the family mainly associated with glucosinolate production. *Isatidis radix*, the sun-dried root of *Isatidis indigotica*, is one of the most used traditional Chinese medicines. The herb has been used in clinical practices to treat viral infections and inflammation for centuries. *Isatidis Radix-derived* glucosinolate isomers and their breakdown products exhibited dose-dependent inhibitory effects against the influenza A virus (H1N1) without toxicity. The antiviral potency of the components was in the order of progoitrin > goitrin > epigoitrin > epiprogoitrin. The attachment of the constituents to the viral envelope was conducive to the mechanism of their antiviral action without disturbing viral adsorption or budding.

Anthraquinone Glycosides Have a relatively limited distribution within the plant kingdom. In Polygonaceae, they are present, for instance, in *Rumex Crispus* and *Rheum* spp. Their primary effect is the induction of the colon's water and electrolyte secretion and peristalsis.

Flavonoids and Proanthocyanidins **Flavonoids** The compounds contain phenol groups involved in an effect as a general antioxidant. Other actions are diverse; several structures reduce inflammation or carcinogenicity. The group isoflavones are primarily known as phytoestrogens. Flavonoids and proanthocyanidins are all pigments occurring in an extended range of plant families. Isoflavones are produced by the species of Fabaceae, the bean family.

Isoprene A five-carbon building block, synthesizes mono- and sesquiterpenoids and phenylpropanoids. Monoterpenoids consist of two isoprene units and sesquiterpenoids of three units. They are referred to as low-molecular-weight terpenoids and represent the most diverse plant constituents, with more than 25,000 individuals and fifteen compounds identified. The less diverse phenylpropanoid are based on a nine-carbon skeleton and are synthesized by another pathway. Compounds of all three groups are lipophilic and also tend to volatilize readily. They have strong odors and flavors. Their actions vary greatly, a range of which has been utilized in herbal remedies. Of particular importance are antineoplastic, antibacterial, antiviral effects, and gastrointestinal stimulation. They are not associated with toxicity unless they are concentrated as volatile oils. The plant family best known for those compounds is Lamiaceae, but it is also present in other families.

Diterpenoids Composed of four isoprene units (20 carbons). They are very lipophilic and tend to have intense flavors. They are not volatile and, thus, odorless. Much less toxicological information is available on the diterpenoids than on the lower molecular terpenoids. Several of the compounds possess antineoplastic activity. Diterpenoids are found in several plants, among them *Coffea arabica*. Diterpenoids are also typically present in resins.

Alkaloids

The alkaloids are heterocyclic, nitrogen-containing compounds, usually with potent activity and bitter taste. They are of limited distribution in the plant kingdom. The various groups have diverse clinical properties. Two spermidine alkaloids have been identified in *Cannabis*. In 1975, the first spermidine alkaloid was isolated from a methanolic extract of *Cannabis* roots from a Mexican variant grown in Mississippi, and it was identified as cannabissativine by X-ray crystallography. Later the same year, the same compound was isolated from an ethanolic extract of the dry leaves and small stems of a Thailand variant. The ethanolic extract was extracted and acid–base partitioning, as well as subjected to column and thin-layer chromatography followed by crystallization of the alkaloid from acetone.

A year after cannabissativine was reported, the second spermidine alkaloid, anhydrocannabissativine, was isolated from the dry leaves and small stems of *Cannabis* of the Mexican variety grown in Mississippi. The compound was isolated through a series of acid–base extractions and silica-gel chromatography. The identity was proven by spectral data analysis and by the conversion of cannabissativine to anhydrocannabissativine. In 1977, the Mississippi group also identified anhydrocannabissativine in 15 different *Cannabis* variants using TLC eluted with chloroform:acetone:ammonia (1).

Tropane Alkaloids Exist in Solanaceae, for instance, *Atropa belladonna*, *Datura* spp., and *Hyoscyamus niger*. The compounds have anticholinergic activity

(muscarine receptor antagonists) and are used medically to reduce smooth muscle spasms, hypersecretion, and pain.

Pyrrolizidine Alkaloids Produced by Asteraceae, particularly in *Senecio* spp. and Boraginaceae. Their adverse effects in man and animals are hepatotoxicity after bioactivation. Polyhydroxylated pyrrolizidine alkaloids can interact with human immunodeficiency virus (HIV) activity. Australine and alexine, isolated from *Castanospermum australe* and *Alexa leiopetala*, are examples of polyhydroxylated pyrrolizidine alkaloids in concentrations between 0.1 and 10 mM inhibited the activity of glycosidases, particularly the nitrogen-linked glycosylation process of HIV [151]. The event ultimately reduced cell fusion with the virions and, consequently, restricted syncytium formation [152].

A study with alexine and four other pyrrolizidine alkaloids isolated from *A. leiopetala* and *C. australe*, respectively, also produced inhibitory activity against HIV-1 [156]. The positive results were obtained with 7,7a-diepialexine, and an IC₅₀ of 0.38 mM was found. The anti-HIV activity was correlated with the inhibition of pig kidney α -glucosidase 1 and the diminished cleavage of the precursor HIV-1 glycoprotein gp [149].

Isoquinoline Alkaloids Produced by Papaveraceae and Berberidaceae families, isoquinoline alkaloids possess biological properties, including acetylcholinesterase inhibitory effects and antiproliferative, antiviral, and antiplasmodial activities. Traditionally, the *Corydalis* species have a history of treating gastric and duodenal ulcers, dysmenorrhea, rheumatism, and cardiac arrhythmia. So far, the traditional background has proven pharmacological activities, isolating more than one hundred isoquinoline alkaloids from the genus. They have a range of biochemical effects relevant to medical uses, such as inhibition of conditions such as pain, cancer cells, and bacteria, and stimulation of bone marrow leucocytes and myocardial contractility.

Tea leaves produce organic compounds that may protect plants against invading pathogens, including insects, bacteria, fungi, and viruses. The metabolites include polyphenolic compounds, the six so-called catechins, and the methyl-xanthine alkaloids caffeine, theobromine, and theophylline. Methylxanthine alkaloids are produced mainly by *Coffea arabica*, *Camellia sinensis*, and *Theobroma cacao*. Methylxanthines, to some extent, bind to adenosine receptors and elicit neurological effects, which may be considered stimulating at low to moderate intake. In rodents, high intakes of methylxanthines show reduced sperm production and testicular atrophy.

Pseudoalkaloids Compounds with chemical properties close to alkaloids and are produced by species in Apiaceae, such as *Cicuta virosa* and *Conium maculatum*, and in Taxaceae instance, *Taxus baccata*. The pseudoalkaloids in *Cicuta virosa* and *Conium maculatum* affect the central nervous system, and taxine in yews like *T. baccata* inhibits the ion transport of the heart.

Tannins A class of astringent, polyphenolic biomolecules that bind indiscriminately to proteins and precipitate the proteins and various other organic compounds, including amino acids and alkaloids. There are two distinct types of tannins. Condensed tannins, polymers of flavonoids, and hydrolyzable tannins are polymers composed of a monosaccharide core, most often glucose, with catechin derivatives attached. The tannins have the most properties in common, but hydrolyzable tannins are less stable and have a more significant potential to cause toxicity. Their solubility in water is restricted and decreases with the size of the tannin molecule. The more prominent tannins are used as astringents in diarrhea, skin abrasions, and transudates. Hydrolyzable tannins have been shown to be effective antagonists against viruses, bacteria, and eukaryotic microorganisms.

Tannins are widely distributed in the plant kingdom, especially those of the Fagaceae and Polygonaceae families. The antimicrobial properties of several secondary metabolites occurring in plant tissues have been discovered. Hydrolyzable tannins, secondary metabolites usually referred to as vegetable tannins, have been widely studied over the years. Vegetable tannins are water-soluble and have a molecular weight between 500 and 5000 Da. They usually give a phenol reaction and express unique properties such as precipitate alkaloids and proteins. A significant group of hydrolyzable tannins includes esters of gallic acid with glucose, their oxidation and polymerization products, and other oxidized and polymerized derivatives. Chemically, hydrolyzable tannins differ from condensed tannins, derivatives of catechins, and tara tannins, which are esters of gallic acid with quinic acid.

Lignans

Although not classified as dietary fibers, they share some of the chemical characteristics of lignin, an insoluble fiber. Lignans most commonly distributed in foods are lariciresinol, matairesinol, pinoresinol, and secoisolariciresinol. Other lignans are present in some foods, including medioresinol in sesame seeds, rye, and lemons; syringaresinol in grains; and sesamin and the lignan precursor sesamol in sesame seeds. Other lignans in foods include arctigenin, cyclolariciresinol (isolariciresinol), 7'-hydroxymatairesinol, and 7-hydroxysecoisolariciresinol. Some cyclolariciresinol occurs naturally, and some are formed from lariciresinol during extraction and analysis under acidic conditions. The nutritional significance of lignans is unknown.

Lignans comprise two phenylpropanoid units forming an 18-carbon skeleton with various functional groups. They are generally lipophilic and have structural functions within the plant cell membranes. Lignans are present at the highest concentrations in oilseeds and found in other parts of an extended range of plants of different families. Lignans have clinical activities such as phytoestrogenic, cathartic, and antineoplastic.

Dibenzylbutyrolactones Dibenzylbutyrolactones, also known as lignans- β - β 0-lactones (lignanoides), are based on dibenzylbutanes with 9–90 epoxy C9

carbonyls. Lignanoides are often found in the same plants as their monodihydrogenated or dehydrogenated compounds and corresponding derivatives. The representative compounds with antiviral activities in this subclass are arctigenin (ATG), Latin, and hinokinin. The relationship between the aryl-naphthalene and aryltetralin subclasses is of interest due to their deceptive structural similarities. The compounds are based on dibenzylbutanes and formed by cyclizing six sites in one C6–C3 unit and seven sites in another C6–C3 unit. Their subtle structural difference lies in whether the B ring consists of benzene or a six-membered ring. Arylnaphthalenes are also named benzene tetrahydronaphthalene, which means that the B-ring structure consists of six-membered rings. Aryltetralins are named benzene naphthalene because the B-ring structure consists of benzene. The representative compounds of aryl-naphthalene are diphyllin, 6-deoxyglucose-diphyllin (DGP), and podophyllotoxin represents aryltetralin.

Diphyllin A natural component of plants with naphthalene and one hydroxyl lignan. It exists in *Haplophyllum Alberti-regelii*, *H. bucharicum*, and *H. perforatum* (Rutaceae). It showed broad-spectrum antiviral activity as a potent vacuolar ATPase (V-ATPase) inhibitor [131]. For example, it blocked ZIKV infection in HT1080 cells with an IC_{50} of $\sim 0.06 \mu M$ [132] and also altered the cellular susceptibility to IAV by inhibiting endosomal acidification, thus interfering with downstream virus replication [133]. There are more reports regarding the antiviral effect of glycosylated diphyllin. DGP, also known as patentiflorin A, was first isolated from *Justicia gendarussa* (Acanthaceae) [134]. The glycosylated diphyllin exhibited anti-ZIKV activity both in vitro and in vivo, and it displayed broad-spectrum antiviral activity against other flaviviruses. MOA showed that DGP inhibits ZIKV fusion with cellular membranes and infection by preventing the acidification of endosomal/lysosomal compartments in the target cells [132]. Besides, it also displays potent activity against a broad spectrum of HIV strains with IC_{50} values in the range of 15 to 21 nM [135]. MOA indicated that it acts as a potential inhibitor of HIV-1 reverse transcription [136].

Podophyllotoxin One of the best-characterized lignans, a type of aryltetralin lignan lactone. It was initially found in *Dysosma Verspiellii Rhixoma*, American mandarin, or mayapple; all belong to Berberidaceae. One of the research interests of podophyllotoxin is focused on anticancer activities. It was first cited in 1942 as a topical treatment for venereal warts, *Condyloma acuminatum*, caused by papillomavirus. The clinical randomized controlled trial data with forty-five cases showed that podophyllotoxin 0.5% solution benefits anogenital warts. It was effective and safe for untreated anogenital warts in immunocompetent individuals [146]. ATG was initially isolated from *Arctium lappa* L.

The research on antiviral activity has been mainly focused on IAV and HIV. The erythrocyte agglutination test showed that ATG could inhibit the replication of the IAV in vitro. The inhibition was shown to be 100% with a concentration of 26.8 mM based on hemagglutination titer. In vivo, ATG can reduce lung index, increase the

survival rate of the infected mice, and induce the interferon levels of normal mice, which suggests that mechanistically ATG can induce the production of interferon [144]. ATG and its glycoside arctiin were also shown to be orally effective but less than oseltamivir. The results indicated that it is an excellent choice to combine arctiin with oseltamivir for IAV in immunocompromised mice infected with IAV [144].

ATG inhibited the expression of protein P17 and P24 of the HIV-1 in vitro. MOA indicated that it targets reverse transcription [145]. Studies on the structure–activity relationship indicated that the structure of lactones is necessary and the number and arrangement of phenolic hydroxyl groups were significant for the activity of lignanols [146].

Yatein Yatein was isolated from *Chamaecyparis obtuse* (Cupressaceae). The anti-inflammatory and antimicrobial activities and the cytotoxicity of the compounds have been extensively studied. MOA showed that yatein could inhibit HSV-1 alpha gene expression, including the expression of the ICP0 and ICP4 genes, by arresting HSV-1 DNA synthesis and structural protein expression in HeLa cells [148]. It could significantly suppress HSV-1 replication in HeLa cells without apparent cytotoxicity [147].

Hinokinin Hinokinin was first isolated from the ether extract of *Chamaecyparis obtusa* in 1933, and it was also found in different species of *Phyllanthus* (Euphorbiaceae), *Aristolochia* (Aristolochiaceae), *Piper* (Piperaceae), *Virola* (Myristicaceae), and *Linum* (Linaceae). It produced antiviral activities against human HBV [139], HIV [140], SARS-virus (SARS-CoV) [141], and human cytomegalovirus (HCMV) [142].

Resins

Resins are complex lipid-soluble mixtures, usually both nonvolatile and volatile compounds. The nonvolatile fraction may consist of diterpenoid and triterpenoid compounds, and mono- and sesquiterpenoids predominate in the volatile fraction. Typically, resins are secreted by wood structures, but resins are also present in herbaceous plants. They are usually sticky, and the fluidity depends on the contents of volatile compounds. When exposed to air, they harden. Most resins are antimicrobial and possess wound-healing effects; their actions depend on the composition of the chemical mixture. Resins are generally safe, but contact allergies may occur.

Major components of the *Cannabis* resin are the cannabinoids and terpenes. Variations of *Cannabis* terpene profiles contribute much to the different flavor and fragrance phenotypes that affect consumer preferences. A major problem in the *Cannabis* industry is the lack of metabolic characterization of the cultivars. Foliar terpene profiles of plants grown from thirty-two seed sources were characterized and revealed large variations within and among sets of plants labeled as the same cultivar. Five plants representing different cultivars with contrasting terpene profiles

were selected for clonal propagation, floral metabolite profiling, and trichome-specific transcriptome sequencing.

Sequence analysis of the five cultivars and the reference genome of cv Purple Kush revealed thirty-three different *Cannabis* terpene synthase (*CsTPS*) genes, variations of the *CsTPS* gene family, and differential expression of terpenoid and cannabinoid pathway genes among the cultivars. The annotation of the cv Purple Kush reference genome identified nineteen complete *CsTPS* gene models and tandem arrays of isoprenoid and cannabinoid biosynthetic genes. An updated phylogeny of the *CsTPS* gene family showed three *Cannabis*-specific clades, including a clade of sesquiterpene synthases within the TPS-b subfamily that typically contains mostly monoterpene synthases. The *CsTPS*s characterized include thirteen that had not been previously characterized and collectively explain a diverse range of *Cannabis* terpenes.

Asafetida is an oleo-gum-resin obtained from the roots of different *Ferula asa-fetida* used as a culinary spice and traditional medicine to treat various diseases, including asthma and gastrointestinal disorders, and intestinal parasites. The oleo-gum-resin has been known to possess antifungal, antidiabetic, anti-inflammatory, antimutagenic, and antiviral activities. Various chemical compounds have been isolated from this plant, including sugars, sesquiterpene coumarins, and polysulfides. Recent studies have shown promising antiviral sesquiterpene coumarins in resins.

Saponins

Saponins are triterpene glycosides. They are organic chemicals that have a foamy quality when agitated in water. The aglycones consist of either pentacyclic triterpenoids or tetracyclic steroids. They are structurally distinct but have main functional properties in common. The saponin glycosides are large molecules with a hydrophilic glycone and a 14-hydrophobic aglycone, which give emulsifying properties and can be used as detergents. Saponins show immune-modulating and antineoplastic effects. A common in vitro effect is the hemolysis of red blood cells. However, this effect does not seem to be an in vivo problem. Some saponins induce photosensitization and jaundice. Saponins are present in a range of plant families. Among them is Liliaceae with the toxic plant *Narthesium ossifragum*.

Saponin production is part of the normal growth and development process in plants. Saponins are sugar-conjugated compounds with biological activities, such as antimicrobial antiviral activities. Saponins are divided into two groups: steroidal saponins, which occur as glycosides in certain pasture plants such as *Brachiaria decumbens* and *Panicum* sp., and triterpenoid saponins, which occur in soybeans and alfalfa.

Many pharmacological activities have been reported about saponins, such as antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory, and antiulcer. They have also been investigated for their membrane-permeabilizing, immunostimulant, hypocholesterolemic, and anticarcinogenic properties. The structurally

diverse compounds have also been observed to kill protozoans and mollusks as antioxidants, impair the digestion of protein and the uptake of vitamins and minerals in the gut and cause hypoglycemia.

The awareness about the health benefits and nutritional properties of *Cannabis* seed oil has encouraged researchers to focus on the innovative application of the oil. The balanced ratio of omega-6 to omega-3 fatty acids (from 2.1 to 3.1) in *Cannabis* seed oil is considered optimal for human health; it triggers several physiological reactions in the body, including vasodilation, proaggregator, and inflammatory responses.

Small quantities of nonpsychoactive cannabinoids such as cannabidiol and cannabigerol in *Cannabis* seed oil have exhibited anti-inflammatory, antinausea, anti-convulsive, and antiepileptic properties effects. *Cannabis* seed oil has also shown antimicrobial activity against some bacteria, including *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Escherichia coli*. Moreover, the hydrophilic and hydrophobic properties make *Cannabis* seed oil suitable as the oil phase for various emulsion systems. The presence of γ -linolenic acid, stearidonic acid (18:4, n-3), terpenoids, β -sitosterol, and antioxidants, including phenolics and flavonoids, depicts the potential of *Cannabis* oil for application in oil-based food and pharmaceutical formulations.

Saponins have shown great potential against various infectious agents, including viruses such as SARS-CoV, and they do not show any adverse side effects. They could be potentially valuable for treating viral diseases such as COVID-19, HIV, HSV, and rotavirus.

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Chapter 3

Chemical Constituents of *Cannabis*



Chemical Constituents

(ppm unless otherwise indicated)

Acetaldehyde: Pl [1]
Acetone: Pl [1]
Actinidiolide, dihydro: EO [2], Pl [1]
Alanine: Pl [1]
Phenylalanine: Pl [1]
Aldotetronic acid, 2-C-methyl: Pl [1]
Aldotetronolactone, 2-C-methyl: Pl [1]
Anethole, cis: EO [2], Pl [1]
Anethole, trans: EO [2], Pl [1]
Apigenin glycoside: Pl [1]
Apigenin-7-O-para-coumaroyl-glucoside: Pl [1]
Arabinic acid: Pl [1]
Arabinose: Pl [1]
Arabitol: Pl [1]
Arachidic acid: Pl [1], Sd [3]
Arginine: Pl [1]
Aromadendrene, allo: Pl [1]
Aspartic acid: Pl [1]
Azelaic acid: Pl [1]
Behenic acid: Pl [1]
Benzaldehyde, para-ethyl: EO [2], Pl [1]
Benzene, 1-methyl-4-iso-propenyl: Pl [1, 4]
Benzo-(A)-anthracene: Lf Smoke 3.3 µg/100 cig [5]
Benzo-(A)-pyrene: Lf Smoke 4.2 µg/100 cig [5]
Benzo-(F)-fluoranthene: Lf Smoke 3.0 µg/100 cig [5]

Benzo-(G-H-I)-perylene: Lf Smoke 0.7 µg/100 cig [5]
Benzo-(K)-fluoranthene: Lf Smoke 1.1 µg/100 cig [5]
Benzoic acid, 4-hydroxy: Pl [1]
Benzoic acid, 4-hydroxy methyl ester: Pl [6]
Benzoic acid, 4-hydroxy n-propyl ester: Pl [6]
Benzoxocin-5-methanol, 2-(H)-1, 3-4-5-6-tetrahydro, 7-hydroxy- α -2-trimethyl-9-n-propyl-2-6-methano: Pl [1]
Benzyl acetate: EO [1, 2]
Benzyl acetate, para-ethyl: EO [2], Pl [1]
Bergamotene, α : Lf EO [7]
Bergamotene, α , trans: Lf EO [8], Resin [9], Inflores [10], Pl [1]
Betaine, iso-leucine, L-(+): Pl [1]
Bibenzyl, 3-4-dihydroxy-5-5-dimethoxy-3-(3-methyl-but-2-enyl): Aer [11]
Bibenzyl, 3-3-dihydroxy-4-5-dimethoxy: Lf 5 [12], Aer [13]
Bibenzyl, 3-4-5-trihydroxy: Resin 0.05965% [14]
Bibenzyl, 3-4-dihydroxy-5-methoxy: Aer [11], Lf 2 [12]
Bisabolene: Pl [4]
Bisabolol, α : Pl [1], Fl EO [7]
Borneol: Resin [9], EO [2]
Borneol acetate: Pl [1], EO [2]
Borneol, (-): Pl [4]
Bornesitol, D, (+): Pl [1]
Butylamine, iso: Pl [1]
Butylamine, n: Pl [1]
Butylamine, sec: Pl [1]
Butyraldehyde, iso: Pl [1]
Cadaverine: Pl [1]
Cadinene, δ : Pl [1], EO [2]
Cadinene, γ : Pl [1], EO [2]
Calamenene: Pl [1], Lf EO [8]
Campest-4-en-3-one: Pl [1]
Campest-5-en-3- β -ol-7-one: Pl [1]
Campestanol: Sd [15]
Campesterol: Sd [15], Callus Tiss [16], Rt [17]
Camphene: Inflores EO [10], Lf EO [8], Resin [9]
Camphene hydrate: EO [2], Pl [1]
Camphor: Lf EO [8], Pl [1]
Cannabispiran: Lf [18]
Cannabamine: Lf [19]
Cannabamine B: Lf [19]
Cannabamine C: Lf [19]
Cannabamine D: Lf [19]
Cannabicclovatin: Pl [1]
Cannabichromanone: Resin 59 [20]

Cannabichromanone, C-3: Resin [21]
Cannabichromene: Rt [22], Resin [9], Aer [24, 25]
Cannabichromene, propyl: Resin [26]
Cannabichromenic acid: Inflores [27], Resin [28], Lf [29]
Cannabichromevarin: Pl [1]
Cannabichromevarinic acid: Pl [1]
Cannabicitran: Pl [1]
Cannabicumaronic acid: Resin 84 [20]
Cannabicumaronone: Pl [1]
Cannabicyclol: Aer [30], Lf [31], Fl [32], Resin [33]
Cannabicyclolic acid: Inflores [27]
Cannabidihydrophenanthrene: Lf 20 [34]
Cannabidiol: Lf [35], Aer [36], Resin [37], Inflores [38]
Cannabidiol monomethyl ether: Pl [1]
Cannabidiol, C-4: Pl [1]
Cannabidiol, propyl: Resin [26]
Cannabidiolic acid: Pl [1]
Cannabidiolic acid-tetrahydro-cannabitril ester: Pl [1]
Cannabidiorcol: Pl [1]
Cannabidivarin: Pl [1]
Cannabidivarol: Pl [1]
Cannabidivarol: Pl [39]
Cannabidivarolic acid: Pl [39]
Cannabielsoic acid A: Pl [1], Resin [40]
Cannabielsoic acid B: Pl [1], Resin [40]
Cannabielsoic acid B, C-3: Pl [1], Resin [21]
Cannabielsoic acid, C-3: Pl [1]
Cannabielsoin: Pl [1]
Cannabielsoin I, dehydro: Resin [41]
Cannabielsoin, C-3: Resin [21]
Cannabifuran: Pl [1], Resin [41]
Cannabifuran, dehydro: Resin [41], Pl [1]
Cannabigerol: Resin [42], Pl [43]
Cannabigerol monomethyl ether: Pl [1]
Cannabigerolic acid: Lf 40 [44], Pl [1], Inflores [27]
Cannabigerolic acid monoethyl ether: Lf 20 [45], Pl [1]
Cannabigerovarin: Pl [1]
Cannabigerovarinic acid: Pl [1]
Cannabinerolic acid: Lf 7.6 [44]
Cannabinodiol: Resin [46]
Cannabinodivarin: Pl [1]
Cannabinol: Resin [23], Sd 8 [47], Fr 0.1275% [48], Pl [49]
Cannabinol methyl ether: Resin [50]
Cannabinol monomethyl ether: Pl [1]
Cannabinol, delta-6(A)-10(A)-tetrahydro, 8-9-dihydroxy (DL): Aer [51]

Cannabinol, delta-6(A)-10(A)-tetrahydro, 10-oxo: Resin [41], Pl [1]
Cannabinol, delta-6(A)-10(A)-tetrahydro, 9-10-dihydroxy (DL): Aer [51]
Cannabinol, delta-6(A)-10(A)-tetrahydro, 9-hydroxy-10-ethoxy: Aer [52]
Cannabinol, delta-6(A)-10(A)-tetrahydro, cis: Pl [1]
Cannabinol, delta-8-tetrahydro: Pl [49], Resin [28], Rt [22], Inflores [27]
Cannabinol, delta-8-tetrahydro, trans: Pl [1]
Cannabinol, delta-8-tetrahydro, trans (-): Aer [30]
Cannabinol, delta-9-tetrahydro: Pl [53], Resin [54], Bk [55], Sd 16.5 [56], Call Tiss
65 [57], Fr 0.5653% [84], Fl tops 4.0% [58], Lf [59], Rt [22]
Cannabinol, delta-9-tetrahydro, 6(A)-7-10(A)-trihydroxy: Pl [1]
Cannabinol, delta-9-tetrahydroxylic acid: Resin [60]
Cannabinol, delta-9-tetrahydro, cis: Lf 2 [61], Lf/Fl [32]
Cannabinol, delta-9-tetrahydro, methyl ether: Resin [26]
Cannabinol, delta-9-tetrahydro, propyl: Resin [26]
Cannabinol, delta-9-tetrahydro, trans: Fl/Lf [32], Pl [1], Resin [26]
Cannabinol, delta-9-tetrahydro, trans (-): Pl [1]
Cannabinol, hexahydro: Aer [30]
Cannabinol, propyl: Resin [26]
Cannabinol, tetrahydro: Pl [62]
Cannabinol, tetrahydro, iso: Resin [26]
Cannabinol, tetrahydro, iso, propyl: Resin [26]
Cannabinol-C-4: Pl [1]
Cannabinol-C-4, delta-9-tetrahydro, trans: Pl [1]
Cannabinolic acid: Lf [45], Resin [28], Inflores [27], Pl [34]
Cannabinolic acid A, delta-9-tetrahydro: Lf [45]
Cannabinolic acid A, delta-9-tetrahydro, trans: Pl [1]
Cannabinolic acid A, delta-9-tetrahydro, trans (-): Aer [30]
Cannabinolic acid B, delta-9-tetrahydro: Resin [28]
Cannabinolic acid B, delta-9-tetrahydro, trans: Pl [1]
Cannabinolic acid B, delta-9-tetrahydro, trans (-): Aer [30]
Cannabinolic acid, delta-1-tetrahydro: Lf 1.43% [44]
Cannabinolic acid, delta-8-tetrahydro: Inflores [27]
Cannabinolic acid, delta-8-tetrahydro, trans: Pl [1]
Cannabinolic acid, delta-9-tetrahydro: Inflores [27], Lf [63, 64]
Cannabinolic acid, tetrahydro: Lf [64], Pl [53], Pollen [65]
Cannabinolic acid-C-4, delta-9-tetrahydro, trans: Pl [34]
Cannabidiol, delta-6(A)-10(A)-tetrahydro, 9-10-dihydroxy (DL): Pl [1]
Cannabiorcol: Pl [1]
Cannabiorcol, delta-9-tetrahydro, trans: Pl [1]
Cannabiorcolic acid, delta-9-tetrahydro, trans: Pl [1]
Cannabipinol: Pl [1]
Cannabiprene: Lf 26.8 [18]
Cannabiripsol: Pl [1], Aer [66]
Cannabisativine: Rt 2 [67]
Cannabisativine, anhydro: Pl [1]

Cannabiscoumaranone: Resin 140 [20]
Cannabisin A: Fr 74 [68]
Cannabisin B: Fr 812 [69]
Cannabisin C: Fr 0.267% [69]
Cannabisin D: Fr 59.8 [69]
Cannabisin E: Fr 120 [70]
Cannabisin F: Fr 45 [70]
Cannabisin G: Fr 20 [70]
Cannabisiradienone: Pl [1]
Cannabispirenone B: Lf [71]
Cannabispiradienone: Lf 5-6 [34, 71]
Cannabispiran: Lf 20.0-245.7 [72, 73]
Cannabispiran, dehydro: Lf 2.3 [18, 91]
Cannabispiran, iso: Lf [74]
Cannabispiranol: Lf 18 [12]
Cannabispiranol, alpha: Lf 0.3 [71]
Cannabispiranol, beta: Lf 8-80 [18, 73]
Cannabispirenone: Lf 61 [12], Aer 10 [75]
Cannabispirenone: Lf 210 [71]
Cannabispirenone A, (-): Lf 30 [71]
Cannabispirenone A, (DL): Lf 30 [71]
Cannabisperenone, iso: Pl [1]
Cannabisirol, acetyl: Pl [1]
Cannabispirone: Aer 30 [75], Pl [1], Lf 40 [12]
Cannabistilbene I: Lf 0.4 [76]
Cannabistilbene II: Lf [76]
Cannabitetrol: Pl [77]
Cannabithrene I: Lf 4 [71]
Cannabithrene II: Lf 8 [71]
Cannabitriol: Aer 250 [78]
Cannabitriol, (+): Aer [52], Pl [1]
Cannabitriol, (+): Pl [1]
Cannabitriol, (DL): Lf 2.7 [79]
Cannabitriol, trans (DL): Lf [80]
Cannabivarichromene: Resin [81]
Cannabivarin: Pl [1]
Cannabivarin, delta-9-tetrahydro, trans: Pl [1]
Cannabivarin, delta-9-tetrahydro, trans (-): Pl [1], Aer [30]
Cannabivarin, tetrahydro: Fl/Lf [32]
Cannabivarinic acid, delta-9-tetrahydro, trans: Pl [1]
Cannabivarinic acid, delta-9-tetrahydro, trans (-): Aer [30]
Cannabivarol: Fl tops [82]
Cannabivarol, delta-9-tetrahydro: Pl [39]
Cannabivarol, tetrahydro: Pl [53]
Cannabivarolic acid, tetrahydro: Pl [53]

Cannflavin: Lf 138.5 [83]
Cannflavin A: Aer 190 [84]
Cannflavin B: Aer 30 [84]
Cannflavone 2: Aer [85]
Canniflavone 1: Lf 0.8 [71]
Canniflavone 2: 6.0 [71]
Canniprene: Lf 27-1490 [73, 86], PI [1]
Car-3-ene: Inflores [10], EO [2], PI [1]
Car-4-ene: PI [1, 4]
Carbazole: Lf (smoke) [87]
Carvacrol: Lf EO [8], PI [34]
Carveol acetate, dihydro: PI [1], EO [2]
Carvone: Rt [89], EO [2], PI [1]
Carvone, dihydro: Rt [88], EO [2], PI [1]
Caryophyllene: Lf EO [8]
Caryophyllene alcohol, alpha: EO [2], PI [4]
Caryophyllene epoxide: Lf EO [8]
Caryophyllene epoxide, beta: Lf EO, Fl EO [7]
Caryophyllene oxide: Fl tops [82], Lf EO [89], PI [1]
Caryophyllene, alpha: PI [1, 4]
Caryophyllene, beta: PI [1], Resin [9], Fl EO [7], Inflores [10]
Caryophyllene, iso: PI [1], Inflores EO [10]
Caryophyllenol: PI [1]
Castasterone: Sd [91]
Cedrene, alpha: PI [1], EO [2]
Cellulose, hemi: PI [1]
Cholest-4-en-3-one, 24-methyl: Sd [91]
Cholestan-3-one, 5-alpha, 24-methyl: Sd [91]
Cholesterol: Sd [15]
Choline: Rt, Fl tops [19], Lf [92], PI [1]
Chrysene: Lf (smoke) 5.0 µg/100cig [5]
Cineol, 1-4: PI [1], Lf EO [8]
Cineol, 1-8: Lf EO [8], PI [1]
Cinnamic acid, trans: Lf [93], PI [1]
Cinnamide, n-(para-hydroxy-beta-phenylethyl)-para-hydroxy-trans: PI [1]
Cinnamide, n-(para-hydroxy-beta-phenylethyl)-para-hydroxy-trans(-): PI [1]
Citric acid: PI [1]
Citric acid, iso: PI [1]
Citronellol: EO [2], PI [1]
Copaene, alpha: Lf EO [8], PI [1]
Cosmosioside: PI [1]
Coumaric acid, para: PI [1]
Cubebene, alpha: PI [1], EO [2]
Curcumene: EO [2]
Curcumene, alpha: PI [1], Lf EO [8]

Curcumene, beta: PI [1, 4]
Cyclocitral, beta: PI [1], EO [2]
Cyclohex-5-enone, 2-2-6-trimethyl: PI [1], EO [2]
Cyclohexanone, 2-2-6-trimethyl: EO [2], PI [1]
Cyclolanost-24-methylene-3-beta-acetate: PI [6]
Cymen-8-ol, para: EO [2], PI [1]
Cymene, para: PI [1, 4], EO [2], Inflores [10]
Cystine: PI [1]
Dec-3-en-5-one: EO [2], PI [1]
Decan-1-al: PI [1], EO [2]
Decan-2-one: EO [2], PI [1]
Decane, n: PI [1]
Dibenz-(A-1)-anthracene: Lf (smoke) 0.3 µg/cig [5]
Docosane, n: PI [1]
Dodecan-1-al: EO [2], PI [1]
Dodecan-2-one: PI [1], EO [2]
Dodecane, n: PI [1]
Dotriacontane, 2-methyl: PI [1]
Dotriacontane, n: PI [1]
Edestin: PI [1]
Edestinase: PI [1]
Eicosadienoic acid: PI [1]
Eicosane, n: PI [1]
Eicosenoic acid: PI [1]
Elemene, gamma: EO [2], Fl EO, Lf EO [7], PI [1]
Ereptase (peptidase): PI [1]
Ergostan-3-one, 5-alpha: Call tiss [16]
Ergosterol: PI [1]
Erythritol: PI [1]
Essential oil: Aer 0.09–0.11% [2], Lf 0.15% [8], Inflores [10]
Ethanol: PI [1]
Ethanolamine: PI [1]
Ethylamine: PI [1]
Ethylamine, (DL): PI [1]
Eudesmol, gamma: EO [2], PI [1]
Eugenol: EO [2], PI [1]
Eugenol methyl ether: EO [2], PI [1]
Eugenol, iso: EO [2], PL [1]
Farnesene: PI [1]
Farnesene, alpha: Fl EO, Lf EO [7]
Farnesene, alpha trans, trans: EO [2], PI [1]
Farnesene, beta: Resin [9], Inflores EO [10], EO [2], PI [1]
Farnesene, beta, cis: PI [1]
Farnesene, beta, trans: Fl EO [7], Lf EO [7, 8]
Farnesol: PI [1], EO [2]

Farnesyl-acetone: EO [2], Pl [1]
Fatty acids: Sd [94]
Fenchol: Lf EO [8], Pl [4]
Fenchone: EO [2], Pl [1]
Fenchyl alcohol: Resin [9], EO [2], Pl [1]
Ferulic acid: Pl [1]
Flavocannabiside: Aer [95]
Flavone, 4-5-7-trihydroxy-3-methoxy-6-prenyl: Lf 0.8 [86]
Flavone, 4-5-7-trihydroxy-3-methoxy-6-geranyl: Lf 6 [86]
Flavosativaside: Aer [95]
Friedelanol, epi: Pl [1, 4]
Friedelin: Pl [1, 4, 6], Rt [88]
Friedelinol, epi: Pl [1], Rt [88]
Fructose: Pl [1]
Furfural, 5-methyl: EO [2]
Furo-(1,2-A)-4-n-pentyl-7-7-10-trimethyl-dibenzopyran, 2-3-dimethyl: Lf (smoke) [96]
Furo-(1,2,A)-4-n-pentyl-7-7-10-trimethyl-dibenzopyran, 2-methyl: Lf (smoke) [96]
Furo-(1,2,A)-u-n-pentyl-7-7-10-trimethyl-dibenzopyran: Lf (smoke) [96]
Galactitol: Pl [1]
Galactosamine: Pl [1], Lf/St 1.9% [97]
Galactose: Pl [1]
Galacturonic acid: Pl [1]
Geraniol: Lf EO [8], Pl [1]
Geranyl acetone: Pl [1], EO [2]
Glucaric acid: Pl [1]
Gluconic acid: Pl [1]
Glucosamine: Pl [1]
Glucose, alpha (D): Pl [1]
Glucose, beta (D): Pl [1]
Glutamic acid: Pl [1]
Glyceric acid: Pl [1]
Glycerol: Pl [1]
Glycerol, (D), D-manno-octulose: Pl [1]
Glycine: Pl [1]
Glycoprotein (*Cannabis sativa*): Lf [60]
Grossamide: Fr 8 [68]
Guaiol: Pl [1]
Gurjunene, alpha: Fl EO [7], Resin [9], Pl [1]
Heneicosane, 3-methyl: Pl [1]
Heneicosane, n: Pl [1]
Hentriacontane, 2-methyl: Pl [1]
Hentriacontane, 3-methyl: Pl [1]
Hentriacontane, n: Pl [1]
Hept-2-3n-6-one, 2-methyl: Pl [1]

Hept-5-en-2-one, 6-methyl: EO [2]
Heptacosane, 3-methyl: PI [1]
Heptacosane, n: Aer [99], PI [34]
Heptadecane, 3-6-dimethyl: PI [1]
Heptadecane, 3-7-dimethyl: PI [1]
Heptadecane, n: PI [1]
Heptan-1-al: PI [1], EO [2]
Heptan-2-one: EO [2], PI [1]
Heptatriacontane, n: PI [1]
Heptulose, sedo: PI [1]
Hex-cis-3-en-1-ol caproate: EO [2]
Hex-cis-3-enol caproate: PI [1]
Hexacosane, 2-methyl: PI [1]
Hexacosane, n: PI [1]
Hexadecane-1-ol: PI [1], EO [2]
Hexadecanamide: Resin [99]
Hexadecane, n: PI [1]
Hexan-1-al: EO [2], PI [1]
Hexan-1-ol acetate: PI [1]
Hexan-1-ol butyrate: PI [1]
Hexan-1-ol caproate: PI [1]
Hexan-1-ol iso-butyrate: PI [1]
Hexatriacontane, n: PI [1]
Hexyl acetate: EO [2]
Hexyl iso-butyrate: EO [2]
Histamine: PI [1]
Histidine: PI [1]
Hordenine: Lf [101], PI [1]
Humulene: Lf EO [8], Resin [9]
Humulene oxide: EO [2]
Humulene oxide I: PI [1]
Humulene oxide II: PI [1]
Humulene, alpha: Lf EO, Fl EO [7]
Humulene, beta: PI [1], Inflores EO [10], EO [2]
Indan-1-spiro-cyclohexane, 5-7-dihydroxy: Resin [101]
Indan-1-spiro-cyclohexane, 5-hydroxy-7-methoxy: Resin [101]
Indan-1-spiro-cyclohexane, 7-hydroxy-5-methoxy: Resin [101]
Indole: Lf (smoke) [87]
Inositol, (+): PI [1]
Inositol, myo: PI [1], Fl/Lf [102]
Ionone, beta: EO [1], PI [1]
Kaempferol: Aer [103]
Ledol: PI [1], EO [2]
Leucine: PI [1]
Leucine, iso: PI [1]

Lignanamide I: Fr [105]
Limonene: Pl [1], Fl EO [7], Resin [9], Inflores EO [10]
Linalool: Pl [1], Resin [9], Lf EO [8]
Linalool, cis, oxide: EO [2], Pl [1]
Linalool, trans, oxide: Pl [1]
Linoleic acid: Pl [1], Sd [3]
Linoleic acid methyl ester: Pl [1]
Linolenic acid: Pl [1], Sd [3]
Linolenic acid methyl ester: EO [2]
Longifolene: Pl [1], EO [2], Inflores EO [10]
Longifolene, (+): Pl [4]
Lysine: Pl [1]
Malic acid: Pl [1]
Malonic acid: Pl [1]
Maltose: Pl [1]
Mannitol: Pl [1]
Mannose: Pl [1]
Mentha-1-8(9)-dien-5-ol, meta: Pl [1]
Methanol: Pl [1]
Methionine: Pl [1]
Methyl acetate: Pl [1]
Methylamine: Pl [1]
Methylamine, di: Pl [1]
Muscarine: Pl [1]
Myrcene: Fl EO [7], Resin [9], Lf EO, Inflores EO [10], Pl [4]
Myristic acid: Pl [1], EO [2]
Nerol: EO [8], Pl [1]
Nerolidol: Lf EO [8], Pl [1]
Neurine: Pl [1], Rt [19]
Nonacosane, n: Aer [98], Pl [1]
Nonadecane, n: Pl [1]
Nonan-1-al: Pl [1], EO [2]
Nonan-1-ol: Pl [1], EO [2]
Nonane, n: Pl [1]
Nonatriacontane, n: Pl [1]
Ocimene, beta, cis: Pl [1], EO [2]
Ocimene, beta, trans: Lf EO [8], Pl [1, 4]
Ocimene, cis: Inflores EO [10]
Ocimene, trans: Inflores EO [10]
Oct-1-en-3-ol: Pl [1], EO [2]
Octacosane, 2-methyl: Pl [1]
Octacosane, 9-methyl: Pl [1]
Octacosane, n: Pl [1]
Octadecane, 3-6-dimethyl: Pl [1]
Octadecane, 3-7-dimethyl: Pl [1]

Octadecane, n: PI [1]
Octan-1-al: EO [2], PI [1]
Octan-1-ol: EO [2], PI [1]
Octan-1-ol caproate: PI [1]
Octan-3-ol: EO [2], PI [1]
Octan-3-one: EO [2], PI [1]
Octatriacontane, n: PI [1]
Octyl caproate: EO [2]
Oleic acid: PI [1], Sd [3]
Oleic acid methyl ester: Call tiss [16]
Olivetol: Aer [85]
Orientin: Aer [95], PI [1]
Orientin-2-O-beta-d-glucoside: PI [1], Aer 4.2 [105]
Orientin-7-O-alpha-L-rhamnosyl glucoside: PI [1]
Orientin-7-O-beta-D-glucoside: PI [1]
Oxidase, polyphenol: PI [1]
Palmitic acid: Sd [3], PI [1]
Palmitic acid methyl ester: Call tiss [16], EO [2], PI [1]
Palmitoleic acid: PI [1]
Pectin: PI [1]
Pentacosane, 3-methyl: PI [1]
Pentacosane, n: PI [1]
Pentadecan-2-one: EO [2], PI [1]
Pentadecan-2-one, 6-10-14-trimethyl: PI [1], EO [2]
Pentadecane, n: PI [1]
Pentan-1-al: EO [2], PI [1]
Pentatriacontane, n: PI [1]
Perillene: EO [2], PI [1]
Peroxidase: PI [1]
Perylene: Lf (smoke) 0.9 µg/cig [5]
Phellandrene, alpha: Inflores EO [10], PI [34]
Phellandrene, beta: Lf EO [8], PI [1]
Phenethylamine, beta: PI [1]
Phenol, 2-6-di-tert-butyl-4-methyl: EO [2]
Phenol, 3-[2-(3hydroxy-4-methoxy-phenyl)-ethyl]-5-methoxy: PI [1]
Phenol, 3-[2-(3-iso-prenyl-4-hydroxy-5-methoxy-phenyl)-ethyl]-5-methoxy: PI [1]
Phenol, 3-[2-(4-hydroxy-phenyl)-ethyl]-5-methoxy: PI [1]
Phenol, 3-[2-(3-hydroxy-4-methoxy-phenyl)-ethyl]-ethyl-5-methoxy: PI [1]
Phenol, 4-vinyl: Aer [107]
Phenol, 5-methoxy-3-[2-(3-hydroxy-4-methoxy-phenyl)-ethyl]: Lf 1.9 [73]
Phloriglucinol, beta-D-glucoside: St [107]
Phosphatase, adenosine-5: PI [1]
Phosphoric acid: PI [1]
Phthalate, n-butyl: EO [2]
Phthalate, n-propyl: EO [2]

Phytol: EO [2], PI [1]
Pinene, alpha: Resin [9], Inflores EO [10], Lf EO [8], PI [1]
Pinene, alpha, oxide: EO, PI [1]
Pinene, beta: Lf EO [8], Resin [9], Inflores EO [10], PI [1]
Pinocarveol: EO [2], PI [1]
Pinocarvone: EO [2], PI [1]
Piperidine: Fl top, Lf [19], PI [1]
Piperitenone: Resin [9], EO [2], PI [1]
Piperitenone oxide: EO [2], PI [1]
Piperitone oxide: EO [2], PI [1]
Proline: PI [1]
Proline, L: Rt [19]
Prop-1-ene, 3-phenyl-2-methyl: PI [1], EO [2]
Prospylamine, n: PI [1]
Pulegone: EO [2], PI [1]
Pyrano-(3-4-B)-benzofuran, 1-4 5-hydroxy-7-pentyl-1-(A)-alpha-3-3-trimethyl-ethano-1-(H): Aer [108]
Pyrene: Lf (smoke) 6.6 µg/cig [5]
Pyroglutamic acid: PI [1]
Pyrrolidine: PI [1]
Quebrachitol, (+): PI [1]
Querachitol: Fl/Lf [102]
µQuercetin: Aer [95]
Raffinose: PI [1]
Rhamnose: PI [1]
Ribitol: PI [1]
Ribose: PI [1]
Sabinene: EO [2], PI [1]
Sabinene, trans: PI [1], EO [2]
Safranal: EO [2], PI [1]
Salicylic acid methyl ester: EO [2], PI [1]
Santalene, beta, epi: PI [1], EO [2]
Sativic acid: PI [1]
Scyllitol: Fl/Lf [103]
Selina-3-7(11)-diene: PI [1], Inflores EO [10]
Selina-4(14)-7(11)-diene: Inflores EO [10], PI [1]
Selinene, alpha: PI [1], Inflores EO [10], EO [2]
Selinene, beta: Inflores EO [10], PI [1], EO [2]
Serine: PI [1]
Sitostanol: Sd [15]
Sitosterol, beta: Rt [88], Sd [15], Call tiss [16], PI [1]
Skatole: Lf smoketurner [87]
Sorbitol: PI [172]
Spiro-(cyclohexane-1-3-(4-6-dihydroxy)-indan: Fl top [109]
Spiro-(cyclohexane-1-3-(4-hydroxy-6-methoxy)-indan: Fl top [109]

Spiro-(cyclohexane-1-3-(6-hydroxy-4-methoxy)-indan: Fl top [109]

Stearic acid: Pl [1], Sd [3]

Stearic acid methyl ester: Call tiss [16]

Stigmast-22-en-3-one, 5-alpha: Call tiss [16]

Stigmast-4-en, 3-one: Pl [1], Rt [17]

Stigmast-5-en-3-beta-ol-7-one: Rt [17], Pl [1]

Stigmasta-4-22-diene-3-one: Rt [17], Pl [1]

Stigmasta-5-22-dien-3-beta-ol-7-one: Rt [17]

Stigmasta-7-24(28)-dien-3-beta-ol, 5-alpha: Pl [1]

Stigmastan-3-one, 5-alpha: Call tiss [16]

Stigmasterol: Sd [15], Pl [1]

Stilbene, dihydro 3'-5-dihydroxy-3-4-dimethoxy: Lf [71]

Stilbene, dihydro 4'-5-dihydroxy-3-methoxy: Lf [71]

Succinic acid: Pl [1]

Sucrose: Pl [1]

Terpinen-4-ol: Lf EO [8], Pl [1]

Terpinen-4-ol, alpha: Pl [1]

Terpinene, alpha: Lf EO [8], Pl [1], Inflores EO [10]

Terpinene, gamma: Pl [1], Resin [9], EO [2], Inflores EO [10]

Terpineol, alpha: Pl [1], Resin [9], Lf EO [8], EO [4]

Terpineol, beta: EO [2], Pl [1]

Terpinolene: Fl EO [8], Lf EO [8], Inflores EO [10], Pl [1]

Tetracosane, 2-methyl: Pl [1]

Tetracosane, n: Pl [1]

Tetradecane, 2-6-dimethyl: Pl [1]

Tetradecane, n: Pl [1]

Tetratriacontane, n: Pl [1]

Threonic acid: Pl [1]

Threonine: Pl [1]

Thujene, alpha: Pl [1, 4], EO [2]

Thujol alcohol: Pl [1], EO [2]

Triacontane, 3-methyl: Pl [1]

Triacontane, n: Pl [1]

Tricosane, 3-methyl: Pl [1]

Tricosane, n: Pl [1]

Tridecan-1-al: EO [2], Pl [1]

Tridecane, 3-6-dimethyl: Pl [1]

Tridecane, n: Pl [1]

Trigonelline: Pl [1], FL top [19]

Tritriacontane, n: Pl [1]

Tryptophan: Pl [1]

Tyramine: Pl [1]

Tyramine, feruloyl: Sd 2.5, Rt, Resin, Lf [109, 110]

Tyramine, n-(para-coumaroyl): Fr 111 [68], Sd 111 [111]

Tyramine, n-trans-caffeoyl: Fr 47.1 [68]

Tyramine, n-trans-feruloyl: Fr 78.5 [68]
 Tyramine, para-coumaroyl: Sd 0.5 [111], Rt, Lf, Resin [110, 111]
 Tyrosine: Pl [1]
 Undecan-1-al: EO [2], Pl [1]
 Undecan-2-one: EO [2], Pl [1]
 Undecan-2-one, 6-10-dimethyl: EO [2], Pl [1]
 Undecane, n: Pl [1]
 Valine: Pl [1]
 Vanillic acid: Pl [1]
 Vitamin K: Pl [1]
 Vitexin, iso, 7-O-alpha-L-rhamnosyl-glucoside: Pl [1]
 Vitexin, iso, 7-O-beta-D-glucosyl-arabinoside: Pl [1]
 Vitexin, iso: Pl [1]
 Vitexin-2-beta-D-glucoside: Pl [1], Aer 4 [106]
 Vitexin-7-O-beta-D-(6-glucoside): Pl [1]
 Vomifoliol: Pl [1]
 Vomifoliol, dihydroxylan: Pl [1]
 Xylitol: Pl [1]
 Xylose: Pl [1]
 Zeatin: Pl [1]
 Zeatin nucleoside: Pl [1]

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Chapter 4

The Bioactive Components of *Cannabis*



Cannabinoids (CBDs) represent a group of C₂₁ or C₂₂ terpenophenolic compounds predominantly produced by *Cannabis* but have also been found in plants from the *Radula* and *Helichrysum* genera. There are about 100 different cannabinoids, although some of them are metabolites. They are generally classified into ten subclasses [1–3].

The phytocannabinoid acids are non-enzymatically decarboxylated into their corresponding neutral forms, which occur both within the plant and, to a much larger extent, upon heating after harvesting [5]. The predominant compounds are delta-9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabinolic acid (CBNA), followed by cannabigerolic acid (CBGA), cannabichromenic acid (CBCA), and cannabinodiolic acid (CBNDA) [1]. THCA is the major cannabinoid in drug-type *Cannabis*, while CBDA predominates in fiber-type hemp. Cannabichromenic acid dominates in the cannabinoid fraction of young plants and declines with maturation [4].

Two cannabinoids related to delta-9-tetrahydrocannabinol, dronabinol and nabilone, were approved by the US Food and Drug Administration (FDA) for chemotherapy-induced nausea and vomiting in 1985. Dronabinol gained a second indication for appetite stimulation in wasting conditions, such as AIDS, in 1992. A third cannabinoid, CBD, was approved by the FDA in 2018 to treat two forms of pediatric epilepsy, Dravet syndrome, and Lennox–Gastaut syndrome. Beyond the four indications for which cannabinoids are FDA-approved, the evidence appears to be for chronic pain, such as neuropathic pain and muscle spasticity resulting from multiple sclerosis.

Cannabinoids accumulate in the secretory cavity of the glandular trichomes, which largely occur in female flowers and most aerial parts of plants. They have also been detected in low quantity in other parts of the plants, including the seeds [6], roots [7], and the pollen to an extent depending on the drug or fiber type of *Cannabis* [8]. The concentration of the compounds depends on tissue type, age, variety, and growth conditions such as nutrition, humidity, light level, and harvest time and

storage conditions [9]. The phytocannabinoids in hempseeds and hempseed oil are very low as the kernel contains only a trace of delta-9-tetrahydrocannabinol and cannabidiol [6, 10]. Cannabinoids in the leaves have been shown to decrease with age. The highest levels are observed in the leaves of the uppermost nodes. Cannabinoid contents in the stem are low [11].

Phytocannabinoids stimulate cannabinoid receptors. Delta-9-tetrahydrocannabinol is the most psychoactive and the most famous of the phytocannabinoid substances. However, other cannabinoids, such as cannabidiol, cannabinol, and tetrahydrocannabivarin (THCV), are gaining interest due to their various medicinal properties. With the discovery of the endocannabinoid system, it has been revealed that other plants have cannabinoid-like molecules that act as cannabinoids, including beta-caryophyllene, being designated a dietary cannabinoid. Foods found with cannabinoids include cacao, black pepper, basil, and black truffles.

The measurement of circulating endocannabinoids has enhanced the endocannabinoid system's role in humans; many studies have indicated that 2-Arachidonoylglycerol (2-AG) is circadian, and there are differences in response to stress among men and women and, potentially, among races. Careful consideration of the sampling time and the determination of endocannabinoids at multiple time points or after a stress or exercise challenge could provide valuable information that cannot be obtained.

The Endocannabinoid System

Cannabinoid receptors are present throughout the body, embedded in cell membranes, and are believed to be more numerous than any other receptor system. When cannabinoid receptors are stimulated, a variety of physiologic processes ensue. Researchers have identified two Cannabinoid receptors: CB1 is present predominantly in the nervous system, connective tissues, gonads, glands, and organs. CB2 is found predominantly in the immune system and its associated structures. Many tissues contain CB1 and CB2 receptors, each linked to a different action. It is speculated that there may be other cannabinoid receptors to be discovered. Endocannabinoids are the substances that the body makes to stimulate those receptors. The two well-understood endocannabinoids are the molecules anandamide and 2-Arachidonoylglycerol. They have synthesized on-demand from cell membrane arachidonic acid derivatives. They have a local effect and a short half-life before being degraded by fatty acid amide hydrolase (FAAH) enzymes and monoacylglycerol lipase (MAGL).

Anandamide was the first endocannabinoid discovered by Dr. Raphael Mechoulam in 1992, two years after the announcement of the discovery of an endocannabinoid receptor system. Anandamide is closely mimicked by delta-9-THC, which means “*joy, bliss, and delight*” in Sanskrit. Dr. Mechoulam said:

It has been known since 1988 that THC acts on a specific receptor in the brain. The specific receptor was not built by the brain just for tetrahydrocannabinol – a compound present in a plant – which is foreign to the receptor. Presumably, the receptor is present because it has a function that has nothing to do with a plant constituent. We considered that a compound possibly activates this particular receptor in the brain. Two years ago, we identified such a compound in the pig brain. We called it anandamide. It binds to this Cannabinoid receptor. Anandamide differs completely from THC in its structure. THC is an aromatic compound, while anandamide is a fatty acid derivative. It also has a nitrogen atom, which is unusual for fatty acid derivatives.

Endocannabinoids modulate and play a regulatory role in various physiological processing, including appetite, pain sensation, mood, memory, inflammation, insulin, sensitivity, and fat and energy metabolism [14, 15]. Most of the biological properties of cannabinoids rely on their interactions with the eCB system in humans. The psychoactive decarboxylated form of THCA, delta-9-THC, is a partial agonist of both CB1 and CB2 receptors but has a higher affinity for the CB1 receptor, which appears to mediate its psychoactive properties. In addition to being present in the central nervous system and throughout the brain, CB1 receptors are also found in the immune cells and the gastrointestinal, reproductive, adrenal, heart, lung, and bladder tissues, where cannabinoids can also exert their activities.

CB2 receptors have immunomodulatory effects and regulate cytokine activity. Delta-9-THC has more molecular targets than just CB1 and CB2 receptors and exhibits potent anti-inflammatory, anticancer, analgesic, muscle relaxant, neuro-antioxidative [14], and antispasmodic activities [16]. Delta-9-tetrahydrocannabinol is associated with side effects, including anxiety, cholinergic deficits, and immunosuppression [17]. CBDA is the most prevalent cannabinoid in fiber-type hemp and the second most important in the drug chemotypes. CBD (decarboxylated CBDA) presents a large array of pharmacological properties that has been downplayed for many years compared to delta-9-THC [18]. CBD is an important entourage compound as it can reduce the side effects of delta-9-THC and may thereby increase the safety of *Cannabis*-based extracts [19].

CBD provides strong antifungal and antibacterial properties and potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) [21]. CBD has been shown in in vitro and in vivo studies to possess antianxiety, antinausea, antiarthritic, antipsychotic, anti-inflammatory, and immunomodulatory properties [18]. CBD has the potential as a therapeutic agent in preclinical models of central nervous system diseases such as epilepsy, neurodegenerative diseases, schizophrenia, multiple sclerosis, affective disorders, and the central modulation of feeding behavior [20].

After delta-9-THC and CBD, cannabichromene (CBC) is the third most prevalent cannabinoid. CBC presents notably anti-inflammatory [22], sedative, analgesic [23], antibacterial, and antifungal properties [24]. CBC is also a potent inhibitor of anandamide uptake, an endogenous ligand of CB receptors [14]. CBN is a degradation product of delta-9-THC and is mostly found in aged *Cannabis*. CBN has a two-fold lower affinity for CB1 receptors and a three-fold higher affinity for the CB2 receptors than delta-9-THC. It affects cells of the immune system more than the central nervous system [25]. Cannabinoid-based treatments are limited to

special cases, i.e., spasticity associated with multiple sclerosis in adult patients, to treat nausea/vomiting linked to cancer therapies, and stimulating appetite in HIV-positive patients. After highlighting the beneficial effects of CBG on murine colitis, it has been suggested that this cannabinoid should also be considered for clinical use in patients affected by inflammatory bowel disease [26–28].

The Entourage Effect of the Phytochemicals

The health benefits of fruits, vegetables, and other plant foods are due to the synergy or interactions among the different bioactive compounds or other nutrients present in whole foods and not the action of a sole compound [29]. *Cannabis*-based therapeutics also exert their pharmacological effects in humans via synergistic or antagonistic interactions among its phytochemicals. The interactions occur through various mechanisms, including bioavailability, interference with cellular transport processes, activation of prodrugs or deactivation of active compounds to inactive metabolites, the action of synergistic partners at different points of the same signaling cascade, or inhibition of binding to target [30]. One example is the stronger muscle-antispastic effect of a *Cannabis* extract compared to pure delta-9-tetrahydrocannabinoid in treating multiple sclerosis [31].

Non-delta-9-THC cannabinoids positively influence the side effects induced by delta-9-THC, such as antianxiety activities. Cannabinoids may also reduce the induced cognitive and memory deficits in subjects smoking *Cannabis* [32]. CBD affects the pharmacokinetics of delta-9-THC through different mechanisms such as fluidizing the membranes, increasing the penetration of delta-9-THC in muscle cells, and inhibiting the P450-mediated hepatic drug metabolism which is involved in the degradation and elimination of the molecule [33].

Terpenes also alter the pharmacokinetics of delta-9-THC by increasing the blood–brain barrier permeability. The characteristic has been used for transdermal patches, which deliver cannabinoids into the bloodstream using terpene as a permeation agent [34]. Terpenes may also modulate the affinity of delta-9-THC for the CB1 receptor and interact with neurotransmitter receptors, which support the contributions of terpenes on cannabinoid-mediated analgesic and psychotic effects [17, 25]. Flavonoids also modulate the pharmacokinetics of delta-9-THC via inhibition of the hepatic P450 enzymes, 3A11 and 3A4 [17]. The potential of phytocannabinoid-terpene synergy could be tailored for treatments such as CBD-terpene extracts to be used against acne, MRSA, depression, anxiety, insomnia, dementia, and addiction.

There is a predator-targeted synergy between terpenes and phytocannabinoids in the *Cannabis* plant. On the one side, the specific mixture of monoterpenes and sesquiterpenes determines viscosity and, thereby, the stickiness of *Cannabis* exudations necessary to trap the insects. On the other side, the phytocannabinoid acid acts as potent insecticidal molecules [17, 35].

The biological properties of cannabinoids rely mostly on their interactions with the endocannabinoid system in humans. The endocannabinoid system includes two

G protein-coupled cannabinoid receptors, CB1 and CB2, and two endogenous ligands, anandamide and 2-arachidonoylglycerol. Endocannabinoids modulate or regulate physiological processing, including appetite, pain sensation, mood, memory, inflammation, insulin, sensitivity, and fat and energy metabolism [14, 15].

The psychoactive decarboxylated delta-9-tetrahydrocannabinolic acid (THCA), delta-9-THC, is a partial agonist of both CB1 and CB2 receptors but with higher affinity for the CB1 receptor, which mediates its psychoactive properties. In addition to being present in the central nervous system and throughout the brain, CB1 receptors are also found in the immune cells and the gastrointestinal, reproductive, adrenal, heart, lung, and bladder tissues, where cannabinoids can exert their activities. CB2 receptors have immunomodulatory effects and regulate cytokine activity. Delta-9-THC has more molecular targets than just CB1 and CB2 receptors and exhibits potent anti-inflammatory, anticancer, analgesic, muscle relaxant, neuro-antioxidative metabolism [8], and antispasmodic activities [16].

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CBD is an important entourage compound in reducing the side effects of delta-9-THC and thereby increases the safety of *Cannabis*-based extracts. In vitro and animal studies have shown CBD to possess antianxiety, antinausea, antiarthritic, antipsychotic, anti-inflammatory, and immunomodulatory properties [19]. CBD is promising as a therapeutic agent in preclinical models of central nervous system diseases such as epilepsy, neurodegenerative diseases, schizophrenia, multiple sclerosis, affective disorders, and the central modulation of feeding behavior [20].

Cannabinoid-based treatments are limited to special cases such as spasticity associated with multiple sclerosis in adult patients, to treat nausea/vomiting linked to cancer therapies, and stimulating appetite in HIV-positive patients. After highlighting the beneficial effects of CBG on murine colitis, it was indicated for patients affected by inflammatory bowel disease [26–28].

Biosynthetic Pathways

The precursors of cannabinoids originate from two distinct biosynthetic pathways: the polyketide pathway, giving rise to olivetolic acid (OLA), and the plastidal 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway, leading to the synthesis of geranyl diphosphate (GPP) [12]. Olivetolic acid is formed from hexanoyl-CoA, derived from the short-chain fatty acid hexanoate, by aldol condensation with three molecules of malonyl-CoA [7]. This reaction is catalyzed by a recently discovered polyketide synthase (PKS) enzyme and an olivetolic acid cyclase (OAC) [13].

The geranylpiphosphate/olivetolate geranyltransferase catalyzes the alkylation of OLA with GPP leading to the formation of CBGA, the central precursor of

various cannabinoids [66]. Three oxidocyclases will then be responsible for the diversity of cannabinoids: The THCA synthase (THCAS) converts CBGA to THCA, while CBDA synthase (CBDAS) while CBDA synthase forms CBDA, and CBCA synthase (CBCAS) produces CBCA [64, 65]. Propyl cannabinoids (cannabinoids with a C3 side-chain instead of a C5 side-chain), such as tetrahydrocannabivarinic acid, synthesized from a divarinolic acid precursor, have also been reported in *Cannabis* [5].

Cannabinoid Receptors

Circulating endocannabinoids are found in multiple organs and tissues, including the brain, muscle, and adipose tissues. There are indications that endocannabinoids in the body accomplish specific purposes. For example, they are involved in regulating energy intake following exercise. In other cases, the endocannabinoids enter the circulation because of their lipophilic tendencies and can easily move from organs and tissues into circulation.

Endocannabinoids are found in all tissues, organs and bodily fluids so far investigated. The two well-studied endocannabinoids defined as endogenous ligands for cannabinoid receptors are the arachidonic acid derivatives, *N*-arachidonylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG). Both AEA and 2-AG are endogenous agonists of the canonical cannabinoid receptors, CB1R and CB2R; G protein-coupled receptors have widespread distribution throughout the body. Additionally, endocannabinoids can bind and activate the vanilloid receptor (TRPV1) and AEA as an agonist for several subtypes of the peroxisome proliferator-activated receptor (PPAR) family.

The biosynthetic and catabolic pathways for the endocannabinoids have been studied and revealed that 2-AG is produced from activating phospholipase C (PLC) in cells expressing diacylglycerol lipase [67]. Diacylglycerol lipase converts the PLC product diacylglycerol to 2-acylglycerols, including 2-AG and 2-oleoylglycerol (2-OG). The concentration of 2-OG in human serum is typically 5 to 10 times greater than 2-AG. It has been indicated that 2-OG is an endogenous ligand for the orphan receptor GPR119, a receptor that plays an important role in coupling food intake to the release of incretin hormones [68].

2-AG is catabolized by hydrolysis of the ester bond, resulting in the formation of glycerol and free arachidonic acid. The enzymes capable of hydrolysis are monoacylglycerol lipase (MAGL), alpha-beta hydrolase domain protein (ABHD)-6, and ABHD-12 [69]. In the metabolism of arachidonic acid by MAGL, 2-AG is a precursor for forming other arachidonic acid derivatives in the vasculature [70] and the brain [71, 72]. In the brain, MAGL activity plays a role in regulating neuroinflammation through this mechanism [72]. 2-AG can also serve as a substrate for enzymes that metabolize free arachidonic acid, including cyclooxygenase 2 (COX2) and some subtypes of lipoxygenase [73].

N-arachidonylethanolamine and other *N*-acyl ethanolamines (NAEs) are synthesized from low abundance phospholipids, *N*-acyl-phosphatidylethanolamine (NAPE), through several different pathways [67]. NAPE is synthesized via enzymatically mediated transfer of an acyl chain from the sn-1 position of donor phosphatidylcholine to the amine of phosphatidylethanolamine (PE). The NAEs formed relative abundance reflects the acyl chains found in the sn-1 position of the donor phospholipids; thus, concentrations of palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) are generally higher than AEA in biological samples, including serum and plasma.

Palmitoylethanolamide and OEA exert anti-inflammatory and anorexigenic effects due to interactions with PPAR α [74]. The NAEs are catabolized by hydrolysis of the amide bond through the actions of fatty acid amide hydrolase (FAAH) [75] and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA), which is found primarily in peripheral tissues [76]. *N*-arachidonylethanolamine can also serve as a substrate for COX-2, lipoxygenase, and P450 enzymes that utilize arachidonic acid as substrate [73].

Endocannabinoids in the Circulation

Endocannabinoid concentrations are typically measured in lipid extracts of serum or plasma obtained from venous blood using isotope-dilution, high-pressure liquid chromatography-mass spectrometry. Although the endocannabinoids can be measured in both plasma and serum, there is evidence that AEA concentrations are higher in serum than in plasma [77], and 2-AG concentrations are more reliably measured in serum [78].

Blood cells can release and inactivate AEA [79, 80]; thus, the concentrations of AEA measured in serum or plasma depend on the time between the blood draw and the separation of cells from serum or plasma [81]. It is the best practice to keep the time between blood draw and serum or plasma harvest as consistent as possible to minimize sample differences in ex vivo generation or loss of AEA. AEA concentrations are stable in serum or plasma after it has been frozen [82]. The arachidonic acid in 2-AG readily migrates to the one position under most storage and extraction conditions [83]. 2-AG and 1-AG migrate at different rates in the standard chromatography used to separate the endocannabinoids. Most methods assume that 2-AG and 1-AG originate from biological 2-AG and add those peaks to determine the total 2-AG.

Several methods have been published to extract endocannabinoids and related lipids from liquid matrices such as serum and plasma [84]. A liquid-liquid extraction method has been used in which the sample is mixed with methanol and chloroform with repeated vortexing [85]. Acetonitrile can also be used as the organic solvent, with sonication as the method of mixing. Solid phase extraction using C18 columns of the samples that have been made 15% in ethanol, followed by elution with ethyl acetate, is also an effective and reproducible method for endocannabinoid

extraction from biological fluids [86]. A modification of that approach utilizes C18-containing pipette tips to reduce the solvent and time for extraction [87].

Quantifying the deuterated endogenous endocannabinoids is usually accomplished with high-pressure liquid chromatography utilizing a C18 column, followed by mass spectrometry. With the availability of quadrupole tandem mass spectrometers, species identification and measurement precision are enhanced due to the abundance of ions rather than just the measured parent ions. One method described the use of online sample trapping and microflow liquid chromatography coupled with a tandem mass spectrometer that allowed for the detection of the endocannabinoids in 50 μ L of serum with the use of much less solvent than the conventional methods [88].

Serum AEA usually falls between 1 and 5 nM, while serum 2-AG concentrations range from 10 to 500 nM [78]. Some likely reasons for the range of endocannabinoids in healthy individuals include the time of day and whether the individual has exercised or eaten recently. AEA can bind to the plasma protein albumin [89]. However, the equilibrium dissociation constant of bovine serum albumin for AEA is 55 nM at 37 °C [90], suggesting that most AEA in the circulation is not protein bound. The binding of 2-AG to plasma proteins is likely similar to AEA, and arachidonic acid would render it a low-affinity ligand for albumin binding sites.

Endocannabinoids have been quantified in other human biological samples, including saliva [91], hair [92], semen [93], breast milk, and amniotic fluid [77]. Endocannabinoid measurements in biological samples are less frequently determined than measurements in circulation; however, they could have important advantages. For example, measurement in the hair could provide a retrospective picture of endocannabinoids. From saliva, it is easier to obtain than from blood, particularly in a nonhospital setting.

Endocannabinoids and Food

Endocannabinoids play a very important role in regulating metabolism in the systems and cellular levels [94]. The endocannabinoid system exerts regulatory control on every aspect of intake, metabolism, and storage of calories. Activation of CB1R signaling by the endocannabinoids results in increased consumption of food. In the hypothalamus, tonic endocannabinoid-CB1R signaling functions to maintain food consumption and is suppressed by the homeostatic appetite regulator, leptin [95]. CB1 receptors in hypothalamic nuclei modulate energy homeostasis by interacting with ghrelin [96]. Activation of CB1R signaling enhances reward circuits through effects in the mesolimbic system [97–99], potentiating hedonic (reward-driven) consumption of highly palatable foods in particular. Functional imaging of humans performing a reward-driven task following delta-9-THC indicates significant attenuation of feedback to the reward circuits compared to placebo administration suggesting a particular role for CB1R signaling in feedback inhibition of reward seeking [100].

CB1R is present in peripheral organs involved in energy metabolism, including adipose tissue, the endocrine, the pancreas, muscle, and the liver [101–103]. Increased CB1R signaling increases energy storage by stimulating fat mass expansion, glucose uptake, adipogenesis in adipocytes [104], induction of lipogenesis in the liver [105], and increased insulin secretion from the pancreas [106]. CB1R signaling increases the processes that reduce energy utilization, including decreased brown fat thermogenesis and glucose uptake into skeletal muscle [107, 108]. CB1R signaling is thus coordinated throughout the body to maximize the intake and storage of energy. The function of the endocannabinoid-CB1R system has great implications for obesity and other components of metabolic syndrome in humans. The CB1R antagonist, rimonabant, was found to have significant, beneficial effects in humans to reduce body weight, improve lipid profiles, and increase insulin sensitivity before it was removed from clinical use [109]. One study identified the expression of CB1R in the subfornical organ of the hypothalamus, a brain region responsive to changes in circulating signals of metabolic status [110]. Endocannabinoids in circulation could contribute to the information utilized by the brain to alter behavior and physiological responses.

The pattern of change in circulating AEA concentrations surrounding food consumption is consistent with its role as an orexigenic factor in homeostatic (hunger-driven) feeding. Presentation of food (prior to consumption) results in a significant increase in circulating AEA in normal-weight and obese individuals [111], followed by a small but reproducible fall in circulating concentrations of AEA over several hours following food consumption [111–113]. In normal-weight individuals, the postmeal reduction in circulating AEA is independent of the hedonic value of the meal [112]. Intravenous glucose administration also reduces circulating AEA for several hours following infusion in normal-weight individuals [114].

Oral consumption of 250 mL of grape juice (41 g sugar) significantly elevated blood glucose levels and reduced circulating 2-AG but did not affect AEA concentrations [115], suggesting that there is a dose dependence on the effects of sugars on endocannabinoids in the circulation. The suppressive effects of food and glucose on circulating AEA concentrations could be mediated by increased insulin since the exogenous insulin infusion also reduces circulating AEA concentrations in the same time frame. The effect of glucose to reduce circulating AEA was lost in insulin-insensitive individuals [113]; however, one study found that a hyperinsulin/euglycemic clamp decreased circulating AEA concentrations in insulin-sensitive and insulin-resistant obese individuals [116]. In vitro data demonstrate that normal but not insulin-resistant adipocytes respond to insulin with significant reductions in intracellular concentrations of both AEA and 2-AG [117].

The response of 2-AG to food is affected by its perceived hedonic value, suggesting that 2-AG is involved in reward-driven (hedonic) feeding. When normal-weight individuals are presented with a favorite compared to nonfavored food, circulating 2-AG concentrations are significantly higher at food presentation and during and after its consumption [112, 113]. Sleep deprivation promotes increased hedonic eating, particularly in the later part of the day [118–120], and also increases circulating 2-AG concentrations at mid-day [121]. Those findings suggest that circulating 2-AG

concentrations reflect the activation of reward circuits in the brain and could be a mediator of hedonic eating.

Neither underweight nor weight-restored individuals with *anorexia nervosa* exhibited an increase in 2-AG in response to the presentation or eating a favorite food [113], consistent with hypotheses that dysregulated evaluation of rewarding stimuli occurs in anorexia [122]. That circulating 2-AG is related to perceived reward. Circulating concentrations of AEA declined after favorite and nonfavorite food consumption in both subgroups of anorexic individuals, patterns that did not differ from normal-weight individuals [113]. The findings are consistent with the notion that was circulating AEA concentrations involve homeostatic and not hedonic feeding processes.

The olfactory threshold discrimination identification capacity is significantly lower in obese women than in normal weight and is inversely related to circulating 2-AG concentrations [123]. It is counterintuitive since preclinical studies suggest that CB1R signaling in the olfactory epithelium is associated with enhanced sensitivity to odorants [124], and delta-9-THC exposure can enhance the sensory aspects of food consumption in humans [125]. However, the human data reflect the down-regulation of olfactory CB1 receptor signaling in response to high 2-AG concentrations.

Food-induced changes in circulating endocannabinoids have been characterized in obese individuals to examine the hypothesis that high endocannabinoid tone is associated with obesity. The consumption-induced reduction in circulating AEA concentrations seen in normal-weight individuals is blunted in insulin-insensitive, obese individuals. The magnitude of the decrease in AEA following eating is inversely correlated with waist circumference [111]. Since the individuals were insulin insensitive and obese, insulin sensitivity loss may underlie differences in waist circumference and the lack of AEA decrease following food consumption. The premeal increase in AEA was intact in obese individuals, suggesting that the mechanisms underlying the premeal increase and postmeal decrease in AEA were different. It was also discovered that obese individuals exhibit a loss in the decline in AEA following food consumption, but the change occurs when favorite but not nonfavorite foods are consumed [126].

Endocannabinoids and Body Mass Index

It has been determined that concentrations of 2-AG in blood, harvested in the morning after an overnight fast, were higher in men and women with body mass index (BMI) values in the obese range compared to normal-weight individuals. Although obese individuals with subcutaneous fat distributions have elevated 2-AG compared to normal weight, those with a visceral fat distribution were even higher [127]. Furthermore, circulating concentrations of 2-AG were positively correlated with BMI, total body fat, particularly measures of visceral fat mass [127], and intra-abdominal adipose (IAA) tissue [128]. There was a positive correlation between

BMI and circulating AEA concentrations in individuals with a range of BMI from 18 to 45 [129]. Women's studies found that AEA and 2-AG are higher in obese than normal-weight individuals [111, 130].

Conversely, a study of obese men with high amounts of IAA found that AEA concentrations in the morning were lower than normal or obese men with low IAA [128]. It is possible that differences in the relative distributions of visceral and subcutaneous fat stores between men and women could underlie the sex differences [131]. The case that identified an increase in 2-AG in obese individuals studied a cohort without high visceral fat depots, which further supports the visceral fat as the source of 2-AG in obesity [114]. The findings suggest that 2-AG enters the circulation from visceral abdominal fat stores, so its concentration is highest in individuals with the most visceral fat. Insulin-resistant obese had higher circulating 2-AG concentrations than insulin sensitive; however, the insulin-resistant group also had a significantly higher BMI, which could underlie the higher circulating 2-AG [116]. The roles of 2-AG to increase fat storage in adipose and liver [94] and its ability to promote the consumption of highly palatable foods through effects on the brain suggest that 2-AG-CB1 receptor signaling participates in a feed-forward cycle to promote other food consumption and increased body weight gain in the obese [132].

The circulating endocannabinoid concentrations in mice, made obese through long-term exposure to exogenous glucocorticoids, had almost twice as much circulating AEA in the obese mice compared to controls [133]. 2-AG concentrations in the circulation were unchanged by the treatment, suggesting that obesity, as a result of excess glucocorticoids, was mediated by AEA rather than increased 2-AG.

The expression of FAAH in adipose tissue biopsies in relationship to the circulating endocannabinoid concentrations in postmenopausal women, adipose FAAH expression was reduced in obese women compared to normal weight and was negatively correlated with circulating AEA [130]. Interestingly, in both men and women, adipose FAAH expression was negatively correlated with morning 2-AG concentrations [127]. Although 2-AG can serve as a substrate for FAAH *in vitro* [134], pre-clinical studies in mice without FAAH expression indicated no changes in 2-AG content, leading to the assumption that FAAH does not catabolize 2-AG *in vivo* [135]. The correlation between adipose FAAH expression and circulating AEA was stronger than with circulating 2-AG [130]. It is possible that reduced FAAH expression was a causative factor for the elevated AEA, which primes the system to increase caloric intake and fat storage through CB1 receptor activation. Adipose fat stores increased, increasing circulating 2-AG, leading to the feed-forward cycle. There was no significant relationship between adipose FAAH activity and BMI or any other measure of metabolic disorder; BMI was positively correlated with adipose MAGL activity in obese without diabetes [136]. The rates of catabolism of AEA and 2-AG in adipose do not contribute to their circulating concentrations.

Preclinical findings suggest that a source of circulating endocannabinoids is the small intestine [137]. The lipids entered the portal system and contributed to elevations in the circulating concentrations. Lipid feeding reduced the catabolism of both AEA and 2-AG, resulting in increased tissue contents. Sham-feeding rats with a lipid meal increased endocannabinoid contents in the jejunum via a signal carried

by the vagus nerve. Peripherally restricted CB1R antagonists reduced hyperphagia in mice fed a Western diet (i.e., high in carbohydrates and fat), which supports a role for peripheral CB1R signaling in hyperphagia [138].

Although not endocannabinoids, preclinical studies indicated that the NAE and OEA have important effects on satiety and feeding behavior. The administration of OEA to mice reduced food intake and body weight gain, likely through PPAR-alpha in the GI tract [139] and or through actions in the area postrema [140]. Enterocytes of the small intestine synthesize OEA in response to fat intake [141]. According to the preclinical findings, hypercholesterolemic patients with diets enriched in oleic acid exhibited significantly higher plasma OEA concentrations and a negative relationship between plasma OEA and percent body fat [142]. The finding that circulating OEA and AEA concentrations do not change in parallel in response to eating supports the hypothesis that circulating OEA concentrations are regulated by a mechanism that differs from that regulating the concentration of AEA [126].

Endocannabinoid and Circadian Rhythm

Serum 2-AG concentrations exhibited a significant circadian rhythm in humans under standardized sleep timing and caloric intake [143]. The average low point (nadir) of 2-AG concentration occurred at 0400 h, 5 h after bed, and 9 h after the last food consumption. 2-AG concentrations rose steadily, followed by an extended period of maximum concentrations between 1200 and 1500 h, encompassing the midday meal at 1400 h. The return to lower, nocturnal 2-AG concentrations began in the late afternoon/early evening, with a small plateau in 2-AG concentrations occurring between 1700 and 2000 h, which surrounded the evening meal at 1900 h. A remarkable threefold change in circulating 2-AG concentration occurred between the nadir and acrophase.

Reducing the number of hours of sleep to four while maintaining the same sleep period mid-point produced a significant increase in the amplitude and peak concentrations of 2-AG [121]. The time of the acrophase was shifted significantly from 1230 to 1430 h, and the mean 2-AG concentrations between 1400 and 2100 h trended higher. Restricted sleep increased and extended the peak 2-AG concentrations in the afternoon following sleep restriction. The sleep restriction did not affect patterns of 2-AG in the morning and did not affect the nadir concentrations or timing. Instead, the effect of restricted sleep on 2-AG was delayed until later in the day.

The circulating endocannabinoids, particularly 2-AG, are in the pantheon of molecules that alter metabolism and are circadian. 2-AG fills a unique role because it is an orexigenic mediator that exhibits its highest concentrations at midday and lowest during sleep. Neither the hormones controlled by the HPA axis nor adipokines and gut hormones reached peak concentrations in the middle of the normal feeding period [144]. The increases in 2-AG and 2-OG began in the middle of the sleep episode, thus preceding breakfast, awakening, and morning light exposure.

The timing of food intake, transition from sleep to wakening, and light/dark cycles are circadian regulators. However, the increases in circulating 2-acylglycerols precede the events and are not likely driven by them. Glucocorticoids also rise in the morning, and data obtained in rodents indicated that glucocorticoids could rapidly increase 2-AG synthesis in the hypothalamus [145]. While morning changes in 2-AG paralleled the rise in cortisol, the nadir and acrophase for cortisol were 3 to 4 h earlier than for 2-AG [143]. Thus, 2-AG concentrations appeared to have an endogenous circadian rhythm that differs from other metabolic regulators.

The CB1R signaling provides a highly efficacious orexigenic signal, and the timing of the peak in circulating 2-AG has been hypothesized to engage CB1Rs throughout the body to promote eating during the middle of the active period [102, 143]. The circulating 2-AG is, therefore, involved in hedonic rather than homeostatic eating. One hypothesis is that midday 2-AG rise promotes food intake for storage rather than immediate use. In this regard, insulin sensitivity exhibits a robust decrease from morning to evening in healthy human volunteers, consistent with increased 2-AG-mediated CB1R signaling over that period [146, 147]. The elevation of 2-AG in situations that could significantly affect future metabolic needs, such as chronic loss of sleep, could protect against starvation by promoting an increase in fat stores. That hypothesis was supported by the findings that sleep deprivation increased feelings of hunger and drive to eat high-calorie foods in the afternoon and increased and shifted the 2-AG acrophase to later times [121]. The mechanisms that underlie the circadian changes in 2-AG and the shift in the pattern that occurs with sleep deprivation are not known. However, given that sleep deprivation is a significant stressor, 2-AG concentrations may be sustained due to stress-induced glucocorticoid elevation.

Endocannabinoids and Exercise

The fact that circulating endocannabinoids coordinate a system-wide response in seeking, consuming, and storing energy leads to the hypothesis that increased energy utilization results in increased circulating endocannabinoids to replenish energy stores. In support of this hypothesis, multiple studies demonstrated that 30–90 min of moderate exercise increased circulating concentrations of AEA and other NAEs immediately after the exercise period [148–150]. The study did not reveal an immediate increase in AEA [151]. AEA increases reached significance when heart rate was increased to about 75% of maximum [152]; neither lower (i.e., walking) nor near-maximum exercise intensities affected AEA concentrations [149, 152], indicating a “U-shaped” relationship between exercise and AEA mobilization.

Well-trained healthy volunteers who participated in a strenuous hike at an altitude below 2100 meters, and those who hiked and ascended approximately 2000 meters in altitude, exhibited increases in circulating AEA concentrations following the exercise period [153]. Hypoxic stress of exercise at high altitudes appeared to

potentiate the increase in AEA. A preclinical study utilizing hepatic ischemia-reperfusion injury also found that 2-AG was increased in response to ischemia, which could reflect the regulation of endocannabinoids by the state of oxygenation of tissues [154].

A study of normal-weight and obese women indicated that moderate–vigorous physical activity monitored over six days was positively correlated with circulating AEA concentrations [155]. High circulating AEA concentrations are also associated with high BMI. On the other hand, no differences in basal concentrations of AEA and 2-AG were found between active and sedentary normal-weight men [156].

The effects of exercise on circulating 2-AG concentrations have been less consistent. Studies that employed 30–90 min of sustained exercise observed increases in AEA and did not find an increase in 2-AG [148–150]. One study employing an exercise regimen known to induce exercise-induced hypoalgesia, 3 min of isometric contractions of the forearm, found that 2-AG increased significantly, in addition to the NAEs [157]. Another study found an increase in 2-AG measured in blood collected 15 min after 30 min of exercise [151]; however, the subjects in that study were not given food on the morning of the experiment, which could have resulted in a more physiologically stressful paradigm than exercise alone. Thus, the data are most consistent with a hypothesis that moderate levels of sustained (i.e., more than 30 min) exercise increased AEA and other NAEs in the circulation, while 2-AG increased in response to short bursts of activity, perhaps specifically in response to isometric contractions.

The increased endocannabinoids in the circulation following exercise come from skeletal muscle. Messenger RNAs encoding NAPE-specific phospholipase D and diacylglycerol lipase were present in skeletal muscle [158, 159]. PEA and another NAE stearoylethanolamide were increased by exercise in microdialysates of the trapezius muscle in individuals with chronic neck/shoulder pain [160], providing additional support for the hypothesis that endocannabinoids can be produced in the muscle. It is well established that exercise increases circulating glucocorticoid concentrations [161], and exogenous glucocorticoid treatment produces a rapid increase in brain AEA concentrations suggesting a possible link between glucocorticoid receptor activation and AEA synthesis [158].

Endocannabinoid mobilization during exercise is consistent with the role of CB1R signaling in regulating metabolism. Activation of CB1R in the liver and adipose move the system toward energy storage and thus can both restrain energy use and contribute to the recovery of energy stores following exercise [162]. AEA and other NAEs could produce positive metabolic effects locally in skeletal muscle, including enhanced glucose uptake, improved insulin action, and mitochondrial biogenesis via activation of PPAR γ m [163].

Although endocannabinoids can also act centrally to increase food consumption, a recent study found that exercise-induced increases in endocannabinoids were not correlated with feelings of subjective hunger, indicating any metabolic role for the circulating endocannabinoids is likely to be more peripheral than central [151]. A study in healthy cyclists demonstrated a positive correlation between the increase in circulating AEA and an increase in the neurotrophic factor, BDNF, following a

90-min exercise period [148]. Although the relationship's directionality is unknown, acute administration of delta-9-THC to healthy, non-*Cannabis*-using humans also increased serum BDNF concentrations [164], suggesting that BDNF synthesis/release is evoked by CB1R activation.

Preclinical studies in the CNS are also consistent with the CB1R regulation of BDNF [165, 166]. The data suggest that endocannabinoids could be the mediators of exercise-induced increases in BDNF [167] and, thus, of the hypothesized effects of exercise on cognitive function and mood [168].

Endocannabinoids and Inflammation

Circulating endocannabinoids were significantly increased in several conditions with systemic inflammations. Both 2-AG and AEA are folds-higher in the serum of patients with endotoxic shock than in control serum [169]. Circulating endocannabinoids were increased in patients with cirrhosis [170], chronic hepatitis C infection [171], exposure to solar ultraviolet radiation [172], congestive heart failure [173], and atherosclerosis [174]. Similarly, rats with acute pancreatitis also exhibited elevated circulating AEA concentrations, particularly in animals with severe necrotizing pancreatitis [175]. When activated, macrophages [80, 176], platelets [80], and T and B cells released endocannabinoids *ex vivo* and could be a source of circulating endocannabinoids [177].

On the other hand, patients undergoing hematopoietic stem cell transplantation (HCT) indicated that bone marrow-derived immune cells might not be an important source of 2-AG [178]. No change in 2-AG concentrations occurred between the period in which the HCT patient's immune system was ablated and after immune system reconstitution. Preclinical studies suggested two alternative hypotheses. First, nonimmune cells may respond to inflammatory mediators with increased endocannabinoid release, as shown in inflamed hepatocytes [179]. Second, inflammation reduces 2-AG catabolism by splenic carboxylesterase 2 *g* in the spleen [180], suggesting that the rise in 2-AG during inflammation could result from spillover from the spleen, which is enhanced as a result of decreased 2-AG hydrolysis in the tissue.

2-AG is an agonist of the CB2 receptor [181]; a GPCR expressed constitutively by human B and T cells, macrophages, and to a lesser extent, neutrophils [182]. CB2R activation is associated with a reduced proinflammatory cytokine release [171, 183] and reduced migration of T cells into the brain [184]. The indications that 2-AG can be elevated during inflammation and that it activates an anti-inflammatory receptor suggest the hypothesis that endocannabinoids function as a feedback system to dampen inflammation. A recent preclinical study highlighted this relationship. In that study, antigen activation of macrophages and dendritic cells resulted in increased 2-AG, and CB2R antagonist treatment increased antigen-specific immune responses in mice, suggesting that 2-AG/CB2R activation suppresses innate and adaptive immune responses in mice [185].

Several studies have indicated positive correlations between circulating 2-AG concentrations and concentrations of the proinflammatory cytokine interleukin-6 (IL-6) [129, 178]. If 2-AG is anti-inflammatory, even to a small extent, one would expect a negative relationship between IL-6 and 2-AG. In this regard, 2-AG potentiates LPS-stimulated IL-6 expression in a macrophage cell line [186]. On the other hand, plasma concentrations of 2-AG and the cytokine tissue necrosis factor- α were found to be negatively correlated [187].

The relationships between 2-AG and cytokines in the circulation need further study, as does the fundamental question of whether circulating endocannabinoids regulate inflammation in humans. Conditions in which circulating endocannabinoids are elevated also result in inflammation, including acute bouts of exercise [188] and obesity [189]. Data support the possibility that there are connections between endocannabinoids and inflammation. Activation of CB1R signaling in the context of inflammation can contribute to fibrosis and other detrimental effects of chronic inflammation [190]. Circulating concentrations of AEA were elevated in patients with cirrhosis, and their concentrations were positively correlated with negative indices of liver function [191].

The Endocannabinoids and Stress

Exposure to physical and psychological stressors resulting in coordinated behavioral, endocrine, and neuronal changes increases the chances for survival if an injury happens. Acute stress responses include activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. Stress exposure also produces consistent and reproducible increases in inflammation systemically and in the brain, likely as a downstream consequence of sympathetic activation [192]. Acute psychological stress resulted in an immediate increase in natural killer T cells and interleukin 1 β (IL-1 β) [193] and a 90 min delay increase in IL-6 [194]. Sympathetic activation resulted in the migration of monocytes and macrophages into the circulation and triggered a release of IL-1 β and other cytokines [193].

Preclinical data support the hypotheses that endocannabinoid signaling is altered by stress and mediates or modulates stress responses [195]. Endocannabinoid/CB1R signaling inhibits the activation of the HPA axis by stress [197] and enhances recovery following stress offset [198]. Evidence obtained in the brain demonstrates that 2-AG mobilization can occur downstream of glucocorticoid receptor activation, while AEA is elevated by corticotrophin-releasing hormone receptor activation secondary to inhibition of FAAH [196].

Preclinical studies demonstrated that CB1R signaling in the nucleus tractus solitaries (NTS) potentiates the sympathoinhibitory responses to activate the baroreceptor reflex [202–204]. CB1R is present on sympathetic nerve terminals, and their activation resulted in sympathomimetic effects in preclinical studies [199, 200]. However, AEA infused intra-arterially in the human forearm failed to affect blood flow, indicating that CB1R activation may not affect release from sympathetic nerve

terminals in healthy humans [201]. On the other hand, CB1R activation in the periaqueductal gray (PAG) increased the activity of the SNS [205].

Activation of the SNS as a result of inducing postural hypotension with a head-up tilt is accompanied by a significant increase in 2-AG concentrations [206]. However, associations between 2-AG concentrations and measures of sympathetic activation were not indicated. In that study, circulating concentrations of AEA were positively correlated with the ability of patients to tolerate the procedure without fainting. The result indicated a positive relationship between AEA and SNS activity.

Vestibular activation of the SNS triggers nausea and vomiting [207], and parabolic flight maneuvers that trigger motion sickness produced an immediate increase in circulating AEA concentrations and a delayed (recovery phase) increase in 2-AG [208]. Increases in 2-AG and AEA concentrations were associated with less motion sickness, consistent with preclinical and human data that exogenous activation of CB1R signaling reduces nausea and vomiting [209].

Cyclic vomiting syndrome (CVS) is a functional disorder characterized by relapsing and remitting bouts of prolonged nausea and vomiting. Hyperemesis is significant physiological stress and is accompanied by increases in both SNS and HPA axis activation. Since endocannabinoids are elevated during stress and can reduce nausea and vomiting, circulating endocannabinoids were compared between sick (active vomiting) and well phases in individuals with CVS [210]. Concentrations of both AEA and 2-AG were elevated in the sick compared to good phases, but the increases did not reach statistical significance. Concentrations of both PEA and OEA were significantly increased and positively correlated with the amount of distress. No significant associations between circulating endocannabinoid concentrations and circulating cortisol or salivary amylase were found in the study.

Acute exposure to psychological stress also elevates circulating endocannabinoid concentrations. A commonly used procedure to evoke psychological stress in humans in a laboratory setting is the Trier Social Stress Test (TSST). There are various forms of the TSST, but most include public speaking and solving difficult arithmetic problems [211]. The TSST activated the SNS and HPA axis and increased perceived anxiety. Administration of a 17-min version of the TSST resulted in a significant increase in 2-AG concentrations immediately afterward that returned to basal concentrations 30 min after the end of the stressed period [198]. Serum AEA concentrations were not significantly different from baseline at either time.

However, a second study found that a shorter stress period of 5 min produced no significant change in 2-AG concentrations and significantly increased circulating AEA concentrations immediately after the stress [212]. There was preliminary evidence of differences in the endocannabinoid response among males, and females in each phase of their menstrual cycles, with males exhibiting a greater increase from baseline than females, particularly females in the follicular phase. The study also found preliminary evidence of differences in the endocannabinoid response to stress between African and European descent. The change in PEA from baseline to after stress was positively correlated with the change in cortisol measured in the same samples; the change in AEA concentration was not significantly associated with cortisol.

A study in which imagery of a previous stressful experience was administered found that AEA concentrations were not changed from baseline but were significantly lower than baseline 75 min after the end of the stressed period, which is reminiscent of the effects of food consumption on circulating AEA concentrations [213].

Circulating endocannabinoid concentrations are sensitive to acute psychological stress, and their relationships are complex, time-dependent, and affected by sex. Circulating concentrations of AEA and 2-AG can be affected by acute stress. However, associations with the primary stress effector systems, the HPA axis, and SNS do not consistently indicate a direct relationship between the activation of the systems and concentrations of endocannabinoids in the circulation. Preclinical studies indicated that the function of elevated endocannabinoids during stress is to buffer the stress response and hasten return to homeostasis through inhibition of the HPA stress response. Thus, if endocannabinoids in the circulation are downstream and upstream of the HPA axis, the relationships between endocannabinoid and cortisol concentrations would likely be nonlinear and dependent upon timing and individual responsiveness to the systems. It is also possible that changes in endocannabinoid concentrations occur with a time delay; cortisol concentrations or measures of SNS were not related when examined simultaneously.

Endocannabinoids and Chronic SNS or HPA Axis Activation

There is evidence that circulating endocannabinoids are associated with any measure of SNS activation at baseline, and AEA is associated with SNS-mediated physiological effects in various pathological conditions. AEA concentrations are higher in individuals with sleep apnea and positively correlate with systolic and diastolic blood pressure independent of BMI and insulin resistance [214]. Depressed women also exhibit a positive correlation between circulating AEA concentrations and diastolic blood pressure, with a trend toward a significant association with systolic pressure [215]. Intravenous injections of AEA in experimental animals reduced blood pressure, indicating that the relationship underlying the correlation between circulating AEA concentrations and blood pressure is that high blood pressure results in increased AEA [216]. Increased blood pressure in rats elevated AEA concentrations in the NTS [203]. On the other hand, circulating concentrations of AEA were significantly reduced in individuals with preeclampsia, a condition associated with hypertension in late pregnancy, indicating that increased blood pressure alone is not adequate to elevate circulating AEA concentrations [217].

Circulating endocannabinoid concentrations were monitored in individuals isolated from family and friends for more than 500 days in a study simulating a trip to Mars [218]. The individuals exhibited reductions in positive emotions and increased SNS activation. Circulating concentrations of 2-AG, but not AEA, were reduced at the end of the chronic stress. 2-AG concentrations were positively correlated with EEG alpha activity, suppressed by the isolation, and negatively correlated with circulating cortisol. The results suggested that 2-AG is brain-derived and driven by

excitatory signaling, consistent with the finding that glutamate increased 2-AG synthesis [67]. The results were also consistent with the role of 2-AG in reducing HPA axis activation [219]. The circulating 2-AG concentrations and measures of positive emotion were not associated in the study, given the evidence that 2-AG is negatively associated with symptoms of depression.

Endocannabinoids in Reward, Anxiety, and Cognition

CB1R signaling regulates mood and modulates reward-seeking behavior, hedonia, and behavioral responses to threats and stress. Studies in which circulating endocannabinoids were measured examined their relationships to mood, anxiety, and perception indices. The underlying assumption was that circulating concentrations were affected by synaptic endocannabinoid tone, particularly in limbic brain regions. However, preclinical studies that have tested this directly dispute the assumption [220]. Rats exposed to chronic, unpredictable stress had reduced endocannabinoid concentrations throughout the limbic regions of the brain, while plasma concentrations of AEA and 2-AG were significantly increased. However, data from human studies have found correlations between circulating endocannabinoids and measures of mood and anxiety, suggesting that CNS function is translated into alterations in circulating endocannabinoids.

Circulating AEA concentrations are inversely related to cognitive and somatic measures of anxiety [86, 212]. Carriers of FAAH C385A have increased circulating AEA concentrations, and anxiety evoked by guided imagery of trauma decayed more rapidly than those homozygous for C at that position [221]. However, touch massage did not alter circulating endocannabinoids, a procedure that reduced feelings of anxiety, although the procedure significantly increased concentrations of PEA [222].

Circulating endocannabinoids were altered in individuals diagnosed with post-traumatic stress disorder (PTSD). The circulating concentrations of 2-AG were significantly lower in individuals with PTSD due to the World Trade Center incident [223]. The 2-AG decrease remained significant after correction for sex, alcohol use, and depression. Lower circulating 2-AG concentrations, without a difference in AEA, were also found in a study of individuals with PTSD due to childhood sexual abuse compared to controls [224]. The concentrations of OEA in hairs were significantly reduced in war survivors with PTSD compared to survivors without the diagnosis and negatively correlated with symptom severity [225]. The measurement in the hair could be more stable and reliable, particularly for a chronic disorder, than a single time point measurement in the blood.

A study of alcoholics with PTSD found that the FAAH C385A SNP is associated with reduced hyperarousal [221]. Although no group effects were identified, AEA concentrations were negatively associated with the number of intrusive symptoms in those with chronic PTSD [223]. War refugees with average scores of greater than ninety on the clinically administered PTSD scale (CAPS) and more than eight

episodes of trauma exhibited increased concentrations of 2-AG and AEA compared to trauma-exposed individuals without PTSD and controls [226]. The contrast suggests that the amount of distress, not just the diagnosis of PTSD, could be an important determinant of endocannabinoid regulation.

Increased circulating 2-AG concentrations are associated with enhanced hedonic food consumption, perhaps due to CB1R-mediated activation of reward seeking. Exercise has beneficial effects on mood, feelings of vigor, and anxiety, effects mediated by the CNS. Exercise-induced increases in AEA were positively correlated with increased positive effects on the Positive and Negative Affect Schedule (PANAS), indicating that recruitment of AEA-mediated signaling could contribute to the rewarding or reinforcing effects of exercise [149]. Exercise-induced increases in AEA were positively correlated with circulating concentrations of BDNF, which is also associated with mood elevation [148].

The acute effects of drugs associated with increased pleasurable feelings on circulating endocannabinoid concentrations have been investigated. Consumption of 250 mL of red wine with low sugar content reduced AEA and 2-AG at 20 and 45 min after consumption [115]. Healthy volunteers receiving an intravenous dose of 0.1 mg/kg delta-9-THC responded with small increases in 2-AG and AEA in the plasma at 30 min, followed by marked reductions in both 5 h after the delta-9-THC administration [230]. The effects of delta-9-THC indicated that the endocannabinoid system is regulated by cannabinoid receptor ligand concentrations so that endocannabinoid concentrations are reduced in the face of exogenous agonist consumption.

Guided imagery procedures that produce alcohol desires and cravings in social drinkers increased circulating concentrations of AEA, which were positively correlated with alcohol craving and increased heart rate [213]. Baseline concentrations of AEA in circulation were significantly lower in abstinent alcoholics than in social drinkers. The relationship between AEA and alcohol craving was lost in recently abstinent alcoholics, suggesting that endocannabinoid signaling within the reward circuit is dysregulated during abstinence.

Circulating AEA concentrations were significantly lower in runners who met the criteria for exercise dependence compared with runners who carry out similar amounts of activity but do not exhibit exercise dependence [227]. Individuals who met the exercise dependence criteria exhibited greater mood disturbance and lower basal levels of AEA when deprived of exercise than runners who did not exhibit exercise dependence [228]. The data indicated deficient endocannabinoid signaling that could contribute to dysphoria during abstinence; resumption of exercise resulted in the elevation of mood without an increase in circulating AEA, which does not support the hypothesis. A large number of NAEs and monoacylglycerol (MAGs) in plasma from abstinent cocaine addicts found that NAEs increased while MAGs decreased [229]. However, neither AEA nor 2-AG was affected in the study.

Circulating concentrations of 2-AG and AEA were altered in women diagnosed with depression. 2-AG concentrations were lower in those newly diagnosed with major depression than in controls, with a negative relationship between circulating 2-AG and the duration of depressive symptoms, supporting the hypothesis that

endocannabinoid deficiency contributes to depressive symptoms [86]. AEA concentrations were increased in women diagnosed with minor depression, which could be a compensatory mechanism to maintain the elevated mood. Baseline concentrations of both AEA and 2-AG were lower than controls [198]. 2-AG mobilization in response to the TSST was not different between control and depressed women, indicating that the mechanisms involved in the endocannabinoid response to acute stress and their role in depression differ [198]. Individuals undergoing HCT were negatively associated with circulating 2-AG concentrations and depressive symptoms [178].

In patients with osteoarthritis, circulating 2-AG concentrations were positively associated with depression scores on the Hospital Anxiety and Depression Scale and negatively correlated with memory performance scores [231]. It was unclear if circulating endocannabinoid concentrations were related to specific depression symptoms or clusters of symptoms.

Cannabinoids and Cognitive Functions

Cannabis use in humans is associated with impaired cognitive functions, including reduced behavioral flexibility [232]. A study of 150 healthy women indicated a negative correlation between 2-AG and cognitive flexibility performance when assessed using the Wisconsin Card Sorting Test (WCST) [233]; is consistent with the hypothesis that excessive CB1R activation, likely in the prefrontal cortex, reduces executive functioning. The same study indicated a positive correlation between circulating AEA concentrations and behavioral flexibility in the WCST and the Iowa Gambling Task.

The roles of cannabinoids and endocannabinoid signaling in memory formation, retrieval, and extinction have been studied extensively in preclinical models. While it is clear that memory is regulated by CB1R signaling, there is considerable variability in pharmacological studies related to dose, timing, and differences in model systems. Circulating 2-AG concentrations are negatively correlated with memory in individuals with osteoporosis [231]. Conversely, plasma 2-AG concentrations are higher in individuals diagnosed with Alzheimer's disease (AD) compared to matched controls and were positively correlated with memory and attention in those with AD [234]. However, there were no differences in circulating endocannabinoid concentrations between patients with AD and controls [187].

The general anesthetic, propofol, has efficacy as a FAAH inhibitor and elevates brain AEA concentrations in mice [235]. In rats, propofol treatment, after training in an inhibitory avoidance task, increased latencies 48 h later, which is consistent with stronger memory consolidation [236]. The effect of propofol was blocked by rimonabant, consistent with a CB1R-mediated enhancement of memory. However, humans tend to exhibit amnesia with propofol treatment, and humans do not exhibit an increase in circulating AEA after propofol treatment [237–239].

Cognitive deficit is a symptom of schizophrenia, and the degree of cognitive deficiency is a primary factor in functional impairment [240]. Chronic heavy use of *Cannabis* is associated with some of the cognitive deficits in schizophrenia, which suggests the hypothesis that endocannabinoid signaling is dysregulated in schizophrenia [241]. Concentrations of AEA in the cerebrospinal fluid (CSF) of first-episode schizophrenics are significantly higher than in the control [242]. Contrary to the hypothesis that dysregulated endocannabinoid signaling contributes to disease symptoms, CSF concentrations of AEA negatively correlate with psychotic symptoms in unmedicated patients [243]. Similarly, individuals in the initial, prodromal state of psychosis with the lowest CSF AEA concentrations had the highest risk of converting to a full psychotic episode [244].

The findings suggest the hypothesis that the elevation of CSF AEA reflects the beneficial recruitment of CNS AEA signaling. No differences in circulating AEA were detected between schizophrenics and controls, in which robust differences in CSF concentrations were found [245]. However, other studies have found increased circulating AEA concentrations in people with schizophrenia compared to controls [85, 246]. Serum AEA concentrations were increased in response to treatment with cannabidiol, which also alleviated the symptoms of schizophrenia [247]. Circulating AEA and 2-AG concentrations were significantly higher in patients diagnosed with borderline personality disorder, a psychiatric disorder with some of the positive symptoms of schizophrenia, compared to controls [224].

The Endocannabinoids and Pain

Preclinical and human studies support the hypothesis that CB1R and CB2R activation reduce pain perception [248]. It has been indicated that PEA is an anti-inflammatory and analgesic [249]. There are mechanisms by which 2-AG and AEA could increase pain sensation; 2-AG serves as a precursor for arachidonic acid [250] and is converted by COX-2 to oxygenated products that increase pain perception [251]. AEA activates pronociceptive TRPV1 channels [252]. Thus, endocannabinoids can potentially increase or decrease pain perception, depending upon the mechanism of action.

A relatively common single nucleotide polymorphism in the gene for FAAH that results in elevated circulating concentrations of AEA, rs324420260, is associated with lower sensitivity to cold pain, indicating that higher circulating AEA concentrations seen in chronic pain could reduce pain perception [255]. Increased circulating AEA concentrations occur in patients with chronic pain syndromes, such as fibromyalgia [253] and complex regional pain syndrome [254], compared to controls without pain. Increased circulating 2-AG concentrations have also been reported in chronic pain, including neuromyelitis optica (NMO) [256] and osteoarthritis [257]. In NMO, 2-AG is negatively correlated with mechanical pain thresholds, indicating an analgesic role [256], and in osteoarthritis, 2-AG is positively correlated with knee pain, indicating that it contributes to pain perception [257].

In dietary investigations into chronic headache sufferers, increased omega-3 and reduced omega-6 fatty acid consumption were used to alter the circulating concentrations of AEA and 2-AG, which were reduced by the intervention [258]. Circulating concentrations of AEA and 2-AG were positively correlated with the number of daily episodes of severe headaches, indicating their roles in the etiology of headaches. The dietary intervention produced significant increases in the ethanolamide and 2-acylglycerol of docosahexaenoic acid (DHA), which were inversely correlated with the number of episodes of severe daily headaches.

Exercise reduces pain perception, termed exercise-induced hypoalgesia (EIH), which occurs through opioid and nonopioid mechanisms; endocannabinoids could also be responsible for nonopioid EIH. In support of the hypothesis, it was demonstrated that brief periods of isometric exercise produced nonopioid-dependent EIH and increased circulating 2-AG concentrations [157, 259, 260]. Correlational analyses did not support a significant relationship between circulating 2-AG or AEA concentrations and any measure of pain responsivity. However, CB1R is densely expressed on peripheral nerve terminals of A-delta and C primary afferents innervating skeletal muscle [261]. The endocannabinoids were increased by the activity of muscles and acted locally to produce EIH. This could explain the lack of correlation between endocannabinoids in the circulation and EIH.

Terpenes

Terpenes are the largest group of phytochemicals, with approximately 100 molecules identified in *Cannabis* [3, 37]. Terpenes are responsible for the odor and flavor of the different *Cannabis* strains and have likely contributed to the selection of *Cannabis* narcotic strains under human domestication [38]. Two different biosynthetic pathways contribute to the synthesis of the terpenes. The cytosolic mevalonic acid pathway (MVA) is involved in the biosynthesis of sesqui- and tri-terpenes, and the plastid-localized pathway (MEP) contributes to the synthesis of mono-, di-, and tetraterpenes [39]. MVA and MEP are produced through various and distinct steps from two molecules of acetyl-coenzyme A, pyruvate, and d-glyceraldehyde-3-phosphate. They are further converted to isopentenyl diphosphate (IPP) and isomerized to dimethylallyl diphosphate (DMAPP).

In the cytosol, two molecules of IPP and one molecule of DMAPP are condensed to produce farnesyl diphosphate by farnesyl diphosphate synthase. FPP serves as a precursor for sesquiterpenes which are formed by terpene synthases and can be decorated by other enzymes. Two FPP molecules are condensed by squalene synthase at the endoplasmic reticulum to produce squalene, the precursor for triterpenes and sterols, which are generated by oxidosqualene cyclases and are modified by various tailoring enzymes. In the plastid, one molecule of IPP and one molecule of DMAPP are condensed to form GPP by GPP synthase. GPP is the immediate precursor for monoterpenes [40].

Terpenes are classified into diverse families according to the number of repeating units of 5-carbon building blocks (isoprene units), such as monoterpenes with ten carbons, sesquiterpenes with fifteen carbons, and triterpenes derived from a 30-carbon skeleton. Terpene yield and distribution in the plant vary according to numerous parameters, such as processes for obtaining essential oil, environmental conditions, or plant maturity [19, 20].

Mono- and sesquiterpenes have been detected in flowers, roots, and leaves of *Cannabis*, with the secretory glandular hairs as the main production site. Monoterpenes dominate the volatile terpene profile, from 3.1 to 28.3 mg/g of dry flower weight, and include d-limonene, β -myrcene, α - and β -pinene, terpinolene, and linalool. The sesquiterpenes, β -caryophyllene, and α -humulene are present in *Cannabis* extracts at concentrations from 0.5 to 10.1 mg/g of dry flower weight [21]. Triterpenes have also been detected in hemp roots as friedelin and epifriedelanol [22], in hemp fibers as β -amyrin [23], and hempseed oil as cycloartenol, β -amyrin, and dammaradienol [24].

Terpenes and cannabinoids have been used as the chemotaxonomic markers of *Cannabis*; they are considered the main physiologically active secondary metabolites. When grown in standardized conditions, a positive correlation was found between the level of terpenes and cannabinoids; an explanation for the mono- and sesquiterpenes synthesized in the same glandular trichomes in which the cannabinoids are produced [25, 26].

Terpenes are lipophilic compounds that easily cross membranes, including the blood–brain barrier [27]. Terpenes present a wide array of pharmacological properties. The biological activities of D-limonene, also found in *citrus* essential oils, have been described in the literature. It notably exhibits potent anticancer, anxiolytic, and immunostimulating properties in humans [28]. β -myrcene, a terpene found in the hop, is recognized as a potent anti-inflammatory, analgesic, and anxiolytic component [34]. α -Pinene is an acetylcholinesterase inhibitor and may aid memory abilities that could counteract the memory deficits induced by THC [41, 42]. Linalool, found in *Lavandula angustifolia*, possesses similar properties to what is described for its monoterpene counterparts, i.e., analgesic, antianxiety, anti-inflammatory, and anti-convulsant [17].

β -caryophyllene, a well-known active principle of black pepper and *Copaiba balsam*, possesses potent anti-inflammatory and gastric cytoprotector activities [43]. It selectively binds to the CB2 receptor and is technically considered a phytocannabinoid [44]. Pentacyclic triterpenes such as β -amyrin and cycloartenol possess numerous biological activities, including antibacterial, antifungal, anti-inflammatory, and anticancer properties [45, 46]. The triterpenes are key contributors to the pharmacological properties of numerous medicinal herbs [47, 48].

Phenols

Phenolic compounds are produced through the phenylpropanoid pathway and constitute one of the plant kingdom's most widely distributed groups of secondary metabolites. They present more than 10,000 different structures, including phenolic acids, such as benzoic and hydroxycinnamic acids, flavonoids, such as flavonols and flavones, stilbenes, and lignans [49]. In *Cannabis*, about twenty flavonoids have been identified, mainly belonging to the flavone and flavonol subclasses [5]. They include the *O*-glycoside versions of the aglycones apigenin, luteolin, kaempferol, and quercetin, as well as cannflavin A and cannflavin B, which are methylated isoprenoid flavones that are unique to *Cannabis* [8]. Phenolic amides and lignanamides have also been described in *Cannabis* fruits and roots. The lignanamides belong to the lignan class of compounds and include cannabisin-like compounds of the types A, B, C, D, E, F, G, and grossamide [5].

Similar compounds, such as cannabisin D, have been described in *Cannabis* leaves, where it was strongly induced upon the UV-C treatment [50]. Lignans were found in the hydrophilic extract of *Cannabis* seeds. The *Cannabis* seed lignan profile was shown to be dominated by syringaresinol and medioresinol, followed by secoisolariciresinol, lariciresinol, and pinoresinol. *Cannabis* seeds contain about twenty times less total lignans, 32 mg of total lignans per 100 g of dry weight, than flax seeds, well-known lignans.

The lignan content of hulled *Cannabis* seeds represents only 1% of the content in the whole seed [51]. Nineteen stilbenes have been isolated in *Cannabis* with characteristic structural backbones such as spirans, phenanthrenes, and bibenzyls [5]. They include molecules such as cannabistilbene I, IIa, IIb, and dihydroresveratrol. Bibenzyl stilbenes, including the putative 3-*O*-methylbatatasin, were strongly induced in the leaves of *Cannabis* by UV radiations [50].

Phenolic compounds may act as antioxidants and protect plants against oxidative stress under certain physiological conditions. In humans, it has been shown that there is a correlation between dietary phenolic compounds and a reduced incidence of chronic diseases such as cancers and cardiovascular and neurodegenerative diseases [52]. The positive health effects may be partially explained by the phenolic antioxidant properties, as they are poorly bioavailable. Phenolic compounds induce the upregulation of endogenous antioxidant enzymes in vivo due to their ability to act as pro-oxidants and generate reactive oxygen species (ROS) [53]. They also exert their action through nonspecific protein binding interactions [54].

The flavones and flavonols in *Cannabis* exert a wide range of biological effects, including properties shared by terpenes and cannabinoids. Apigenin has been shown to possess anxiolytic [55] and estrogenic properties [56]. The specific cannflavin A and B are potent anti-inflammatory compounds through the inhibition of prostaglandin E2 and 5-lipoxygenase [57]. Health-related studies concerning lignanamides are few but indicated anti-inflammatory effects [58], and cytotoxic activities, in vitro [59].

Lignans indicate many health-promoting properties, including antioxidant, antiviral, antidiabetic, antitumorigenic, and antiobesity. Secoisolariciresinol, lariciresinol, and pinoresinol are converted into enterolignans by the anaerobic intestinal microflora, mammalian estrogen precursors, and phytoestrogens [60]. Due to the structural similarity of enterolignans with mammalian estrogens; the compounds are interesting in combating hormone-dependent cancers. The mechanisms of action of the lignans are complex, with multiple targets involved [61].

Trichomes

Trichomes are epidermal protuberances covering plants' leaves, bracts, and stems. The glandular trichomes can secrete and store secondary metabolites as a defense mechanism. Focus has been on the characterization of the specialized structures since their integrated study can favor the harvest of their biochemical potential. A database, TrichOME, was created to allow researchers to mine data relative to metabolites, genes, and expression profiles. Several procedures for isolating trichomes from the leaves of different plant species have also been explored.

Hemp has different trichomes, which belong to two categories, glandular and nonglandular capitate sessile, capitate-stalked, and bulbous hemp trichomes. *Cannabis* THCA is accumulated in the heads (glands) of both capitate-stalked and capitate sessile trichomes, but in the former, the content is higher. In the textile variety, the cannabinoids CBDA and CBCA occur at high concentrations instead of THCA, while the reverse is true for drug strains [62].

THCA is synthesized in the storage cavity, and the enzyme responsible for THCA production, THCAS, follows a sorting pathway from the secretory cells to the storage cavity. The accumulation in the storage cavity is due to the cytotoxicity of cannabinoids: they induce death via apoptosis when supplied for 24 h to both hemp and tobacco cell suspension cultures. Heterologous expression of THCAS fused to GFP in tobacco leads to the fluorescence of the trichome heads, confirming the localization of the enzyme in the storage cavity [35].

Depending on their color, hemp glandular trichomes show different secretory phases. The mature secreting gland appears translucent when the cannabinoid content is the highest while aging glands are yellow and senescing brown [62].

Cannabinoids are produced via terpenes secreted by plastids in the disk cells and phenols stored in their vacuole. Subsequently, vesicles are released into the cavity together with a fibrillar matrix originating from the cell walls of the disk cells. The fibrillar matrix is transported to the subcuticular cell wall and contributes to its thickening [62]. Analyses using the electron microscope have shown that oily secretions, most likely terpenes, are round in shape and secreted from the plastids, which have the appearance of reticulate bodies.

Besides cannabinoids, *Cannabis* trichomes produce other secondary metabolites, such as terpenes, responsible for the typical plant aroma. Among the *Cannabis* terpenes of low abundance is nerolidol, 0.09% of the total terpene content, which

has antimalarial and antileishmanial effects [17]. The pharmacological interests of the compounds are to devise genetic engineering strategies to boost the secondary metabolism or increase the density of trichomes in *Cannabis*. Such approaches are as in *Artemisia annua*, where the transformation of *A. annua* with the *rolB* and *rolC* genes of *Agrobacterium rhizogenes* led to plants with increased artemisinin content [63]. The *rol* genes are known for their stimulatory action on plant secondary metabolism.

The study on *A. annua* indicated that *rolB* and *rolC* trigger different effects, with *rolB* showing enhanced production for *rolC*. Studies on *A. annua* have shown that the expression of a β -glucosidase from *Trichoderma reesei* increases glandular trichome density and artemisinin production [43]. The hydrolytic enzyme favors the release of active plant growth regulators from the conjugates stored in the plastids, favoring trichome formation, biomass production, and leaf area. Studies on *Cannabis* trichomes will be helpful in the identification of important genes, among which are transcription factors that are involved in trichome formation. It could be interesting to devise an engineering strategy to increase the density of trichomes in *Cannabis* by adopting a similar strategy.

Adverse Effects of Cannabinoids

The recreational and medicinal use of *Cannabis*, delta-9-THC, and other synthetic cannabinoids has been associated with numerous side effects. When adjusted for confounders such as cigarette smoking, the impact of short- and long-term use of *Cannabis* appeared to be similar for the different types of products consumed and is directly linked to the level of delta-9-THC or its synthetic analog. The delta-9-THC content of recreational *Cannabis* has drastically increased, from 3% in the 1980s to almost 20% in 2015, with low levels of other cannabinoids such as CBD. The effects of short-term use include memory and cognitive deficits, impaired motor coordination, and psychosis. Long-term use of delta-9-THC has been associated with an increased risk of addiction, cognitive impairment, altered brain development when initial use was done early in adolescence, and an increased risk of chronic psychosis disorder, including schizophrenia [36].

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Chapter 5

Neurological Effects of *Cannabis*



Cannabis products and *Cannabis* use are inherently variable. Given the difficulty in standardizing *Cannabis* products and *Cannabis* use patterns, studies are often impacted by differences in the participants' exposure to *Cannabis* or even specific cannabinoids, especially in medical use. Although it is clear that chronic recreational use impacts brain function, albeit subtly, future researches exploring moderating factors, including the age of onset, recovery of function after abstinence, frequency and magnitude of *Cannabis* use, high- versus low-potency products, mode of use, and the unique effects of specific cannabinoids, are all needed to understand the impact of *Cannabis* fully. As legalization efforts expand, overall use rates continue to rise, and questions regarding *Cannabis* and public policy measures remain crucial.

Research has been focused on the impact of *Cannabis* for medical purposes, specifically concerning potential cognitive alterations. Studies to directly assess cognition in medical *Cannabis* patients, before and after initiating treatment, reported improvements in cognitive function, specifically on measures of executive function, following 3 months of medical *Cannabis* treatment [110]. The cognitive changes may have been due to a variety of reasons. First, medical *Cannabis* patients tend to be adults beyond the critical stages of neurodevelopment, which may afford some protection from the neurocognitive decrements typically associated with *Cannabis* use. Medical *Cannabis* patients and recreational *Cannabis* users also tend to differ in terms of the products they use and their constituent profiles.

While it is likely that each cannabinoid has a unique effect, it is also important to note the “entourage effect,” which refers to the synergistic action that occurs in the presence of multiple cannabinoids and terpenes, the essential oils contributing flavor and fragrance components to *Cannabis* that share a common precursor [118]. The combination of the constituents is thought to create a unique synergism, which supports anecdotal reports that whole-plant-derived products may be more effective therapeutic agents than isolated or synthetic cannabinoids.

While recreational *Cannabis* products are generally sought for high delta-9-THC levels with the ultimate goal of getting high, medical *Cannabis* patients seek symptom alleviation and often choose products with rich and varied cannabinoid profiles, including constituents other than delta-9-THC. Although delta-9-THC and CBD are the most abundant cannabinoids, many other cannabinoids, including cannabigerol, cannabinol, cannabichromene, and tetrahydrocannabivarin, are present in *Cannabis* and are often present in higher amounts in natural *Cannabis* products. Each of the constituents is posited to have unique properties that may be beneficial. For example, cannabichromene is thought to have anti-inflammatory effects and has been shown to increase the viability of adult neural stem progenitor cells (NSPCs), which are essential for brain plasticity and are suggestive of neurogenesis [116].

Cannabigerol has been shown to inhibit GABA uptake, has anti-inflammatory properties, and is hypothesized to be neurogenic also [117]. One study utilizing fMRI techniques examines the impact of medical *Cannabis* treatment on cognition and brain function using a pre/post model [119]. The results revealed that after 3 months of medical *Cannabis* treatment, the patients exhibited better task performance relative to baseline and apparent normalization of brain activation during the completion of the MSIT, a robust measure of cognitive control.

It is important to note that most investigations have examined the impact of heavy, chronic *Cannabis* use, and although some have begun to examine the impact of light or more casual *Cannabis* use, what is known about the effects of *Cannabis* on the brain is typically reflective of more chronic users. Among studies of heavy users, there is no consensus in terms of the definition of “chronic,” “regular,” or “heavy” use. Criteria are often based on current days of use per week (regardless of the amount of *Cannabis* consumed) or estimated lifetime smoking or use episodes. Unlike alcohol, there is no standardized measure of *Cannabis*.

The magnitude or amount of *Cannabis* consumed is often measured in joints, smokes, or puffs taken. Some researchers attempt to quantify the actual amount in grams and milligrams of *Cannabis* used. While the metrics related to *Cannabis* use are informative, it is imperative to consider other factors that influence overall exposure, including routes of administration and *Cannabis* potency (% delta-9-THC) of products used. For example, in a study of individuals who used vaporized *Cannabis*, one of the main advantages cited was that the user could achieve more effect from the same amount of *Cannabis* relative to smoking [112]. Assessing exposure to *Cannabis* is further complicated by rising levels of *Cannabis* potency, which have increased exponentially over the last decade.

Studies using brain imaging techniques and neuropsychological tests show that, acutely, all drugs create disharmony in the neuropsychological network causing decreased activity in areas responsible for short-term memory and attention. *Cannabis* induces loss of internal control and cognitive impairment, especially attention and memory, to intoxication. Heavy *Cannabis* use is associated with reduced function of the attentional/executive system, as exhibited by decreased mental flexibility, increased perseveration, and reduced learning, to shift and sustain attention [36].

Recent investigations on amphetamine/methamphetamine have documented deficits in learning, delayed recall, processing speed, and working memory. MDMA users exhibit difficulties in coding information into long-term memory, display impaired verbal learning, are more easily distracted, and are less efficient at focusing on complex tasks. The degree of executive impairment increases with the use severity, and the impairments are long-lasting over time. Chronic cocaine users display impaired attention, learning, memory, reaction time, and cognitive flexibility.

Factors Affecting the Impact of *Cannabis* Use on the Brain

Duration of Abstinence

Withdrawal from *Cannabis* has been characterized by mood-related symptoms, including anxiety, aggression, anger, irritability, and restlessness, as well as physical symptoms such as sweating, decreased appetite, stomach pain, shakiness, and sleep problems (e.g., strange dreams, difficulty sleeping); symptoms can vary in their onset (one to 6 days after cessation) and have been shown to peak in severity at different times for the first 2 weeks of abstinence. Studies examining the chronic effects of *Cannabis* have employed a wide range of abstinence thresholds, generally ranging from about 12 h to 1 month. The variability in required abstinence across studies is likely to impact study findings, as heavy *Cannabis* users may experience a range of withdrawal symptoms, each of which can occur during specific abstinence periods.

Variability in abstinence periods can impact findings within a single study, as subjects may choose to abstain for more than the minimum required time, complicating cross-study comparisons given the wide range of abstinence periods employed [105]. While some studies utilize specific periods of abstinence to avoid withdrawal symptoms (e.g., less than 24 h, more than 1 week), other studies may report and control for recency of use and *Cannabis* withdrawal metrics. However, the approach to controlling for abstinence periods is not standard, and effects related to abstinence or withdrawal are often acknowledged as potential confounds or limitations in *Cannabis*-related research studies.

Few studies have investigated the effects of extended periods of abstinence to determine whether long-term abstinence results in recovery of function or normalization. Some preliminary evidence appears promising, as improvements have been observed longitudinally throughout abstinence; for example, short-term memory improvements throughout 3 weeks of *Cannabis* abstinence [106]. *Cannabis* users who abstained for a minimum of 3 months demonstrated cognitive performance similar to healthy controls [107]. The data suggest that altered cognitive performance observed in young *Cannabis* users may begin to normalize after several weeks of abstinence; studies utilizing longer periods of abstinence combined with neuroimaging techniques are needed to more thoroughly examine the extent of recovery of function in *Cannabis* users.

Age of Onset of Cannabis Use

Much research has focused on adolescent users, given public health concerns regarding expanded access to *Cannabis* products and the increased vulnerability of the developing brain. With the expanded legalization of medical and recreational *Cannabis*, older adults are the fastest-growing population of *Cannabis* consumers. Despite the increasing prevalence of *Cannabis* use among older adults for medical purposes, the consequences of *Cannabis* use are relatively unknown in that population, especially compared to literature focused on the impact of *Cannabis* use among adolescent and emerging adult users.

Data indicate that recreational *Cannabis* use during adolescence is related to cognitive decrements. Recent data indicated improved cognitive function in older adults following 3 months of medical *Cannabis* treatment, likely related to participants' age and several factors that differ between those used for medical versus recreational use [110]. A preclinical study reported a reversal of the age-related cognitive decline in mature and old mice treated with low doses of delta-9-THC; the improvements may result from upregulation of the aging endocannabinoid system via increased signaling secondary to low-dose delta-9-THC exposure [111]. The same *Cannabis* exposure resulted in cognitive decrements in young mice. Despite the findings, virtually no studies have systematically assessed the specific impact of *Cannabis* use among older adults.

Frequency and Magnitude of Cannabis Use

Cannabis concentrates, also known as dabs, butane hash oil (BHO), shatter, wax, or budder, contain significantly higher levels of delta-9-THC that often reach or exceed 80% [113]. Although concentrates are growing in popularity; little formal research has been conducted regarding the impact of the products on the brain. Using highly concentrated products is potentially concerning, given that delta-9-THC has been associated with adverse physiological and psychological effects [114]. A study assessed the impact of *Cannabis* potency (determined via self-report) on brain structure. It noted alterations in corpus callosum white matter microstructure in high-potency compared to low-potency users and controls. The findings indicate that high-potency *Cannabis* products/concentrates may negatively affect the brain [115].

Errors in Monitoring Awareness, Reward Processing, and Affective Processing

Monitoring awareness and the decreased ability to learn from errors (utilize feedback) have specifically been examined in *Cannabis* users, as decrements in the domain are thought to be related to a combination of loss of insight and impaired

cognitive control [95]. Using various tasks, including paired associate learning and go/no-go response inhibition tasks; researchers have examined *Cannabis* users' ability to learn from errors.

The studies report that *Cannabis* users exhibit reduced learning from errors and that the failure to utilize feedback is related to hypoactivity in cognitive control and error awareness regions, including the ACC [96]. Some have also utilized monetary incentive delay tasks to examine reward processing in *Cannabis* users. The tasks require individuals to respond quickly to a target stimulus to win or avoid losing monetary rewards [97]. Although frequent *Cannabis* users perform similarly to controls across studies, functional differences have been observed.

The effects of nicotine and *Cannabis* use are difficult to disentangle in studies. Studies suggest that adolescent and adult *Cannabis* users demonstrate striatal hyperactivation, which may indicate an overly sensitive motivation circuitry; however, attenuated activity has been reported in adult *Cannabis* users within the caudate nucleus and nucleus accumbens, brain regions commonly associated with reward anticipation [98]. The inconsistent findings may be related to several factors, including the age of the participants (adolescents versus adults) or length of abstinence. Additionally, nicotine use may have impacted activity in the nucleus accumbens, while *Cannabis* use likely moderated activity in the caudate.

Studies have assessed reward processing during the Iowa gambling task (IGT), an economic decision-making task requiring participants to choose between small immediate gains with small long-term losses versus large immediate gains with larger long-term losses [99]. Despite similar performance between heavy *Cannabis* users and controls, the *Cannabis* group demonstrated higher activation than controls during wins in the left superior temporal gyrus and right orbitofrontal cortex and insula, regions typically associated with decision-making.

The magnitude of *Cannabis* use (grams used) was positively correlated with neural activity related to winning versus loss evaluation in the right insula and the caudate and ventrolateral prefrontal cortex. Task performance and activation during discrete stages of the task were examined, and performance decrements were detected in *Cannabis* users relative to controls at the end of the IGT, despite no difference in task performance during the initial strategy development phase [100]. Interestingly, although no functional differences were noted in response to losses, during the initial phase of the task (before performance differences emerged), *Cannabis* users exhibited attenuated activity in response to losses in several regions, including the ACC, medial frontal cortex, precuneus, superior parietal lobe, occipital lobe, and cerebellum. Activation in response to losses was only associated with task performance over time within the control group. Therefore, the study indicated that *Cannabis* users are less sensitive to negative feedback while developing their initial strategy.

Risky decision-making and reward response were measured utilizing a decision-reward uncertainty task, which contains three conditions: no risk (correct response rewarded 100% of the time), reward risk (correct response rewarded 50% of the time), and behavioral risk (correct response unknown and 50% chance of reward). No performance differences were noted between abstinent adolescents with

Cannabis use disorder (CUD) and controls; however, the CUD group demonstrated hyperactivation in brain regions related to decision-making when making risky decisions and attenuated OFC response to reward [101].

Altered responses to pleasant and negative stimuli have also been observed among *Cannabis* users. Differential activation patterns were detected in adolescent *Cannabis* users and nonusers during an emotion-arousal word task in which the participants were presented with positive, negative, and neutral words [102]. In response to negative words, *Cannabis* users demonstrated attenuated activity in regions linked to emotion processing and integration (insula, PFC, occipital cortex). Viewing positive words also resulted in hypoactivation, but in different regions, namely the right inferior parietal lobe, associated with attentional control. *Cannabis* users also demonstrated lower amygdala activation in response to negative and positive words.

The altered emotional circuitry in heavy *Cannabis*-using adolescents might affect later emotional outcomes. An association was also detected between greater frequency of *Cannabis* use and higher negative emotionality. Some studies have specifically examined affective processing and noting altered functional activation in *Cannabis* users relative to nonusers. In one fMRI study, using masked affective faces presented below the level of conscious awareness, *Cannabis* users exhibited attenuated activity within the ACC and amygdala compared to controls who demonstrated increased activity in those regions while viewing masked faces [103]. During emotional regulation, *Cannabis* users exhibited hyperactivity in a bilateral frontal network, which included the precentral gyrus, superior frontal gyrus, and midcingulate cortex relative to healthy controls.

Emotional regulation was examined in *Cannabis* users when presented with neutral or negative pictures and had to either passively view the photos or try to regulate their emotions. The findings indicated that chronic *Cannabis* users demonstrate altered reward circuitry and effectively process information differently than those who do not use *Cannabis*. *Cannabis* users appear to display a poorer ability to learn from errors and may be less sensitive to negative feedback, which is indicated by altered activation in reward circuitry. Additionally, evidence suggests that *Cannabis* users demonstrate unique activation patterns in response to affective stimuli and emotion regulation [104]. Given that learning from mistakes, accurate and efficient appraisal of affective stimuli, and regulating emotions are critical for successful social interactions, the underlying neural changes could lead to negative consequences for recreational *Cannabis* users.

Functional MRI Studies on *Cannabis*

Through advanced neuroimaging techniques, fMRI studies have elucidated the underlying neural mechanisms associated with the cognitive consequences commonly observed in *Cannabis* users. The studies suggest that chronic, heavy *Cannabis* use is associated with cognitive decrements across various domains. Functional

MRI studies have also revealed that functional alterations are often present even in the absence of significant performance deficits, suggesting that *Cannabis* users may compensate for poorer performance through less efficient neural processing, including recruitment of additional brain regions or activation of regions not typically associated with a cognitive domain or task. Although the findings may appear somewhat inconsistent across studies, they illustrate the range of tasks used to probe underlying neural processes within and across cognitive domains.

Functional MRI studies are often limited by sample size, with most investigations enrolling approximately 12–20 *Cannabis* users and a similarly sized group of healthy controls. While the samples appear large enough to detect between-group differences in brain activation patterns, they may only sometimes be powerful enough to detect subtle differences in cognitive performance that could be obscured by interindividual variability. This explains why many studies report changes in functional activation patterns without detecting performance differences between groups. Additionally, fMRI investigations vary in analytic approaches across studies and employ a range of statistical thresholds. Significance values typically range from $p < 0.05$ to $p < 0.001$ and may or may not be corrected for multiple comparisons. The minimum cluster extent is often derived using various methods and can vary widely across studies. Some investigations utilize whole-brain analyses, while others use a region of interest (ROI) approach based on either a priori hypotheses or differences detected after examining whole-brain results, resulting in a combination whole-brain/ROI analytic model.

It is important to note that studies vary widely about inclusion and exclusion criteria for other substance use. While investigators often include information regarding substance use-related inclusion/exclusion criteria, the methodological factors pose limitations when attempting to summarize the overall effects of *Cannabis* on brain function across studies. Despite the facts, most studies suggest that chronic, heavy *Cannabis* users demonstrate altered brain activation patterns relative to those who do not use *Cannabis*.

Delta-9-tetrahydrocannabinol exerts all of its known central effects through the CB1 cannabinoid receptor. Research on cannabinoid mechanisms has been facilitated by the availability of selective antagonists acting at CB1 receptors and the generation of CB1 receptor knockout mice. Important classes of neurons that express high levels of CB1 receptors are GABAergic interneurons in the hippocampus, amygdala, and cerebral cortex, which also contain the neuropeptides cholecystokinin.

Activation of CB1 receptors inhibits the release of amino acid and monoamine neurotransmitters. The lipid derivatives anandamide and 2-arachidonylglycerol (endocannabinoids (eCBs)) act as endogenous ligands for CB1 receptors. They may act as retrograde synaptic mediators of the phenomenon of depolarization-induced suppression of inhibition or excitation in the hippocampus and cerebellum.

Studies have linked chronic use of *Cannabis* with an increased risk of psychiatric illness. Central effects of cannabinoids include disruption of psychomotor behavior, short-term memory impairment, intoxication, stimulation of appetite, antinociceptive actions (particularly against the pain of neuropathic origin), and antiemetic

effects. Although there are signs of mild cognitive impairment in chronic *Cannabis* users, there is little evidence that such impairments are irreversible or accompanied by drug-induced neuropathology. A proportion of regular users of *Cannabis* develop tolerance and dependence on the drug.

Endocannabinoid-Mediated LTP in Learning

Several links between eCB-LTD and various forms of memories have been woven, such as in habit learning or during critical periods of sensory processing [186]. There is evidence of the involvement of homosynaptic eCB-LTP and eCB-mediated heterosynaptic facilitation of LTP in learning based on studies using electrophysiological recordings and pharmacological or genetic tools modifying eCB-LTP.

At the LPP-dentate gyrus synapses, conveying cue identity to the hippocampus, eCB-LTP is implicated in the memory of both simultaneous and serial two-odor discriminations, acquired after several trials in rats. Systemic injections of CB1R or MAGL antagonists, preventing or enhancing eCB-LTP, had opposite effects on learning the simultaneous two-odor discrimination task. Importantly, MAGL inhibition has led to long-term memory 24 h after six training trials; a protocol that failed to induce efficient controlled learning. Learning performance was correlated with greater expression of pROCK in LPP of trained rats and reduced expression with CB1R inhibitor.

The serial odor discrimination task, testing the encoding of cues embedded in a sequence, reflects episodic memory's constant flow of experience. *Frm1*-KO mice, characterized by impaired 2-AG signaling, reduced NMDAR-mediated transmission, and substantial impairment of eCB-LTP at LPP-dentate gyrus synapses, exhibited learning deficits in the task. Also, systemic injection of MAGL inhibitor or chemogenetic activation of Gq in the entorhinal cortex was sufficient to rescue in vitro homosynaptic eCB-LTP and learning.

Studies highlight the importance of eCB-mediated i-LTD and the regulation by eCBs in the excitation/inhibition balance of memory formation and maintenance, unraveling the role of eCBs in disinhibitory mechanisms during learning [187]. Mice, in which only subsaturating forms of LTP requiring i-LTD expression in vitro were impaired in CA1 pyramidal cells (by a targeted mGluR5 knockout), showed no deficits in spatial memory. However, they performed poorly in trace-conditioning tasks when a 30 s interval separated the two cues.

Although partial occlusion of i-LTD in wild-type mice could be observed ex vivo, LTP could still be induced in CA1 after learning, probably because only a few active synapses had been saturated during the task. The acquisition and retention of the temporally-based associative learning were enhanced by systematic MAGL inhibition, shown to promote i-LTD-mediated LTP. The effect supports a study in which MAGL knockout mice showed improved learning in the water maze and object recognition tasks [188].

A subpopulation of hippocampal interneurons appears critical in controlling the inhibition and CB1R-dependent LTP expression in pyramidal neurons during incidental learning; GABA-CB1R-KO mice with impaired learning and *in vivo* LTP can both be fully rescued by reducing GABAergic transmission. Furthermore, *ex vivo* i-LTD amplitude enhancement in trained mice suggests its involvement in learning. CB1R activation is also required for encoding emotionally salient stimuli at the BLA-medial prefrontal cortex pathway. Notably, BLA pharmacological CB1R activation or anandamide reuptake inhibitor potentiates the formation of associative memories with normally subthreshold footshock, putatively through CB1R-dependent heterosynaptic facilitation of BLA output.

The result leads to enhanced cortical activity and bursting in response to olfactory cues previously paired with footshock [189]. Systemic treatment with CB1R antagonist prevents LTP expression and learning *in vivo*. Astroglial CB1R-knock-out mice do not express NMDAR-LTP at CA3–CA1 synapses *in vivo* and showed impaired performance in the novel object recognition task, which could be rescued by elevating D-serine levels, gating NMDAR activation [190]. The learning of several tasks might initially be associated with an eCB-mediated relief of GABAergic transmission, hence reducing the threshold of LTP induction, and could be followed by an elevation of the inhibitory tone that could participate in long-term memory stabilization.

The Homosynaptic and Heterosynaptic eCB-Mediated Potentiation

The endocannabinoid system is a lipid-based neurotransmitter complex that plays crucial roles in the neural control of learning and memory. The eCB-mediated retrograde signaling model releases eCBs from postsynaptic elements and travels retrogradely to presynaptic axon terminals, where they activate cannabinoid type-1 receptors and ultimately decrease neurotransmitter release on a short- or long-term scale. Evidence has enlarged this view to show that eCBs, besides depressing synaptic transmission, can also increase neurotransmitter release at multiple brain synapses, indicating that eCBs act as bidirectional regulators of synaptic transmission and plasticity.

Studies revealed links between the expression of eCB-mediated long-term potentiation (eCB-LTP) and learning, and its dysregulation and several pathologies. The various forms of eCB-LTP based on their mechanisms, resulting from homosynaptically or heterosynaptically mediated processes and the neuromodulation of eCB-LTP, the behavioral impact on learning and memory, and finally, eCB-LTP disruptions in various pathologies and its potential as a therapeutic target in disorders such as stress coping, addiction, Alzheimer's and Parkinson's diseases, and pain has been examined. In this context, it is critical to understand eCB-mediated signaling in its multifaceted complexity. The bidirectional nature of eCB-based

neuromodulation may offer an important key to interpreting the functions of the eCB system and how *Cannabis* and other drugs impact it.

The homosynaptic from heterosynaptic eCB-mediated potentiation has been distinguished. Using spike-timing-dependent plasticity (STDP), a Hebbian synaptic learning rule relying on paired activity on either side of the synapses, a few numbers of pairings induce eCB-LTP at corticostriatal synapses, which are CB1R- and TRPV1-mediated. 2-AG levels, and subsequent CB1R activation, have a dual effect on eCB-plasticity; high levels of eCBs synthesis and CB1R activation (reached with ~10–15 post-pre pairings) induce eCB-LTP, while low levels (reached with ~50–100 pre-post pairings) induce eCB-LTD. Few pairings promote efficient eCB synthesis (via maximal calcium influx and efflux from voltage-gated calcium channels and TRPV1, and endoplasmic reticulum, respectively); thus, maximal CB1R activation is combined with minimal CB1R desensitization.

Corticostriatal eCB-plasticity relies on presynaptic pKA/calcineurin balance, such that eCB-LTP requires active pKA, whereas eCB-LTD depends on calcineurin activation. Therefore, at corticostriatal synapses, eCB-mediated plasticity is bidirectional, and eCB-LTP or eCB-LTD expression is determined by pre- and postsynaptic activity patterns. A similar homosynaptic and bidirectional eCB-plasticity occurs between neocortical pyramidal cells following a limited number of coincident activities. Interestingly, eCB-LTP is robust to spike-time jittering, contrarily to NMDAR-LTP, and can thus arise in noisy neural network activity.

A homosynaptic CB1R-dependent eCB-LTP was also characterized in hippocampal granular cells of the dentate gyrus resulting from postsynaptic 2-AG synthesis upon high-frequency stimulation of the lateral perforant path. When activated, CB1R, detected presynaptically at LPP terminals using STORM microscopy, engages the presynaptic FAK/ROCK signaling pathway favoring glutamate release. Interestingly, at CA3-CA1 synapses, CB1R is preferentially linked to ERK/Munc18-1, whose activation depresses glutamate release. In both cases, the eCB-LTP magnitude did not reach saturating levels. It could be increased under monoacylglycerol lipase (MAGL) inhibition, the 2-AG degrading enzyme, suggesting that eCB-LTP might serve as priming plasticity accounting for fast learning and episodic memory. Finally, homosynaptic CB1R-mediated eCB-LTP was also observed in stratum oriens interneurons.

By reducing inhibition from GABAergic synapses through a CB1R-dependent short-term depolarization-induced suppression of inhibition (DSI), eCBs were first shown to facilitate NMDAR-LTP induction at hippocampal CA3-CA1 synapses, exclusively in the cell subjected to the subthreshold LTP inducing protocol. Endocannabinoid-mediated facilitation through long-term disinhibition was then observed at various synapses, cell types, and brain regions. In the hippocampus, high- or low-frequency stimulations or theta-burst stimulations of Schaffer collaterals induce LTD of local GABAergic interneurons (i-LTD), which facilitates LTP at excitatory CA3-CA1 synapses. i-LTD, originating from metabotropic glutamatergic receptor (mGluR) activation and subsequent 2-AG release from CA1 pyramidal cells that leads to the activation of CB1R located on GABAergic terminals, causes relief of the GABAergic brake in a restricted dendritic area (~10 μm) when

synaptically induced or on a cell-wide extent following endogenous CA1 pyramidal cell activity. In contrast to the transient LTP facilitation induced by DSI in a single active cell and up to neighboring naïve cells, i-LTD provides long-lasting priming of at most a single cell.

The modulation of CA1-LTP by i-LTD is an example of metaplasticity, i.e., long-lasting neural changes induced by activity at a given time, which modulates subsequently induced plasticity orchestrated by eCBs [170]. The i-LTD is finely tuned by the parallel activation of CB1R on GABAergic or glutamatergic cells and is also accompanied by changes in excitability enhancing the spiking probability in response to a given EPSP, i.e., EPSP-to-spike potentiation both eCB-mediated [171, 172].

A circuit-based synaptic learning rule, consisting of paired stimulation of the perforant path and Schaffer collaterals, induced an input-timing-dependent heterosynaptic LTP at CA3-CA1 but not at cortical-CA1 synapses [173]. Input-timing-dependent LTP depends on CB1R-mediated i-LTD occurring at GABAergic synapses (here cholecystokinin interneurons) [174]. Activation of the cortical-CA1 pathway triggers heterosynaptic calcium transients, boosting eCB signaling originating from the CA3-CA1 pathway, ultimately leading to i-LTD. [175] Similar metaplasticities involving eCB-mediated i-LTD have been reported in the striatum ventral tegmental area, basolateral amygdala (BLA), and spinal cord [176].

Neuromodulators can regulate eCB-LTP expression or magnitude through various mechanisms targeting eCB synthesis and releasing the signaling downstream of CB1R. In the hippocampus, anandamide increases miniature excitatory and inhibitory postsynaptic currents [177]. Anandamide and 2-AG potentiate NMDAR-mediated currents via TRPV1-dependent and TRPV1-independent mechanisms [178]. Although anandamide/2-AG NMDAR-mediated metaplasticity favors hippocampal LTD, it remains to investigate whether the eCB-NMDAR cross-talk exists in other brain areas and, considering the crucial role of NMDAR in synaptic potentiation, could constitute a metaplasticity promoting LTP.

Brain-derived neurotrophic factor (BDNF) modulates eCB-LTD and eCB-LTP. Heterosynaptic eCB-LTP in the hippocampus, neocortex, ventral tegmental area, and striatum, activation of the postsynaptic tropomyosin receptor kinase-B (TrkB) by BDNF increased 2-AG mobilization and, consequently, CB1R activation which allows an eCB-mediated depression of IPSCs and i-LTD, tuning the magnitude of glutamatergic LTP [179]. In the neocortex, eCBs released by dendritic calcium spikes reduce inhibitory transmission, which facilitates postsynaptic calcium spike generation, the calcium-dependent release of BDNF, and, ultimately, the induction of eCB-LTP. For homosynaptic eCB-LTP, TrkB activation facilitates 2-AG synthesis and shapes the expression domain of corticostriatal eCB-LTP [180].

The biological actions of eCBs partly rely on their ability to regulate nitric oxide signaling. At cerebellar parallel fiber-Purkinje cell synapses, low- and high-frequency stimulations induce differential CB1R activation leading to a low and high amount of nitric oxide production, which orientates the plasticity, respectively, toward eCB-LTP and eCB-LTD. [182] Therefore, nitric oxide levels may act as a threshold in the modulation of synaptic strength.

Memory and Cognitive Impairments

Magnetic resonance imaging (MRI) has allowed researchers to examine the underlying neural substrates associated with *Cannabis* use, and many comprehensive reviews have been conducted on the impact of *Cannabis* use on the brain, each focusing on various outcomes or aspects of *Cannabis* use. For example, the behavioral (cognitive, motivational, and psychosis) effects of *Cannabis* use, the impact of *Cannabis* use specifically in adolescent users, and studies utilizing an abstinence period of two or more weeks to examine the long-term effects of *Cannabis* use on neurocognitive function. A review examined the acute and residual impact of *Cannabis* use on cognitive function across various domains through functional MRI (fMRI) investigations.

The effects of *Cannabis* on cognition can be subdivided into effects observed with acute use and those observed with chronic use or dependence. It is known that during the acute intoxication period, episodic memory and attention are negatively impacted while impulsivity increases [10]. A randomized, placebo-controlled, double-blind study demonstrated that acute *Cannabis* use impaired working memory and verbal memory [11]. Working memory, verbal, and visual memory have also been shown to be impacted more during acute intoxication in subjects with the Val COMT allele, which is thought to be a link between *Cannabis* and schizophrenia.

Cognitive impairments observed in *Cannabis* users are especially prevalent in executive function, memory, and attention [6]. The effects of *Cannabis* are often discussed in three main functional domains: cognition, emotion, and reward/motivation [7]. Cognitive impairments associated with *Cannabis* have been correlated with frequency, quantity, and duration of use. *Cannabis* negatively impacts the domains of cognition, emotion, and reward and also has the potential to blur the lines between functional processes. It has been revealed that the domain of emotion in young adults with *Cannabis* use disorder (CUD) has the potential to interfere with the cognitive domain and inhibit normal cognitive control. The hypothesis considers that craving and inhibitory control decreases less in adolescents following intoxication than adults [8].

Functional magnetic resonance imaging activation to emotional stimuli demonstrated that individuals with CUD have impaired segregation between cognitive and emotional processes. The finding underlies the observation that *Cannabis* users have poor cognitive control and decision-making in emotionally-demanding circumstances. The results are supported by the findings of electroencephalography (EEG) studies which demonstrated that *Cannabis* users have decreased delta and increased theta, beta, and gamma power in the resting state [9]. The EEG findings suggest that *Cannabis* users experience increased cortical activation during rest, which may reflect disinhibition of inhibitory processes that interfere with normal cognition and result in a less efficient, “noisy” brain.

One study showed that heavy, chronic abusers of solely *Cannabis* had memory impairment in the Rey–Osterrieth Visual Memory Test [15]. The same study also demonstrated that not only are chronic *Cannabis* abusers inclined to have memory

impairment and are likely to develop significant brain dysfunction that involves the visual-motor system as assessed by the Bender Visual-Motor Gestalt test.

When the cognitive domains of chronic *Cannabis* users were evaluated following short-term (mean 15 h) abstinence and compared to those of chronic tobacco users, *Cannabis* users were found to have poorer overall learning, delayed recall, greater interference, and increased forgetfulness [16]. They also exhibited slower reaction times during information processing and sustained attention tasks.

To further investigate the marked cognitive impairments associated with chronic *Cannabis* use, a meta-analysis of studies was conducted to assess the effects of partial CB1 receptor agonists on spatial and nonspatial memory [17]. Delta-9-THC (1.6–5 mg/kg), a partial CB1 receptor agonist, was shown to significantly impair nonspatial memory in humans, while high delta-9-THC doses (67 mg/kg) additionally impaired spatial memory. Delta-9-THC was also shown to impair visuospatial memory in monkeys and nonhuman primates. Chronic delta-9-THC administration did not significantly impair spatial or nonspatial memory in rodents, but evidence of the effect in humans was inconclusive.

A study on the effects of *Cannabis* use on general cognition and memory has led to an inquiry into how the effects are moderated by abstinence versus decreased use [6]. The treatments for substance use disorders have historically aimed for abstinence as the end goal. It has become increasingly evident that this approach does not capture patients who significantly reduce their substance use without achieving abstinence.

A study of individuals who were currently or formerly in treatment for CUD found that compared to heavy users, individuals who had decreased their use (to less than or equal to 3 days per week) had similarly improved global health, appetite, and depression outcomes to those who became abstinent. Still, only those individuals abstained from *Cannabis* use demonstrated improved cognition per the Reported Outcomes Measurement Information System (PROMIS). Despite the observation that abstinence can improve cognitive function in former *Cannabis* users, the hypothesis that the administration of an adenosine A2A (A2AR) receptor antagonist could revert the effects of synthetic cannabinoids on recognition memory was tested [18]. The A2AR receptor antagonist istradefylline was shown to revert memory deficits induced by chronic cannabinoid exposure, likely via mitigation of synaptic plasticity impairment that occurs in the CA1 area of the hippocampus following *Cannabis* exposure.

It has been shown that subtle cognitive deficits are evident even 7 days following heavy *Cannabis* use. It is thought that *Cannabis* use also has lasting effects on brain structure and function [19]. Structural neuroimaging studies have continued to show that *Cannabis* users exhibit abnormalities in hippocampal volume and gray matter density. There is also evidence of damage to white matter resulting from *Cannabis* use, as it is where CB1 receptors is highly concentrated [20]. It remains unclear if the observations of abnormal brain structure and activity are a consequence of or a risk factor for *Cannabis* use. There is limited evidence supporting the use of *Cannabis* over standard gold therapy for restoration of the central nervous system in conditions such as epilepsy, schizophrenia, or multiple sclerosis.

In a study of 75 adults with longitudinal assessments of *Cannabis* use, early age of onset of *Cannabis* use was associated with reduced posterior parietal cortex (PPC) activation on fMRI [21]. Posterior parietal cortex activation was shown to mediate reaction times during the spatial working memory task significantly. In chronic *Cannabis* users, greater cumulative *Cannabis* use was associated with increased dorsolateral prefrontal cortex (DLPFC) activation. Interestingly, the changes observed in PPC activation did not differ between individuals with single reported use and those with repeated use, suggesting that the effects represent substance use risk factors rather than exposure effects.

Further investigation using meta-analysis found that in adult *Cannabis* users, brain activation was increased in the superior and posterior transverse temporal and inferior frontal gyri [22]. Activities in the striate area, insula, and middle temporal gyrus were decreased in adults. In adolescents, activation was increased in the putamen and inferior parietal gyrus. It is thought that the alterations in neural activation during cognitive activation tasks reflect compensatory neuroadaptive changes that occur in users of *Cannabis*. The compensatory changes reflect less efficient neural strategies used by *Cannabis* users to achieve the same results as nonusers.

The negative neurocognitive effects of *Cannabis* have been explored disproportionately more than the potential positive effects of the drug [23]. A study was conducted on *Cannabis* users exposed to inhaled-*Cannabis* without CBD, *Cannabis* with CBD, and a placebo. When the response of the individuals to music was measured using fMRI, it was determined that *Cannabis* without CBD decreased response to music in the bilateral auditory cortex, the right hippocampus/parahippocampal gyrus, and the right ventral striatum. The ventral striatum is known to be involved in processing reward and positive emotion, suggesting that *Cannabis* may decrease pleasure associated with listening to music. Interestingly, the administration of CBD with *Cannabis* improved connectivity between the ventral striatum and auditory cortex. This observation was in line with the hypothesis that CBD balances out certain negative effects of *Cannabis*.

Cannabis is known for its behavioral and psychological effects, such as feelings of happiness, and relaxation, changes in perception of time, decreased concentration, and learning disabilities [1]. The results can be divided into short-term and long-term. Short-term effects include anxiety, panic attacks, memory loss, and an increased risk of symptoms of mental disorders. The long-term effects of *Cannabis* are usually physical effects, including coughing, excessiveness of chest sputum, and severe mental, behavioral, cognitive, and depressive disorders [2]. Studies have shown that people with mental illness are 2.7 times more likely to experience substance abuse than others.

Cannabis is associated with cognitive and behavioral disorders, and its progressive nature in mental, physical, social, economic, and cultural life endangers the health of the individual, family, and society. In almost all addiction research, personality factors lead a person to addiction – prominent features of an addict's inadequacy and difficulty establishing warm and long-lasting social relationships. About 2% of drug addicts have a psychiatric disorder. The most common comorbid psychiatric diagnoses include major depressive disorder, alcohol-related disorders,

antisocial personality disorder, schizophrenia, bipolar disorder including psychopathy, depression, feelings of tension, insecurity, feelings of and anxiety disorder, and about 5.9% of drug addicts attempt suicide at least once in their lifetime [3].

A review discussing the most recent evidence for the short-term and long-term effects of *Cannabis* on cognition indicated that *Cannabis* intoxication is associated with short-term impairment across several basal cognitive domains, including learning and (episodic) memory, attentional control, and motor inhibition, which is increasing. However, evidence regarding the effects of long-term heavy *Cannabis* use on cognition remains equivocal. *Cannabis* research suffers from difficulties in measuring *Cannabis* exposure history, poor control over potential subacute effects, and heterogeneity in cognitive measures and sample composition.

A pilot study compared cognitive function and emotional functioning among adults age sixty and older who were regular, primarily recreational *Cannabis* users ($n = 28$) and nonusers ($n = 10$) [44]. A bimodal distribution was observed among *Cannabis* users such that they had either initiated regular use more recently (“short-term” users; ≤ 7 years, $n = 13$) or earlier in life (“long-term” users; ≥ 19 years, $n = 15$). Nonusers, short-term, and long-term users were not different in depression, anxiety, emotion regulation, or alcohol use. Nonusers scored significantly higher than long-term users in executive function. Short-term users scored significantly higher than long-term users in executive function, processing speed, and general cognition.

The findings indicate that short-term recreational *Cannabis* use does not result in differences in cognitive performance compared to nonusers, which may indicate that short-term use is relatively benign in older adults. However, longer duration of use is associated with poorer processing speed and executive functioning, and more recent *Cannabis* use is associated with poorer working memory, which may impact older adults’ overall cognitive functioning. Additionally, greater recent *Cannabis* use frequency was negatively associated with working memory.

***Cannabis* on Attention and Working Memory**

Studies have specifically examined attention and working memory in *Cannabis* users using task-related fMRI techniques. The attention network task (ANT) requires that participants indicate the direction of an arrow with a key press. However, the task can parse the activity of three distinct aspects of attention: alertness, orienting to stimuli, and executive control. In a second, more difficult task, participants were rapidly presented with nouns and must provide a use for that noun (e.g., *pen/write*).

The results indicate behavioral and functional differences between adolescent chronic *Cannabis* users and healthy controls, with *Cannabis* users demonstrating poorer performance and increased activation within the right PFC while engaging in tasks requiring executive attention [81]. Although both groups activated the executive network, *Cannabis* users demonstrated increased activation, suggesting that the

executive network may be less efficient in those who use *Cannabis*, given their decreased performance.

Extensive evidence indicates that the mammalian endocannabinoid system plays an integral role in learning and memory. Our understanding of cannabinoids' influence on memory comes predominantly from studies examining cognitive and emotional memory systems mediated by the hippocampus and amygdala, respectively. However, evidence suggests cannabinoids also affect habit or stimulus-response (S-R) memory mediated by the dorsal striatum.

Studies implementing various maze tasks in rats indicate that systemic or intra-dorsolateral striatum infusions of cannabinoid receptor agonists or antagonists impair habit memory. In mice, cannabinoid 1 receptor knockdown can enhance or impair habit formation, whereas delta-9-THC tolerance enhances habit formation. Studies on *Cannabis* users also suggest an enhancement of S-R/habit memory. A tentative conclusion is that acute endocannabinoid disruption with either agonists or antagonists impairs the endocannabinoid system.

Cannabinoid 1 receptor is required for multiple forms of striatal synaptic plasticity implicated in memory, including short-term and long-term depression. Interactions with the hippocampus-dependent memory system may also have a role in some of the observed effects of cannabinoids on habit memory. The impairing effect often observed with acute cannabinoid administration supports cannabinoid-based treatments for human psychopathologies associated with a dysfunctional habit memory system (e.g., posttraumatic stress disorder and drug addiction/relapse) [48]. In contrast, chronic cannabinoid exposure enhances dorsal striatum-dependent S-R/habit memory. In addition, the enhancing effect of repeated cannabinoid exposure on habit memory suggests a novel neurobehavioral mechanism for *Cannabis* addiction involving the dorsal striatum-dependent memory system.

Investigation of visual attention uses a task in which participants mentally tracked multiple digital targets (one to four balls) among ten balls that moved randomly and collided. Although abstinent (delta-9-THC-negative) and active chronic *Cannabis* users demonstrated similar behavioral performance relative to healthy controls, the *Cannabis*-using groups demonstrated different brain activation patterns relative to nonusing controls. Specifically, *Cannabis* users exhibited decreased activity in several regions within the attentional network but greater activation in several smaller clusters within additional regions, including the frontal, posterior parietal, occipital, and cerebellar areas; increased activation in the regions may reflect compensatory function necessary for *Cannabis* users to achieve similar levels of task performance to control subjects [82].

Attention and working memory were examined in a small sample of adolescent *Cannabis* users. During the first task, individuals were required to memorize a string of five letters and indicate when one of those letters was present in subsequent trials containing ten-letter stimuli. In the second task, a visuoauditory selective-attention task, participants either detected tones with a higher or lower pitch than a baseline tone or detected larger or smaller dots than a baseline dot. Additional analyses revealed that frontal and cerebellar activation patterns appeared to normalize during abstinence.

The *Cannabis* group and controls demonstrated similar performance across both tasks and similar patterns of overall brain activity in the executive attention system (ACC and DLPFC). However, a specific region of interest (ROI) analysis revealed increased left superior parietal activity in *Cannabis* users. As that region is also implicated in working memory, it is posited that *Cannabis* users may experience minor alterations in the attention network even though large disruptions may not always be evident [83].

Most fMRI studies examining working memory in *Cannabis* users have specifically examined spatial working memory (SWM). A short-delay response task that required participants to look at a series of three dots and then, after a 3-s delay, determine whether a target stimulus was placed in the same location as one of the dots was utilized. It revealed that adult long-term *Cannabis* users exhibited similar task performance but a greater expanse of activation relative to controls. Specifically, compared to control subjects, *Cannabis* users demonstrated increased activation in regions typically associated with completing SWM tasks (PFC and ACC) and also activated additional regions not typically associated with SWM (e.g., basal ganglia), suggestive of neural compensation [84].

Similar task performance among young adult *Cannabis* users and controls produced altered activation in *Cannabis* users during the completion of a visuospatial 2-back task, which requires participants to indicate whether a target (letter “O”) was presented in the same position that it was in two presentation screens prior. *Cannabis* users demonstrated hyperactivation in regions typically associated with SWM (inferior and middle frontal gyri) and regions not typically associated with SWM (right superior temporal gyrus) [85].

Examination of the SWM in adolescent *Cannabis* users after a 28-day abstinence period, the participants completed a task in which they had to indicate whether a figure appeared in the same location as a previous figure. Similar task performance was observed among *Cannabis* users and healthy controls, and differential brain activation patterns emerged. It is hypothesized that since *Cannabis* users exhibited similar task performance to controls, they may have altered neural functioning during visuospatial working memory tasks, which is subsequently compensated for by the recruitment of additional brain regions.

Cannabis users demonstrated hyperactivation in the right basal ganglia, often associated with skill learning and increased activity in parietal areas, which are related to several processes, including attention, spatial perception/encoding, organization, and working memory [86]. The results provide evidence of potential neurocompensation in *Cannabis* users to achieve the same level of performance as controlled subjects.

Additionally, decreased activation was observed within the right DLPFC, a region implicated in the executive demands of SWM, suggesting that *Cannabis* users rely upon more basic strategies, including rehearsal and attention, rather than employing more complex executive processes [87]. A follow-up study of adolescent *Cannabis* users with a shorter duration of abstinence (2–7 days) revealed increased activity in frontal areas, highlighting the impact of recency of *Cannabis* use on neural response [88].

Cortical activation was examined in adult *Cannabis* smokers with high and low frequencies of *Cannabis* use (determined via median split) during the completion of a verbal n-back task. During n-back tasks, the participants must indicate when a letter is identical to the one presented in the preceding one, two, or three trials, depending on the condition. Despite similar task performance, increased activation in the left parahippocampal gyrus was noted among the high-frequency group relative to low-frequency users, suggesting reduced neural efficiency during working memory tasks [89].

No differences were found in verbal working memory network functional connectivity during the completion of an n-back test among young adult *Cannabis* users, even after assessing the relationship between network function and *Cannabis* use patterns (e.g., onset, duration, lifetime use, weekly use, and problems) [90]. However, null findings may be partially due either to the relatively late average age of onset of use (18.8 years old) within the sample or to ceiling effects in n-back performance, which may have obscured working memory network alterations related to *Cannabis* use.

Neurophysiologic differences appear common among *Cannabis* users even when no differences in task performance are detected. Neural alterations may depend on the type of task utilized, specific brain regions under investigation, or heterogeneity within samples of *Cannabis* users (i.e., chronological age, age of onset, frequency or magnitude of use, and length of abstinence). Although findings are somewhat mixed across investigations of attention and working memory in *Cannabis* users, all but one fMRI study revealed altered activation patterns, regardless of whether the performance was similar or altered relative to non-*Cannabis* users [90]. More specifically, among *Cannabis* users, hyperactivation during attention/working memory tasks is often noted in regions implicated in task performance. The findings from attention and working memory studies highlight the importance of utilizing neuro-imaging techniques to supplement traditional neuropsychological assessments.

Several investigations have explored functional correlates of memory, which is known to be significantly impacted by *Cannabis* use. Using a virtual water maze task to measure spatial learning and memory (participants must virtually navigate to either visible or hidden platforms across trials). While chronic *Cannabis* users demonstrated similar task performance on learning trials, a trend indicating subtle differences in memory retention was detected between *Cannabis* users and healthy controls [91]. Imaging results indicated hypoactivation in the parahippocampal and cingulate gyri among *Cannabis* users; the parahippocampal gyrus is related to landmark-based memory, while the cingulate may be related to the attentional demands of this task. Accordingly, attenuated activation in the cingulate could reflect a poorer ability to meet the attentional demands of this task.

Associative learning and memory are often studied in the context of abused drugs. The type of memory process is influenced by repetitive drug use and influences subsequent drug use, as stimuli associated with rewards may eventually cue drug behavior. Implicit associative memory in heavy *Cannabis* users and nonusing controls between ages 18–25 was examined using a *Cannabis*-specific implicit associate task (IAT) [92].

The participants were asked to categorize items that fit a certain category and those that did not (e.g., “*Cannabis* pictures,” “other pictures,” “relaxed words,” and “neutral words”). Trials were divided into compatible trials with implicit associations (e.g., “*Cannabis* pictures + relaxed” versus “other pics + neutral”) and incompatible trials (e.g., “*Cannabis* pictures + neutral” versus “other pictures + relaxed”). The results revealed that *Cannabis* users demonstrated greater bilateral activity during compatible trials in the caudate and putamen, regions typically associated with habit formation. The results suggest that users’ experience with *Cannabis* may be able to complete trials comprising concepts associated with *Cannabis* more easily than healthy controls. In contrast, healthy controls only showed greater activation than *Cannabis* users in the right inferior frontal gyrus, a region implicated in planned, purposeful behaviors. As a result, *Cannabis* users may not engage regions associated with more deliberative processes to the same extent as nonusers.

Associative learning in frequent *Cannabis* users was examined using a pictorial memory task designed to activate the hippocampal formation reliably. During the task, the participants learn to associate pairs of pictures and must later identify the pairs. Despite similar performance between *Cannabis* users and controls, *Cannabis* users exhibited decreased activation compared to nonusers in brain regions implicated in associative learning, particularly parahippocampal regions and the right DLPFC [93].

As task performance was not related to neural response, hypoactivation might not have been related to cognition but other behavioral or physiological variables, such as vigilance or mental attitude during the task. In a similar study, cortical and parahippocampal activity were evaluated in current *Cannabis* users and healthy controls during a face-number associative learning paradigm [35].

For the task, participants were presented with faces, each with a unique number (encoding condition); they were later asked to recall the digits that matched the faces over several trials (recall condition). As with previous studies, no differences in behavioral performance were observed, and decreased activation was noted in frontal regions: right superior temporal gyrus, right middle frontal gyri, and bilateral superior frontal gyrus.

In contrast to previous studies, *Cannabis* users exhibited increased activation within the right parahippocampal gyrus during learning trials. The increased parahippocampal activity might represent neurocompensation that could account for the decreased frontal activity involvement during the encoding condition. Variations in findings across associative learning studies might be related to different task demands. For example, a study required participants to recall digits associated with faces and, in some cases, required the participants to recall pairs of faces.

Cannabis use and the administration of delta-9-tetrahydrocannabinol have been associated with both acute and long-term deficits in working memory (WM). The effects appear to be related to the disruption of synaptic synchrony within the cortico-basalganglio-thalamic circuits that are part of a broader network subserving WM [34]. The circuitry includes the striatum, globus pallidus, and thalamus, their reciprocal connections to the dorsolateral prefrontal cortex, and densely expresses *Cannabis* type 1 receptors. A study was conducted to determine whether a remote

Cannabis use disorder (CUD) was associated with morphological differences in the basal ganglio-thalamic circuit and whether such differences were associated with WM deficits and a history of *Cannabis* use [33].

Large-deformation high-dimensional brain mapping with magnetic resonance imaging was used to obtain surface-based representations of the striatum, globus pallidus, and thalamus, compared across groups and correlated with WM and CUD history. Groups were matched on demographics that include 44 healthy controls, 10 subjects with a CUD history, 28 schizophrenia subjects with no history of substance use disorders, and 15 schizophrenia subjects with a CUD history. Surface maps were generated to visualize morphological differences.

There were significant *Cannabis*-related parametric decreases in WM across groups. Similar *Cannabis*-related shape differences were observed in the striatum, globus pallidus, and thalamus in controls and schizophrenia subjects. *Cannabis*-related striatal and thalamic shape differences correlated with poorer WM and younger age of CUD onset in both groups. Schizophrenia subjects demonstrated *Cannabis*-related neuroanatomical differences that were consistent and exaggerated compared with *Cannabis*-related differences found in controls. The cross-sectional results suggest that both CUD groups characterized WM deficits and subcortical neuroanatomical differences.

In a cross-sectional observational study, the association between *Cannabis* exposure and working memory impairment and its neural substrates was investigated [37]. The relationship between frequency of exposure to *Cannabis*, working memory performance, and regional brain volumes, whether lower volumes of cortical and subcortical structures mediate the association between *Cannabis* exposure and working memory deficit, were studied using the Human Connectome Project.

Data from 234 individuals with self-reported *Cannabis* exposure and 174 individuals unexposed to *Cannabis* were tested. The relationship between self-reported frequency of *Cannabis* exposure and list-sorting working memory task performance (total number of correct responses), T1-weighted MRI-derived regional gray-matter volumes, and working memory task performance, and between frequency of *Cannabis* exposure and brain volumes after controlling for potential confounders were determined. Mediation analysis was carried out to test if the deficit in working memory performance associated with *Cannabis* use was mediated by its association with lower gray-matter volume. The participants who reported a higher frequency of *Cannabis* use tended to have a lower number of correct responses in the list-sorting working memory task and lower bilateral hippocampal volumes. The association between the severity of *Cannabis* exposure indexed by frequency of *Cannabis* use and impairment in working memory was mediated by lower left hippocampal volume in *Cannabis* users. The evidence supports the left hippocampus volume-mediated working memory impairment associated with recreational *Cannabis* exposure.

Working memory is a component of cognitive control involved in developing and maintaining an addiction. A study evaluated the association between *Cannabis* use and WM (load)-related brain activity to assess gender effects in the association [46]. The brain activity of 104 frequent *Cannabis* users (63% men) and 85 controls (53%

men) was recorded during an N-back WM task. Behavioral results revealed a significant interaction between WM load and group for accuracy and reaction time, with *Cannabis* users showing a relatively larger decrease in performance with increasing WM load. *Cannabis* users, compared to controls, showed a relatively smaller reduction in WM (load)-related activity in the precuneus and posterior cingulate cortex at a higher WM load.

An exploratory analysis showed higher WM-related activity in the superior frontal gyrus in men compared to women. The WM load-related activity was not associated with either performance or *Cannabis* use or related problems. While *Cannabis* users showed higher WM (load)-related activity in central nodes of the default mode network, it was not directly attributable to the group-specific worsening of performance under higher cognitive load.

***Cannabis* and Spatial Working Memory**

The effects of repeated delta-9-tetrahydrocannabinol on reinforcement-related learning and performance of spatial working memory tasks of varying difficulty were examined in adolescent monkeys [150]. Seven pairs of male adolescent rhesus monkeys, matched for baseline cognitive performance, received vehicle or delta-9-THC intravenously 5 days/week for 12 months. Performance on four-item spatial WM trials was assessed throughout the 12-month study period. During the 6 month, more difficult and distractor eight-item, spatial WM trials were added. Throughout the study, the study determined residual effects on performance 23 or 71 h after delta-9-THC or vehicle administration.

Relative to vehicle-exposed animals, repeated delta-9-THC exposure was initially associated with significantly slower performance accuracy on 4-item spatial WM trials; however, the performance difference gradually diminished such that accuracy did not significantly improve by month 12 differed between vehicle and delta-9-THC groups. Similarly, for the novel and distractor eight-item trials introduced at month 6, performance accuracy improved more slowly in the delta-9-THC than in the vehicle group, despite comparable performance between groups on the four-item task during the same period. The findings indicated that delta-9-THC exposure during adolescence impairs the reinforcement-related learning process required for improved performance on spatial WM tasks compared to vehicle exposure. However, this impairment might be overcome with continued training, even after ongoing delta-9-THC exposure.

Adult male rhesus monkeys were trained on tasks that assess spatial working memory, visuospatial associative memory and learning, and motivation for a food reward [152]. The monkeys were subsequently challenged with 0.1–0.3 mg/kg delta-9-THC, intramuscularly, in randomized order and evaluated on the behavioral measures. In studies, monkeys have not shown consistent evidence of memory-specific effects of delta-9-THC on recognition tasks.

The performance of both Visuospatial Paired Associates Learning and Self-ordered Spatial Search tasks was impaired by delta-9-THC in a dose and task-difficulty-dependent manner. It is concluded that delta-9-THC disrupts cognition in a way that is consistent with a direct effect on memory. There was evidence for interference with spatial working memory, visuospatial associative memory, and incremental learning in the latter task. The results and the lack of a specific effect of delta-9-THC in prior visual recognition studies imply spatial memory processing and working memory sensitivity to endocannabinoid perturbation.

The relationship between change in *Cannabis* use and changed cognitive performance was evaluated over 8 years by a survey methodology with a cohort design [139]. An Australian community sample aged 20–24 years at baseline was assessed for cognitive performance with the California Verbal Learning Test (CVLT) (immediate and delayed), Spot-the-Word test (STW), Symbol Digit Modality test (SDMT), and Digit Backwards (DB).

Groups of *Cannabis* users were defined from self-reports across three waves as: “never” ($n = 420$), “remain light” ($n = 71$), “former light” ($n = 231$), “remain heavy” ($n = 60$), “former heavy” ($n = 60$), and “always former” (since the start of the study) ($n = 657$). Planned contrasts within the mixed model repeated-measures analysis of variance were used for longitudinal analysis with an adjusted alpha of 0.01. Data were obtained from 2404 participants, with 1978 (82.3%) completing wave 3. At baseline, there were significant differences between *Cannabis* groups on CVLT (immediate and delayed) and SDMT. However, after controlling for education, gender, gender $\times$ group, and gender $\times$ wave, there were no significant between-group differences.

Only CVLT immediate recall reached adjusted statistically significant longitudinal change associated with changed *Cannabis* use (group $\times$ wave $p = 0.007$). Specifically, former heavy users improved their performance relative to remaining heavy users (estimated marginal means: former heavy 6.1–7.5: remains heavy 6.4–6.6). Cessation of *Cannabis* use appears to be associated with an improvement in the capacity to recall information that has just been learned. No other measures of cognitive performance were related to *Cannabis* after controlling for confounds.

Cannabis users display a constellation of withdrawal symptoms upon drug discontinuation, including sleep disturbances, irritability, and possibly memory deficits. In cannabinoid-dependent rodents, the CB1 antagonist, rimonabant, precipitates somatic withdrawal. It enhances forskolin-stimulated adenylyl cyclase activity in the cerebellum, an effect opposite that of acutely administered delta-9-tetrahydrocannabinol. A test was conducted to determine whether delta-9-THC-dependent mice undergoing rimonabant-precipitated withdrawal would display short-term spatial memory deficits when assessed in the Morris water maze. Rimonabant precipitation of adenylyl cyclase superactivation was also evaluated in hippocampal and cerebellar tissue from delta-9-THC-dependent mice [160].

Rimonabant significantly impaired the spatial memory of delta-9-THC-dependent mice at lower doses than those necessary to precipitate somatic withdrawal behavior. In contrast, maze performance was near perfect in the cued task, suggesting sensorimotor function and motivational factors were unperturbed by the

withdrawal state. Finally, rimonabant increased adenylyl cyclase activity in the cerebellar but not in hippocampal membranes.

The disruptive memory effects of delta-9-THC undergo tolerance following repeated dosing, while the withdrawal state leads to a rebounding deficit in memory. The results establish spatial memory impairment as a particularly sensitive component of cannabinoid withdrawal, an effect that may be mediated through compensatory changes in the cerebellum.

***Cannabis* and Executive Function**

Executive function is a higher-order, multifaceted cognitive construct that involves controlling and executing goal-directed behaviors. Executive functioning encompasses many important skills: planning, reasoning, inhibitory processing, self-monitoring, and problem-solving. Several studies have utilized Stroop paradigms to study executive functioning and inhibitory processing in chronic *Cannabis* smokers.

During the traditional Stroop color-word test, participants must complete three conditions in which they either name colors of blocks; read words printed in black ink; or, when presented with words printed in a different colored ink from what they spell, must name the color of the ink, thereby inhibiting the automatic tendency to read. Altered activation patterns were observed in *Cannabis* users relative to controls within the cingulate cortex and DLPFC; frontal regions typically activated during the Stroop interference condition completion. Specifically, *Cannabis* users demonstrated reduced anterior cingulate but increased midcingulate and a more diffuse bilateral pattern of DLPFC activity relative to non-*Cannabis* users.

Although *Cannabis* users and controls performed within normal limits on the task, the *Cannabis*-using group exhibited higher rates of commission errors (incorrect responses) relative to controls, which were associated with activation in different brain regions [73]. Poorer performance on the Stroop task among *Cannabis* users relative to control subjects was accompanied by reduced, but more diffuse, activation throughout the cingulate cortex.

A modified Stroop task containing a mix of congruent (e.g., “green” printed in green ink) or incongruent trials (e.g., “red” printed in blue ink) was used to assess functional activation patterns in treatment-seeking *Cannabis* users. Among the *Cannabis* users, those with earlier onset of regular *Cannabis* use (onset prior to age 16) demonstrated activation in anterior regions of the cingulate cortex. In contrast, those with late-onset exhibited activation in more posterior cingulate regions, similar to the healthy control group [74]. Despite similar activation patterns during congruent trials, relative to healthy controls, *Cannabis* users exhibited poorer task performance coupled with reduced activity across multiple regions, including the prefrontal cortex (PFC), striatum, amygdala/parahippocampal gyrus, thalamus, and midbrain regions during incongruent trials.

The counting Stroop test has also been employed in young adult *Cannabis* users (19–21 years old). The variation of the traditional color-word Stroop task presents

individuals with sets of one, two, three, or four words and then requires them to report the number of words presented; during congruent trials, they are presented with names of animals, but during incongruent trials, they are presented with number words (e.g., “one,” “two,” “three,”) that are incongruent with the correct response. The counting Stroop has primarily been shown to increase activity in the ACC but also activates a range of networks involved in attention, response selection, motor planning, and motor output [75].

Although another study revealed that *Cannabis* users demonstrated similar task performance as healthy controls, hyperactivation was observed during the completion of the task within the cingulate gyrus and additional regions, including the right rolandic operculum, postcentral gyrus, cerebellar tonsil, and right supplementary motor area, suggesting that recruitment of additional brain regions may have been necessary in order for *Cannabis* users to achieve adequate performance [76].

Studies using traditional Stroop paradigms primarily report reduced activation and poorer task performance in *Cannabis* users relative to healthy control subjects. One study using an alternate version of the task (e.g., counting Stroop) revealed increased activation and similar task performance between *Cannabis* users and controls that may be due, at least in part, to the fact that the traditional Stroop interference condition requires verbally mediated responses that produce robust inhibitory effects. In contrast, the counting Stroop requires a button press, utilizing different response outputs and related neural circuitry.

The multisource interference task (MSIT) is a measure of cognitive control processing and response inhibition shown to reliably activate the cingulo-fronto-parietal (CFP) cognitive/attention network. The MSIT uses aspects from the Stroop as well as other well-established measures of cognitive interference (e.g., Simon and Flanker tasks); subjects are presented with sets of three numbers and must indicate the identity of the number that is different regardless of their position within the set, which is consistent with its position on the button box in congruent trials, yet inconsistent with its button box position during interference trials. The MSIT found that *Cannabis* users exhibited more diffuse patterns of ACC activation than healthy controls despite similar task performance [77].

Connectivity analyses indicated altered connectivity within the response inhibition circuit, which was correlated with *Cannabis* use. The *Cannabis* groups had significantly different durations of abstinence across studies, ceasing use the night before the scan versus 28 days of abstinence, recency of use likely affected neural response across the investigations. Nonetheless, the results suggest that *Cannabis* use affects brain circuitry related to executive functioning and response inhibition.

Studies on the impact of *Cannabis* on cognitive function in adolescents and adults demonstrated that those who use *Cannabis* regularly exhibit poorer cognitive performance across domains relative to non-*Cannabis* users, primarily attributed to delta-9-THC. The studies suggest that executive functioning and memory are most strongly affected by regular *Cannabis* use [50]. and processing speed is also adversely impacted. The findings are more inconsistent with regard to IQ. A meta-analysis concluded that among studies citing negative effects of *Cannabis* use on cognition are because the effect sizes are often small, raising the question of the

clinical importance of the decrements. In contrast, others have not reported decrements among *Cannabis* consumers [51].

In examining the acute effects of delta-9-THC on memory/working memory function, 6 mg of delta-9-THC was administered to occasional *Cannabis* users via a vaporizer, followed by three subsequent 1 mg delta-9-THC doses to maintain stable delta-9-THC levels throughout the scanning [52]. In the first study, the participants completed a pictorial memory task containing both encoding and recall conditions to assess associative memory.

Although delta-9-THC administration did not affect task performance, imaging analyses demonstrated that it was correlated with attenuated activity in the right inferior frontal gyrus, right insula, and left middle occipital gyrus during encoding. In contrast, during recall trials, acute delta-9-THC exposure appeared to result in a network-wide increase in activity, most notably in the bilateral cuneus and precuneus. Functional activation was inversely correlated with performance during placebo recall conditions, suggesting more efficient processing, but no association between activation and performance was detected during the delta-9-THC recall condition.

A second study specifically assessed working memory function using a Sternberg Item Recognition task where the participants were instructed to encode strings of 1, 3, 5, 7, or 9 letters; they were then presented with individual letters and must identify each letter that appeared in the preceding string via a button press. In that study, delta-9-THC negatively impacted task performance accuracy. Delta-9-THC also affected the relationship between working memory load and overall activity within the working memory neural network, specifically mediating activity in the left dorsolateral prefrontal cortex (DLPFC), inferior temporal gyrus, inferior parietal gyrus, and cerebellum [53]. ROI analyses examining the working memory network revealed linear increases in activity as the working memory load increased under placebo conditions. At the same time, delta-9-THC exposure was associated with increased activity only for low working memory loads.

The two studies revealed alterations across distinct, nonoverlapping brain regions during the performance of memory tasks, likely because the tasks assess different aspects of memory (visual memory versus working memory), resulting in different patterns of activation and performance differences between placebo and delta-9-THC conditions were only noted in the second study. The findings suggest that delta-9-THC can impact various aspects of memory processing, even without performance decrements.

Neuropsychological Functioning in First-Episode Psychosis

The importance of neuropsychological functioning in First-Episode Psychosis (FEP) has led to several studies. One study pursued three goals: First, to examine verbal and visual memory in a sample of Child and Adolescent FEP; second, to evaluate the effect of other cognitive domains on verbal and visual memory; and

finally, to examine the relationship between performance in the cognitive dimension and the age of *Cannabis* use [38].

A sample of 41 FEPs and 39 healthy subjects were evaluated. Verbal and visual memory, attention, working memory, processing speed, mental flexibility, verbal fluency, motor coordination, planning ability, and intelligence were assessed. The results found impairment of short and long-term recall of verbal memory and short-term visual memory in early psychosis. Relationships between cognitive dimensions, such as visual memory and intelligence, and motor coordination were also found. A paradoxical effect was found in patients who used *Cannabis*, as the FEP consumers performed the visual memory test better than those who had not used it.

The patients showed impairment in short and long-term recall of verbal information and short-term visual reproduction. In the second place, motor coordination and intelligence influenced short-term visual memory in patients in the early stages of the illness. Third, the use of *Cannabis* in patients with FEP was associated with better performance in the test that evaluated short-term visual memory, measured by task completion time, that is, efficiency in performing the test. However, their visual memory was similar to the controls when measured by task execution accuracy.

A sequential multiple-assignment randomization treatment pilot study examined: (a) adding working memory training to contingency management (CM) for youth with *Cannabis* use disorder and (b) switching nonresponding youth to higher magnitude CM incentives boosts outcomes. In Phase 1, youth with CUD ($n = 59$, M age = 16, male = 71%) attending an intensive outpatient program were randomly assigned to 14 weeks of CM only or CM plus working memory training (WMT) [39]. In Week four, a Phase 2 treatment was assigned. Those with negative urine drug tests (responders) continued in their Phase 1 treatment. The drug-positive (nonresponders) were randomly assigned to remain in their Phase 1 treatment or to higher magnitude CM.

Zero-inflated negative binomial models comparing those assigned to CM versus CM + WMT indicated no differences in the likelihood of having ≥ 1 week of continuous abstinence or longer abstinence duration. Those assigned to WMT showed greater but not significant improvements in working memory ($n = 35$; $\beta = 0.69$, $p = 0.06$). Working memory improvements were associated with achieving any abstinence (odds ratio = 3.50, 95% CI [1.01, 12.10], $p = 0.05$). Phase 2 randomization to higher magnitude CM did not boost outcomes. The results suggest that WMT appears promising, but the sample size was small, attrition was high, and replication was crucial.

***Cannabis* and Olfactory Neuroepithelium Cells**

Cannabinoid receptors and serotonergic 2A receptors (5HT2AR) form heteromers in the brain of mice, mediating the cognitive deficits produced by delta-9-tetrahydrocannabinol. The expression levels and functionality of CB1R-5HT2AR

heteromers were investigated in human olfactory neuroepithelium (ON) cells of *Cannabis* users and control subjects. The molecular characteristics through adenylate cyclase and the ERK 1/2 pathway signaling studies were determined [129]. Olfactory neuroepithelium cells from controls and *Cannabis* users expressed neuronal markers such as β III-tubulin and nestin, displayed similar expression levels of genes related to cellular self-renewal, stem cell differentiation, and generation of neural crest cells, and showed comparable Na^+ currents in patch clamp recordings. Heteromer expression levels and their correlation with *Cannabis* consumption and cognitive performance in neuropsychological tests were evaluated.

CB1R-5HT2AR heteromer expression was significantly increased in *Cannabis* users and positively correlated with the amount of *Cannabis* consumed and negatively with the age of onset of *Cannabis* use. In addition, a negative correlation was found between heteromer expression levels and attention and working memory performance in *Cannabis* users and control subjects. The findings suggest that *Cannabis* consumption regulates the formation of CB1R-5HT2AR heteromers and may have a key role in cognitive processing. The heterodimers could be potential new targets for developing treatment alternatives for cognitive impairments.

WIN administered during late adolescence prevented stress-induced impairments and reduced anxiety levels. WIN normalized the early life stress-induced upregulation in PFC-GRs and CA1-CB1r in females. In males, WIN normalized the early life stress-induced upregulation in PFC-GR and downregulation in BLA-CB1r. The differences in recognition memory and the expression of GRs and CB1r, the fear circuit, suggest gender differences in the mechanism underlying coping with stress.

***Cannabis* and Audio/Visual/Verbal Memories**

The concentration of delta-9-tetrahydrocannabinol in *Cannabis* has increased by 250% since investigations on the effects of *Cannabis* on short-term memory first appeared in scientific journals. Investigations of short-term memory in long-term *Cannabis* smokers involved adults only. The auditory/verbal and visual/spatial memories of ten *Cannabis*-dependent adolescents were evaluated, and the results were compared with the performance of 17 subjects in two control groups [28]. The control groups included eight adolescent drug abusers who had not been long-term users of *Cannabis* and nine adolescents who had never abused any drug. The three groups were matched on age, IQ, and absence of previous learning disabilities. Adolescents with a history of frequent alcohol or phencyclidine abuse were excluded from entering the study.

Seven neuropsychological tests were initially administered to all subjects, and a parallel test battery was administered 6 weeks later. Significant differences between the *Cannabis*-dependent group and the two control groups were obtained initially on the Benton Visual Retention Test ($F[2,24] = 6.07$) and the Wechsler Memory Scale Prose Passages ($F[2,23] = 7.04$). After 6 weeks of supervised

abstention from intoxicants, the subjects in the *Cannabis*-dependent group showed significant improvement on the Wechsler Memory Prose Passages score and the Benton Visual Retention Test; however, the improvement failed to achieve statistical significance. It was concluded that *Cannabis*-dependent adolescents have selective short-term memory deficits that continue for at least 6 weeks after the last use of *Cannabis*.

In three experiments, various tasks sufficiently sensitive to show differences in visual, verbal, everyday memory, and executive function between controls and *Cannabis* users were examined [41]. A series of three studies explored cognitive function deficits in *Cannabis* users (phonemic verbal fluency, visual recognition, immediate and delayed recall, and prospective memory) in short-term abstinent *Cannabis* users. The participants were selected using snowball sampling, with *Cannabis* users being compared to a standard control group and a tobacco-use control group. The *Cannabis* users, compared to both control groups, had deficits in verbal fluency, visual recognition, delayed visual recall, and short- and long-interval prospective memory. There were no differences in the immediate visual recall. The findings suggest that *Cannabis* use leads to impaired executive function.

***Cannabis* and Motor Skills**

Psychomotor speed and visuomotor processing are important skills, particularly when considering the potential impact of chronic *Cannabis* use on driving. Studies have directly assessed the underlying skills and their functional correlates in chronic *Cannabis* users using finger-tapping tasks. Male but not female chronic *Cannabis* users exhibited slower psychomotor speed across several measures, which was related to increased activation in the superior frontal gyrus and additional regions associated with attention and motor planning, as well as decreased activation in the lingual gyrus, which is typically associated with visual attention [94].

During a bilateral finger-tapping task that did not formally assess performance, *Cannabis* users demonstrated reduced activation in the ACC (Brodmann areas 24 and 32) and supplementary motor cortex (BA6) after 4–36 h of abstinence. One study using the same tapping task revealed hypoactivation of the cingulate in older adolescents with heavy *Cannabis* use compared to controls. Additionally, cerebellar activation, which was decreased in the *Cannabis*-using group relative to controls, was examined and suggestive of disrupted cortico-cerebellar circuits. Furthermore, decreased activation in the cerebellum and cingulate within the sample was associated with higher lifetime *Cannabis* exposure. It is likely that, relative to healthy controls, *Cannabis* users demonstrate activation-related differences during motor control tasks.

Cannabis and the Mismatch Negativity

Cannabis use is associated with the development of psychotic symptoms and increased risk for schizophrenia. The mismatch negativity (MMN) is a brain event-related potential marker of change detection thought to index glutamatergic N-methyl-D-aspartate receptor-mediated neurotransmission, which is known to be deficient in schizophrenia. A study examined auditory MMN in otherwise healthy chronic *Cannabis* users compared with nonuser control subjects [135].

Forty-two chronic *Cannabis* users and 44 nonuser healthy control subjects completed a multifeature MMN paradigm, including duration, frequency, and intensity deviants (6%; standards 82%). The MMN was compared between users and control subjects and between long- and short-term users and age- and gender-matched control subjects. Associations between MMN, *Cannabis* use measures, and symptoms were examined.

The pattern of MMN alterations in *Cannabis* users differed from that typically observed in patients with schizophrenia, indicating overlapping but distinct underlying pathology. The MMN amplitude was significantly reduced to frequency but not duration or intensity deviants in overall *Cannabis* users relative to control subjects. Frequency MMN was similarly attenuated in short- and long-term users relative to control subjects. Long-term users also exhibited reduced duration MMN relative to control subjects and short-term users, correlated with increased duration of exposure to *Cannabis* and increased psychotic-like experiences during intoxication. In short-term users, a younger age of onset of regular *Cannabis* use and greater frequency of use were associated with greater psychotic-like experiences and symptomatic distress. The results indicated impaired sensory memory that might reflect N-methyl-D-aspartate receptor dysfunction in chronic *Cannabis* users.

Mismatch negativity is an auditory event-related potential indicating auditory sensory memory and information processing. The hypothesis that chronic *Cannabis* use is associated with deficient MMN generation was investigated in age- and gender-matched chronic *Cannabis* users ($n = 30$) and nonuser controls ($n = 30$) [149]. The *Cannabis* users were divided into two groups according to duration and quantity of *Cannabis* consumption. The MMNs resulting from a pseudorandomized sequence of 2×900 auditory stimuli were recorded by 32-channel EEG. The standard stimuli were 1000 Hz, 80 dB SPL, and 90 ms duration. The deviant stimuli differed in duration (50 ms) or frequency (1200 Hz). There were no significant differences in MMN values between *Cannabis* users and nonuser controls in both deviance conditions.

Concerning subgroups, reduced amplitudes of frequency MMN at frontal electrodes were found in long-term (≥ 8 years of use) and heavy (≥ 15 joints/week) users compared to short-term and light users. The results indicate that chronic *Cannabis* use may cause a specific impairment of auditory information processing. In particular, the duration and quantity of *Cannabis* use could be identified as important factors of deficient MMN generation.

***Cannabis* and Traumatic Brain Injury**

The prevalence of mild traumatic brain injury is highest among adolescents and can lead to complications, including neuroinflammation and excitotoxicity. Also pervasive in adolescents is recreational *Cannabis* use. Delta-9-Tetrahydrocannabinol has anti-inflammatory properties and serves as a neuroprotective agent against excitotoxicity. The effects of delta-9-tetrahydrocannabinol on recovery were examined when administered either prior to or following repeated mild brain injuries [144].

Male and female Sprague-Dawley rats were randomly assigned to receive delta-9-tetrahydrocannabinol or vehicle either prior to or following repeated injuries. The rats were then tested on a behavioral battery to measure postconcussive symptomology. The hippocampus, nucleus accumbens, and prefrontal cortex were extracted from all animals to examine mRNA expression changes (*Bdnf*, *Cnr1*, *Comt*, *GR*, *Iba-1*, and *Vegf-2R*).

Delta-9-Tetrahydrocannabinol administration after repeated mild traumatic brain injury was beneficial to three of the six behavioral outcomes of injury (reducing anxiety and depressive-like behaviors while mitigating injury-induced deficits in short-term working memory). Delta-9-Tetrahydrocannabinol administration following injury also showed beneficial effects on the expression of *Cnr1*, *Comt*, and *Vegf-2R* in the hippocampus, nucleus accumbens, and prefrontal cortex. There were no notable benefits of delta-9-tetrahydrocannabinol when administered prior to the injury, suggesting that delta-9-tetrahydrocannabinol may have a potential therapeutic benefit on postconcussive symptomology when administered postinjury but not preinjury.

A study was conducted to determine if delta-9-THC could enhance brain repair and improve short-term spatial working memory in mice following controlled cortical impact (CCI) by upregulating granulocyte colony-stimulating factor (G-CSF) and other neurotrophic factors (brain-derived neurotrophic factor [BDNF], glial-derived neurotrophic factor [GDNF]) in the hippocampus (HP), cerebral cortex, and striatum [158].

C57BL/6 J mice underwent CCI and were treated for 3 days with delta-9-THC at a dose of 3 mg/kg intraperitoneally (i.p.). Short-term working memory was determined using the spontaneous alternations test during exploratory behavior in a Y-maze. Locomotor function was measured as falling latency from a rotating drum (rotometry). The behaviors were recorded at baseline and three, seven, and 14 days after CCI. Groups of mice were euthanized at seven and 14 days. The extent of microgliosis, astrogliosis, and G-CSF, BDNF, and GDNF expression was measured at seven and 14 days in the cerebral cortex, striatum, and HP on the side of the trauma. The levels of the most abundant endocannabinoid (2-arachidonoyl-glycerol [2-AG]) were also measured.

Delta-9-THC-treated mice ran on the rotarod longer than vehicle-treated mice and recovered to normal rotarod performance levels at 2 weeks. The delta-9-THC-treated mice exhibited marked improvement in performance on the Y-maze, indicating that treatment with the phytocannabinoid could reverse the deficit in working

memory caused by the CCI. Delta-9-THC-treated mice, compared with vehicle-treated animals, exhibited significant upregulation of G-CSF as well as BDNF and GDNF in the cerebral cortex, striatum, and HP. Levels of 2-AG were also increased in the delta-9-THC-treated mice.

Administration of delta-9-THC promotes significant functional recovery from traumatic brain injury (TBI) in working memory and locomotor function. The beneficial effect is associated with the upregulation of brain 2-AG, G-CSF, BDNF, and GDNF. The latter three neurotrophic factors have been previously shown to mediate brain self-repair following TBI and stroke.

The Cognitive and Emotional Impairments Induced by Carotid Artery Occlusion

An investigation of the effects of cannabidiol on the cognitive and emotional impairments induced by bilateral common carotid artery occlusion (BCCAO) was conducted in mice [147]. A multitiered behavioral testing battery for 21 days revealed that BCCAO mice exhibited long-lasting functional deficits reflected by the increase in anxiety-like behavior (day nine), memory impairments (days 12–18), and despair-like behavior (day 21).

Short-term CBD, at a dose of 10 mg/kg, prevented cognitive and emotional impairments, attenuated hippocampal neurodegeneration and white matter injury, and reduced glial response induced by BCCAO. Ischemic mice treated with CBD increased hippocampal brain-derived neurotrophic factor (BDNF) protein levels. CBD also stimulated neurogenesis and promoted dendritic restructuring in the hippocampus of BCCAO animals. The results demonstrate that short-term CBD treatment results in global functional recovery in ischemic mice and impacts multiple and distinct targets involved in the pathophysiology of ischemic brain injury.

Cannabidiol was investigated as a potential treatment for indications characterized by altered memory processing. An investigation was conducted to determine how CBD influences cerebral blood flow (CBF) in regions involved in memory processing and if CBD's effects on CBF were associated with differences in working and episodic memory task performance. A randomized, double-blind design was used, in which 15 healthy participants were administered 600 mg CBD orally or placebo on separate days [141]. Regional CBF was measured at rest using arterial spin labeling 3 h after CBD ingestion. Working memory was assessed with the digit span (forward, backward) and n-back (0-back, 1-back, 2-back) tasks and a prose recall task (immediate and delayed) to assess episodic memory.

Cannabidiol increased CBF in the hippocampus (mean (95% confidence intervals) = 15.00 (5.78–24.21) mL/100 g/min, $t_{14} = 3.489$, Cohen's $d = 0.75$, $p = 0.004$). There were no differences in memory task performance. However, there was a significant correlation where greater CBD-induced increases in orbitofrontal CBF were associated with reduced reaction time on the 2-back working memory task

($r = -0.73$, $p = 0.005$). The findings indicated that CBD increases CBF in key regions involved in memory processing, particularly the hippocampus. The results identified potential mechanisms of CBD for various conditions associated with altered memory processing, including Alzheimer's disease, schizophrenia, posttraumatic stress disorder, and *Cannabis*-use disorders.

Relapse/Reinstatement of Drug-Seeking Behavior

Cannabis use usually begins in adolescence, and heavy adolescent use is often associated with impaired cognitive function in adulthood. Dissociating innate differences in cognitive function from *Cannabis*-induced deficits is challenging. A study sought to develop a rodent model of adolescent cannabinoid self-administration using the synthetic cannabinoid receptor agonist WIN55212-2 (WIN) to assess relapse/reinstatement of drug-seeking and long-term effects on cognitive function assessed in a delay-match-to-sample working memory task and a spatial recognition task [142]. Adolescent male rats readily self-administered WIN in two- or six-hour sessions/day but did not demonstrate an escalation of intake with 6-h access. The rats exhibited significant cue-induced WIN-seeking reinstatement that increased with 21 days of abstinence (i.e., "incubation of craving").

Cognitive testing occurred in adulthood under drug-free conditions. Two-hour and six-hour adolescent WIN self-administration groups exhibited significantly better working memory performance in adulthood than sucrose self-administration controls. Performance was associated with altered expression of proteins regulating GABAergic and glutamatergic signaling in the prefrontal cortex. Self-administered WIN did not produce either acute or chronic effects on short-term memory. However, experimenter administration of WIN in adolescence, at doses previously reported in the literature, produced acute deficits in short-term memory that recovered with abstinence. Thus, the self-administration of a rewarding cannabinoid in adolescence does not produce long-term cognitive dysfunction.

Considerable neurodevelopmental alterations occur throughout adolescence, and the environmental insult posed by exogenous cannabinoid exposure may alter natural developmental trajectories. Studies indicated that long-lasting deficits in cognitive function occur due to adolescent *Cannabis* use, but considerable variability exists in the magnitude of the effects. A procedure was established for intravenous delta-9-THC self-administration in adolescent rats to determine if volitional delta-9-THC intake produced indices of addiction-related behavior, altered working memory performance in adulthood, or altered the expression of proteins associated with the behaviors across several brain regions [151].

Male and female adolescent rats learned to self-administer escalating delta-9-THC intravenously from PD 32–51. Upon reaching adulthood, they were tested in abstinence for cued reinstatement of delta-9-THC-seeking and working memory performance on a delayed-match-to-sample task. In a separate cohort,

glutamatergic, GABAergic, and cannabinoid receptor protein expression was measured in multiple brain regions.

Male and female adolescents self-administered delta-9-THC and exhibited cue-induced lever pressing throughout abstinence. Delta-9-THC-exposed males exhibited slightly enhanced working memory performance in adulthood, and better performance positively correlated with total delta-9-THC self-administered during adolescence. Adolescent delta-9-THC-exposed rats exhibited reductions in CB1, GABA, and glutamate receptor protein, primarily in the prefrontal cortex, dorsal hippocampus, and ventral tegmental area. The results indicated that delta-9-THC exposure at self-administered doses could produce moderate behavioral and molecular alterations, including sex-dependent effects on working memory performance in adulthood.

Dysregulatory Risk Factor

Cannabis users with a dysregulatory risk factor may be particularly vulnerable to more frequent and problematic *Cannabis* use. Contemporary models of dysregulated behavior suggest that dysregulation emerges due to distinct mechanisms. A study examined the dysregulatory correlates of *Cannabis* involvement, including working memory capacity, delay discounting, impulsivity, and reward sensitivity [40].

The participants were 104 nontreatment-seeking frequent *Cannabis* users (the average participant used *Cannabis* 71% of the days/past 60 days [$SD = 22\%$], with an average of two uses per day [$SD = 1.2$]). The mean age was 21.3 ($SD = 4.3$); 36.5% were female. Working memory was assessed via the Trail-Making Test-B and the Digit Span subtests of the WAIS-III, delay discounting was assessed via a computer-based task, trait impulsivity was self-reported via the Barratt Impulsiveness Scale, and reward sensitivity was self-reported via the Reward Dependence Scale and the Snaith–Hamilton Pleasure Scale.

Structural equation modeling estimated the associations between different facets of dysregulation and *Cannabis* involvement. Results suggest that poor working memory capacity and high trait impulsivity were associated with both use and problem use. Greater delay discounting was associated with problem use rather than with the frequency of use. Low reward sensitivity was associated with frequent *Cannabis* use but not with problem use. The results confirmed that the dysregulatory correlates of *Cannabis* involvement consist of multiple dimensions of functioning.

***Cannabis* and Driving**

One of the most common public health policy concerns is the potential impact of *Cannabis* on driving. In order to understand specific functional changes that may affect driving, driving-related skills were evaluated in occasional *Cannabis* smokers using a visuomotor tracking task during fMRI [54]. The participants tracked a square as it moved along pseudo-random trajectories during the active and passive conditions. The participants were asked to track the square visually in the passive condition. However, in the active condition, the participants tracked the square by keeping it at the center of another square using a joystick. The results revealed that after smoking *Cannabis* (an 11% delta-9-THC joint), the participants exhibited impaired psychomotor skills (i.e., difficulty with the active task condition) and decreased activity in several of the brain's primary cognitive networks (e.g., salience and central executive networks), reflective of less focused on the task.

In contrast, the ventromedial prefrontal cortex (vmPFC) and rostral anterior cingulate cortex (ACC), posited to be part of the default mode network, demonstrated increased activity. The vmPFC and ACC are related to spontaneous self-generated thoughts and therefore concluded that *Cannabis* users' performance may reflect an increase in self-oriented thoughts. If that were the case, intoxicated individuals might be more likely to attend to stimuli related to them and subsequently fail to attend to the task, resulting in poorer task performance which may ultimately correlate with poorer driving performance.

***Cannabis* and Microglia Function**

A study evaluated female rats after chronic administration of increasing doses of delta-9-tetrahydrocannabinol during adolescence (PND 35–45) to determine the effect on microglia function in the long term. The possible contribution of microglia to the development of delta-9-THC-induced alterations in mood and cognition in adult female rats was also explored [153]. The data indicate that delta-9-THC induces a persistent neuroinflammatory state specifically localized within the adult prefrontal cortex (PFC), characterized by increased expression of the proinflammatory markers, TNF- α , iNOS, and COX-2, and reduction of the anti-inflammatory cytokine, IL-10.

The neuroinflammatory phenotype is associated with the downregulation of the CB1 receptor on neuronal cells and the upregulation of CB2 on microglia cells, conversely. Interestingly, blocking microglia activation with ibudilast during delta-9-THC treatment significantly attenuates short-term memory impairments in adulthood, simultaneously preventing the increases in TNF- α , iNOS, COX-2 levels, and the upregulation of CB2 receptors on microglia cells. In contrast, delta-9-THC-induced depressive-like behaviors were unaffected by ibudilast treatment. The findings demonstrate that adolescent delta-9-THC administration is associated with

persistent neuroinflammation within the PFC and provide evidence for a causal association between microglial activation and the development of long-term cognitive deficits induced by adolescent delta-9-THC treatment.

Endocannabinoids and Astrocytes

Endocannabinoids released from a given stimulated CA3-CA1 synapse activated astrocytic CB1R and promoted astrocytic glutamate release via an IP3-induced calcium-release mechanism. This series of events, in turn, induces an NMDAR-mediated short or nitric oxide (NO)-mediated long-term potentiation on neighboring CA1 synapses. The lateral synaptic regulation achieved by astrocytes and eCBs is also reported in the dorsal striatum. It appears as a means of controlling distant synapses by activated ones since astrocytes are interconnected by gap junctions, permeable to calcium and IP3, both involved in the propagation of intercellular calcium waves.

Impairment of working memory is one of the most important deleterious effects of *Cannabis* intoxication in humans, but its underlying mechanisms are unclear. It has been demonstrated that the impairment of spatial working memory (SWM) and in vivo long-term depression (LTD) of synaptic strength at hippocampal CA3-CA1 synapses, induced by acute exposure to exogenous cannabinoids, is fully abolished in conditional mutant mice lacking type-1 cannabinoid receptors in brain astroglial cells but is conserved in mice lacking CB1R in glutamatergic or GABAergic neurons [166].

Blockade of neuronal glutamate N-methyl-D-aspartate receptors (NMDAR) and synaptic trafficking of glutamate α -amino-3-hydroxy-5-methyl-isoxazole propionic acid receptors (AMPA) also abolishes cannabinoid effects on SWM and LTD induction and expression. It was concluded that the impairment of working memory by *Cannabis* and cannabinoids is due to the activation of astroglial CB1R and is associated with astroglia-dependent hippocampal LTD in vivo.

Cannabis and α 2A-Adrenoceptor

The cannabinoid-1 receptor agonist delta-9-tetrahydrocannabinol adversely affects human working memory performance. The α 2A-adrenoceptor (AR) agonist guanfacine improves working memory performance in humans. The effects of short-term (6 days) treatment with guanfacine on the adverse cognitive effects produced by delta-9-THC were examined using a double-blind, placebo-controlled crossover design [154].

The cognitive, subjective, and cardiovascular effects produced by oral delta-9-THC (20 mg) administration were determined twice in the same *Cannabis* users: once after treatment with placebo and once with guanfacine (3 mg/day). Compared

with a performance at baseline, delta-9-THC negatively affected the accuracy on spatial working memory trials while participants were maintained on placebo ($p = 0.012$) but not guanfacine ($p = 0.497$); compared with placebo, accuracy was significantly ($p = 0.003$, Cohen's $d = -0.640$) improved in individuals treated with guanfacine.

Similarly, compared with baseline, delta-9-THC increased omission errors on an attentional task while participants were maintained on placebo ($p = 0.017$) but not on guanfacine ($p = 0.709$); compared with placebo, there were significantly ($p = 0.034$, Cohen's $d = 0.838$) fewer omissions while individuals were maintained on guanfacine. Although delta-9-THC increased visual analog scores of subjective effects and heart rate, the increases were similar during treatment with placebo and guanfacine. Delta-9-THC did not significantly affect the performance of a recognition memory task or blood pressure while individuals were on either treatment. Although preliminary, the results suggest that guanfacine warrants further testing as a potential treatment for *Cannabis*-induced cognitive deficits.

***Cannabis* and Catechol-O-Methyl Transferase**

The catechol-O-methyl transferase enzyme has been implicated in determining dopaminergic tone and the effects of delta-9-tetrahydrocannabinol in the human brain. A study was designed to evaluate the effect of a functional polymorphism and acute pharmacological inhibition of COMT on humans' acute response to delta-9-THC [164]. In group (a), the effect of intravenous (IV) delta-9-THC (0.05 mg/kg) was investigated in 74 healthy subjects genotyped for the COMT rs4680 (Val/Met) polymorphism in a 2-test-day double-blind, randomized, placebo-controlled study. In group (b), COMT rs4680 homozygous subjects (Val/Val and Met/Met) from the group (a) received the COMT enzyme inhibitor tolcapone (200 mg) followed by IV delta-9-THC or placebo on two additional test days. Subjective, behavioral, and cognitive data were obtained periodically on each test day.

The results of individuals from group (a), Val/Val individuals, were most sensitive to delta-9-THC-induced attention and working memory deficits. In contrast, delta-9-THC's psychotomimetic and subjective effects were not influenced by the COMT genotype. In group (b), Tolcapone reduced delta-9-THC-induced working memory deficits but not delta-9-THC's psychotomimetic effects. Tolcapone and COMT genotype (met/met) were associated with an increased reported feeling of "mellow."

The interaction between COMT rs4680 polymorphisms and tolcapone on the cognitive, but not on the psychotomimetic and overall subjective effects of delta-9-THC, suggests that modulation of dopaminergic signaling may selectively influence specific cannabinoid effects in healthy individuals. The role of dopaminergic signaling in the cognitive effects of cannabinoids should be considered in drug development efforts targeting the effects.

Cannabis use can induce cognitive impairments and psychotic experiences. A functional polymorphism in the catechol-O-methyltransferase gene (Val(158)Met) appears to influence the immediate cognitive and psychotic effects of *Cannabis*, or delta-9-tetrahydrocannabinol. A study investigated the moderation of the impact of experimentally administered delta-9-THC by COMT [168].

Cognitive performance and psychotic experiences were studied in participants without a psychiatric diagnosis using a between-subjects design (delta-9-THC vs. placebo). The effect of COMT Val(158)Met genotype on the cognitive and psychotic effects of delta-9-THC, administered intravenously in a double-blind, placebo-controlled manner to 78 participants who were vulnerable to paranoia, was examined.

The results showed interactive effects of genotype and drug group (delta-9-THC or placebo) on working memory, assayed using the Digit Span Backwards task. Specifically, delta-9-THC impaired performance in COMT Val/Val, but not Met, carriers. In contrast, the effect of delta-9-THC on psychotic experiences, measured using the Community Assessment of Psychic Experiences positive dimension, was unaffected by the COMT genotype. The data suggest that the COMT genotype moderates the cognitive, but not the psychotic, effects of acutely administered delta-9-THC.

***Cannabis* and Cyclooxygenase-2**

The CB1 cannabinoid receptor is targeted in the brain by endocannabinoids under physiological conditions and delta-9-tetrahydrocannabinol under *Cannabis* use; its signaling appears to affect cognitive brain processing. The crucial role of cyclooxygenase-2 (COX-2) in the mechanism of intraneuronal CB1 signaling transduction has been highlighted in recent studies. In contrast, it has been indicated that two single-nucleotide polymorphisms (SNPs) (rs1406977 and rs20417) modulate the expression of CB1 (CNR1) and COX-2 (PTGS2) coding genes, respectively. Functional MRI was used to investigate whether the SNPs interact in modulating prefrontal activity during working memory processing in healthy individuals and if this modulation is linked to *Cannabis* use [47].

Two hundred and forty-two healthy subjects were genotyped for CNR1 rs1406977 and PTGS2 rs20417 then performed the N-back working memory tasks during fMRI and interviewed using the *Cannabis* Experience Questionnaire. The interaction between CNR1 rs1406977 and PTGS2 rs20417 is associated with dorso-lateral prefrontal cortex activity such that specific genotype configurations (CNR1 C carriers/PTGS2 C carriers and CNR1 TT/PTGS2 GG) predict lower cortical response versus others despite similar behavioral accuracy.

Furthermore, DLPFC activity in the cluster associated with the CNR1 by PTGS2 interaction was negatively correlated with behavioral efficiency and positively correlated with the frequency of *Cannabis* use in *Cannabis* users. The results suggest that a genetically modulated balancing of signaling within the CB1-COX-2

pathway may reflect on more or less efficient patterns of prefrontal activity during working memory. The frequency of *Cannabis* use may be a factor for further modulation of CNR1/PTGS2-mediated cortical processing associated with the cognitive process.

Neurobiological Alterations During Pregnancy and Breastfeeding

An important concern is an increase in *Cannabis* use among pregnant and breast-feeding women, especially since the content of delta-9-tetrahydrocannabinol is currently around two-fold higher than it was 5–10 years ago. A study reviewed *Cannabis* use during pregnancy and breastfeeding, including epidemiological aspects, therapeutic or preventive strategies, and experimental considerations [5].

The results from animal models of perinatal *Cannabis* exposure analyzed the underlying neurobiological mechanisms and identified new therapeutic approaches. Several reports revealed that perinatal *Cannabis* exposure was associated with low birth weight, reduction in head circumference, cognitive deficits (attention, learning, and memory), and disturbances in emotional response leading to aggressiveness, high impulsivity, or affective disorders, and higher risk to develop a substance use disorder. One report revealed that among pregnant women aged 15–44, *Cannabis* use prevalence was over 4.9%, rising to 8.5% in the 18–25-year-old age ranges. Pre- and postnatal exposure to *Cannabis* may be associated with critical alterations in newborn infants that are prolonged throughout childhood and adolescence.

Furthermore, important neurobiological alterations in different neuromodulatory and neurotransmission systems have been associated with *Cannabis* consumption during pregnancy and lactation. Despite the evidence pointing out the negative behavioral and neurobiological consequences of *Cannabis* use in pregnant and breastfeeding women, there are still limitations to identifying biomarkers that could help to establish preventive or therapeutic approaches. It is difficult to define the direct association with *Cannabis*, avoiding other confounding factors, co-occurrence of other drugs consumption (mainly nicotine and alcohol), lifestyle, or socioeconomic factors.

Cannabis impairs memory in the short run, which becomes more significant with chronic use, and may also be accompanied by poorer effort, slower processing, and impacted attention. It is generally believed that long-term use and earlier age are risk factors for neurocognitive deficits. Neuroimaging studies have shown reduced hippocampal volume and density. Executive functions and memory are worse in adolescent users versus adults. *Cannabis* addiction is different and likely less common than other addictive substances, but up to 10% of users meet the criteria for lifetime *Cannabis* dependence. Addiction patterns may be linked to genetic and epigenetic differences. It is still unclear whether abstinence reverses patterns of addiction. It is becoming clear that use at earlier ages carries a higher risk for long-term deficits.

The association between developmental patterns of *Cannabis* use and adult working memory processes was examined in 75 adults with longitudinal assessments of *Cannabis* use (60 with reported use, 15 with no reported use) and prenatal drug exposure assessment completed a spatial WM task during fMRI at age 28 [21]. All subjects passed a multidrug urine screen on the day of testing and denied recreational drug use in the past week. A fast event-related design with partial trials was used to separate the WM task's BOLD response associated with encoding, maintenance, and retrieval periods.

Behavioral results indicated that the subjects who began using *Cannabis* earlier in adolescence had longer reaction times (RT) than those with later initiation. *Cannabis* age of onset was further associated with the reduced posterior parietal cortex (PPC) encoding BOLD activation, which significantly mediated age of onset WM RT associations. However, *Cannabis* age-of-onset brain-behavior associations did not differ between groups with single reported use and those with repeated use, suggesting the age-of-onset effects may reflect substance use risk characteristics rather than a developmentally-timed *Cannabis* exposure effect.

Within repeated *Cannabis* users, greater levels of total *Cannabis* use were associated with performance-related increases in dorsolateral prefrontal cortex activation during maintenance. This pattern of significant results remained unchanged with the inclusion of demographic and prenatal measures as covariates. However, at the group level, *Cannabis* users generally performed better than participants who reported never using *Cannabis* (faster RT, higher accuracy). The previous investigations were extended by identifying that WM associations with *Cannabis* age of onset may be primary to PPC stimulus encoding activity. At the same time, the amount of *Cannabis* use is associated with DLPFC maintenance processes. The poor performance of participants who reported never using *Cannabis* and the consistency of *Cannabis* age of onset associations across single and repeated users limit the interpretation of direct developmental effects of *Cannabis* on WM in adulthood.

Neurobehavioral and cognitive impairments have been reported in longitudinal studies on children and adolescents prenatally exposed to *Cannabis*, and a link to psychiatric disorders has been indicated. Interestingly, the deleterious effects of prenatal *Cannabis* use are similar to those observed in adult rats prenatally exposed to (L)-kynurenine, the direct bioprecursor of the neuroactive metabolite kynurenic acid (KYNA). An investigation was conducted into whether alterations in KYNA levels in the rat brain might play a role in the long-term consequences of prenatal cannabinoid exposure [130].

The extracellular levels of KYNA and glutamate were determined by in vivo microdialysis in the medial prefrontal cortex of adult animals. Pregnant Wistar rats were treated daily with delta-9-THC [5 mg/kg, p.o.] from gestational day 5 (GD5) through 20 (GD20). Compared to controls, extracellular basal KYNA levels were higher, and basal glutamate levels were lower in prenatally delta-9-THC-exposed rats.

The rats also showed abnormal short-term memory. Following an additional acute challenge with a low dose of kynurenine (5 mg/kg i.p.) in adulthood, the increase in extracellular KYNA levels in the mPFC was more pronounced in

prenatally delta-9-THC-exposed rats. The effects could be related to the cognitive dysfunction seen in prenatally delta-9-THC-exposed rats. In the translational realm, the experiments raise the prospect of preventing KYNA neosynthesis as a promising approach to combat some of the detrimental long-term effects of prenatal *Cannabis* use.

A prospective study examined the effects of prenatal *Cannabis* exposure on the intelligence test performance of 648 children at a 6-year follow-up. Women were interviewed about the amount and frequency of their *Cannabis* use at four and 7 months of pregnancy and delivery [162]. The women were light to moderate users of *Cannabis*. Children were assessed with the Stanford–Binet Intelligence Scale by examiners blind to exposure status. Multiple regressions were applied to examine the effects of prenatal *Cannabis* exposure on children’s intelligence after partialing out the effects of other significant predictors.

There was a significant nonlinear relationship between *Cannabis* exposure and children’s intelligence. Heavy *Cannabis* use (one or more cigarettes per day) during the first trimester was associated with lower verbal reasoning scores on the Stanford–Binet Intelligence Scale. Heavy use during the second trimester predicted composite, short-term memory, and quantitative scores deficits. Third-trimester heavy use was negatively associated with the quantitative score. Other significant predictors of intelligence included maternal IQ, home environment, and social support. The findings indicate that prenatal *Cannabis* exposure significantly affects school-age intellectual development.

The endocannabinoid system has been suggested as a therapeutic target for treating stress- and anxiety-related disorders. Cannabinoids may cause cognitive deficits and exacerbate the effects of early life stress. Early life stress significantly increases predisposition to psychopathologies. A study was conducted to determine if Cannabinoids administration during late adolescence, ages 18–25, could reverse the long-term adverse effects of early life stress on neurocognitive function in adulthood.

Male and female rats were exposed to early life stress during postnatal days P7 to 14, then injected with the cannabinoid CB1/2 receptor agonist WIN55212–2 (WIN; 1.2 mg/kg, i.p.) for 2 weeks during late adolescence (P45 to 60) and tested in adulthood (P90) for working memory, anxiety, and alterations in CB1 receptors (CB1r) and glucocorticoid receptors (GRs) in the stress circuit [hippocampus, prefrontal cortex (PFC), and basolateral amygdala (BLA)]. Early life stress males and females exhibited impaired performance in short-term memory in adulthood in the spatial location and social recognition tasks; males were also impaired in the novel object recognition task [131].

***Cannabis* in Adolescence**

This view is not surprising since the brain, once thought to be fully developed by puberty, actually undergoes critical neurodevelopment throughout adolescence and into at least the mid-to-late twenties, rendering youth more vulnerable to the

negative neural effects associated with *Cannabis* use [109]. Accordingly, the age of onset of *Cannabis* use is a critical variable that must be included in *Cannabis*-related research. The factor may help explain some of the inconsistencies observed across investigations. Some research suggests that increased *Cannabis* use may be a trait characteristic of early-onset users, rendering the individuals even more vulnerable to the negative effects of *Cannabis* use [73].

Working memory is an executive function that develops largely during adolescence. It has been shown that executive function is more impaired in adolescent frequent *Cannabis* users than in adult frequent *Cannabis* users. Analyses examining the age of onset of use revealed that while early onset users (those who initiated regular use prior to age 16) displayed more focal patterns of activation relative to later onset users (regular use after age 16), they tended to make more errors on the task, suggestive of possible neural adaptation despite difficulty with behavioral inhibition [78]. Similar behavioral performance and magnitude of brain activation during the MSIT were observed among *Cannabis* users and healthy controls; however, there was greater functional connectivity between the PFC and occipitoparietal cortex in *Cannabis* users and found that the magnitude of connectivity was positively correlated with age of onset of use.

Some investigations have also utilized go/no-go paradigms to examine response inhibition among *Cannabis*-using adolescents [79]. Similar performance was observed between abstinent *Cannabis* users and healthy controls; however, brain activation patterns were notably different. The *Cannabis* group demonstrated increased activity in frontal, parietal, and occipital areas during inhibition (no-go) trials and poorer task performance among *Cannabis* users and healthy controls, but no differences were detected using ROI analyses [80].

Research efforts have been on the effects of *Cannabis* on adolescents, either by directly assessing adolescents or by examining individuals with the onset of regular *Cannabis* use during adolescence. Although studies suggest that the neurobiological effects are similar in adult and adolescent *Cannabis* users, those who use *Cannabis* during adolescence are more likely to exhibit cognitive alterations [108]. Deficits are more likely to persist in adolescent users and adults with adolescent onset.

Long-lasting cognitive impairment is one of the major negative consequences of exposure to *Cannabis* during adolescence, particularly to delta-9-tetrahydrocannabinol. A study was conducted to compare the protracted effects of adolescent versus late-adolescent chronic exposure to delta-9-THC on short-term memory and plasticity and to examine whether rapamycin, a blocker of the mammalian target of rapamycin (mTOR) pathway, can restore delta-9-THC-induced deficits in memory and plasticity [29].

Male rats were injected with ascending doses of delta-9-THC [2.5, 5, 10 mg/kg; intraperitoneally during adolescence and late-adolescence (postnatal days 30–41 and 45–56, respectively), followed by daily injections of rapamycin (1 mg/kg, i.p.) during the first 10 days of cessation from delta-9-THC. Thirty days after the last injection, the rats were tested for short-term and working memory, anxiety-like behavior, and plasticity in the pathways projecting from the ventral subiculum

(vSub) of the hippocampus to the prefrontal cortex (PFC) and nucleus accumbens (NAc).

Delta-9-THC exposure in adolescence, but not late adolescence, induced long-term deficits in object recognition, short-term memory, and synaptic plasticity in the hippocampal-accumbens pathway. Rapamycin rescued the persistent effects of delta-9-THC administered during adolescence. The findings indicated that some forms of memory and plasticity are sensitive to chronic delta-9-THC administration during adolescence. Rapamycin administered during delta-9-THC cessation may restore cognitive function and plasticity, thus protecting against the possible long-term harmful effects of delta-9-THC.

Convergent findings indicate that *Cannabis* use and variation in the cannabinoid CB1 receptor coding gene (CNR1) modulate prefrontal function during working memory (WM). Other results also suggest that *Cannabis* modifies the physiological relationship between genetically induced expression of CNR1 and prefrontal WM processing. *Cannabis* may exert its modifying effect on prefrontal physiology by interacting with complex molecular ensembles coregulated with CB1. Since coregulated genes are likely coexpressed, genetically predicted coexpression of a molecular network, including CNR1 interaction with *Cannabis* use in modulating WM processing in humans, was investigated [30].

Using postmortem human prefrontal data, a polygenic score (CNR1-PCI) was computed, combining the effects of single-nucleotide polymorphisms (SNPs) on the coexpression of a cohesive gene set CNR1, and positively correlated with such coexpression. Then, in an in vivo study, CNR1-PCI in 88 *Cannabis* users and 147 nonusers, the interaction with *Cannabis* use on brain activity during WM was investigated.

The results revealed an interaction between *Cannabis* use and CNR1-PCI in the dorsolateral prefrontal cortex (DLPFC), with a positive relationship between CNR1-PCI and DLPFC activity in *Cannabis* users and a negative relationship in nonusers. DLPFC activity in *Cannabis* users was positively correlated with the frequency of *Cannabis* use. The results suggest that coexpression of a CNR1-related network predicts WM-related prefrontal activation as a function of *Cannabis* use.

Cannabis affects cognitive performance by activating the endocannabinoid system. Using the novel object-recognition memory test in mice, it was revealed that the main psychoactive component of *Cannabis*, delta-9-tetrahydrocannabinol, alters short-term object-recognition memory specifically involving protein kinase C (PKC)-dependent signaling [32]. The systemic or intrahippocampal pretreatment term memory did not modify such a short-term cognitive deficit. In contrast, systemic pretreatment with mammalian target of rapamycin complex 1 inhibitors, known to block the amnesic-like effects of delta-9-THC on long-term memory, did not modify such a short-term cognitive deficit.

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isoforms expressed in the hippocampus except for PKC. Moreover, delta-9-THC transiently enhanced the phosphorylation of the postsynaptic calmodulin-binding protein neurogranin in a PKC-dependent manner. The data demonstrate that delta-9-THC alters short-term object-recognition memory through hippocampal PKC/neurogranin signaling.

The use of *Cannabis* can impair cognitive function, especially short-term memory. A controversial question is whether long-term *Cannabis* use during the late-adolescence period can cause irreversible deficits in higher brain function that persist after drug use stops. To examine the short- and long-term effects of chronic exposure to cannabinoids, rats were administered chronic treatment with the CB1/CB2 receptor agonist WIN55212-2 (WIN; 1.2 mg/kg) for 2 weeks during the late adolescence period (postnatal days 45–60) and tested for behavioral and electrophysiological measures of cognitive performance 24 h, 10 and 30 days after the last drug injection [31].

The impairing effects of chronic WIN on short-term memory in the water maze and the object recognition tasks, as well as long-term potentiation (LTP) in the ventral subiculum (vSub)-nucleus accumbens (NAc) pathway, were temporary as they lasted only 24 h or 10 days after withdrawal. However, chronic WIN significantly impaired hippocampal-dependent short-term memory measured in the object location task 24 h, 10, 30, and 75 days after the last drug injection. The findings suggest that some forms of hippocampal-dependent short-term memory are sensitive to chronic cannabinoid administration. However, other cognitive impairments are temporary and probably result from a residue of cannabinoids in the brain or acute withdrawal effects from cannabinoids.

Early-onset *Cannabis* use has been associated with later use/abuse, mental health problems, abnormal cognition, and brain function development. During adolescence, ongoing neurodevelopmental maturation and experience shape the neural circuitry underlying complex cognitive functions such as memory and executive control. Prefrontal and temporal regions are critically involved in those functions. Maturation processes leave those brain areas prone to the potentially harmful effects of *Cannabis* use.

A two-site (the United States and The Netherlands; pooled data) functional magnetic resonance imaging (fMRI) study with a cross-sectional design was performed to investigate the effects of adolescent *Cannabis* use on working memory and associative memory (AM) brain function in 21 abstinent but frequent *Cannabis*-using boys (13–19) years of age and compared them with 24 nonusing peers [43]. Brain activity during WM was assessed before and after rule-based learning (automatization). AM was assessed using a pictorial hippocampal-dependent memory task. *Cannabis* users performed normally on both memory tasks. During the WM assessment, the *Cannabis* users showed excessive activity in the prefrontal regions when a task was novel. In contrast, automatization of the task reduced activity to the same level in users and controls. No effect of *Cannabis* use on AM-related brain function was found.

In adolescent *Cannabis* users, the WM system was overactive during a novel task, suggesting functional compensation. Inefficient WM recruitment was not

related to a failure in automatization but became evident when processing continuously changing information. The results confirm the vulnerability of developing frontal lobe functioning for early-onset *Cannabis* use.

A study investigated the acute and long-term behavioral effects of the cannabinoid agonist WIN-55212-2 (WIN) in pubertal and adult rats [133]. Chronic WIN (1.2 mg/kg)/vehicle treatment was extended over 25 days throughout puberty, from postnatal day (pd) 40–65, and for a similar period in adult rats (> pd. eighty). All animals were tested at three-time points for object/social recognition memory, social interaction, and spontaneous social behavior. First, acute cannabinoid effects were investigated directly after the first injection. Additionally, behavioral performance was retested 24 h and 15 days after cessation of WIN treatment.

Chronic pubertal WIN treatment induced persistent object/social recognition deficits, indicating a general impairment in short-term information processing. Lasting disturbances in social behavior, social play, and self-grooming were also found. After acute WIN administration, behavioral deficits were more pronounced in pubertal than in adult rats. The results confirmed that chronic pubertal cannabinoid treatment leads to lasting behavioral alterations in adulthood. They show that acute cannabinoid administration induces more severe behavioral deficits in pubertal rats than in mature animals.

A longitudinal study was conducted to investigate bidirectional associations between adolescent *Cannabis* use (CU) and neurocognitive performance in a community sample of 294 young men aged 13–20 [134]. The results indicated that in early adolescence and prior to initiation to CU, poor short-term and working memory but high verbal IQ were associated with earlier onset of CU. In turn, the age of CU onset and CU frequency across adolescence were associated with (a) a specific neurocognitive decline in verbal IQ and executive function tasks tapping trial-and-error learning and reward processing by early adulthood and (b) lower rates of high-school graduation.

However, the association between CU onset and change in neurocognitive function was accounted for by CU frequency. Whereas the link between CU frequency across adolescence and change in verbal IQ was explained (mediated) by high school graduation, the link between CU frequency and tasks tapping trial and error learning was independent of high school graduation, concurrent *Cannabis*, and other substance use, adolescent alcohol use, and externalizing behaviors. The findings support prevention efforts to delay the onset and reduce the frequency of CU.

Verbal memory difficulties are the most widely reported and persistent cognitive deficit associated with early-onset *Cannabis* use. Nevertheless, it is not known what memory stages are most impaired in those early *Cannabis* use. Forty-eight young adults, aged 18–25, who used *Cannabis* at least once per week and 48 matched nonusing controls completed the California Verbal Learning Test, Second Edition (CVLT-II) [157]. The age of initial use stratified *Cannabis* users: early onset users, who started using *Cannabis* at or before age 16 ($n = 27$), and late-onset *Cannabis* user group, who started using *Cannabis* after age 16 ($n = 21$). Outcome variables included immediate trial recall, total learning, clustering strategies (semantic

clustering, serial clustering, ratio of semantic to serial clustering, and the total number of strategies used), delayed recall, and percent retention.

Learning improved with repetition, with no group effect on the learning slope. Learning improved with greater semantic versus serial encoding but did not vary between groups. Early-onset users learned fewer words than the late-onset *Cannabis* user group or controls. There was no difference between the late-onset *Cannabis* user group and controls in the number of words learned. Reduced overall learning mediated the effect on reduced delayed recall among early-onset users but not controls or the late-onset *Cannabis* user group. Early onset users were not related to delayed recall after adjusting for encoding. Young adults reporting early-onset *Cannabis* use had learning weaknesses, which accounted for the association between early-onset *Cannabis* use and delayed recall. No amnesic effect of *Cannabis* use was observed.

***Cannabis* and Schizophrenia**

There is dissent in medicine about the positive and negative effects of *Cannabis*, and recently, a large research effort has been directed to that domain. The main influencing drug ingredient is delta-9-THC, which acts on the cannabinoid system and binds to the CB1 receptor. The discovery of the receptor led to an endogenous ligand, anandamide, and another receptor-CB2. The researchers discovered that cannabinoids have extensive biological activity, and their short and long-term effects may cause cognitive and emotional deficiencies. Findings show that the short-term effects, such as short-term memory and verbal Learning, are reversible [27].

However, despite the accumulation of evidence about long-term cognitive damage due to *Cannabis* use, it is difficult to find unequivocal results arising from many variables, such as large differences between *Cannabis* users, frequency of use, dosage, and endogenous brain compensation. Besides cognitive damage, current studies investigate how *Cannabis* affects mental illness. A high correlation between *Cannabis* use and schizophrenia was found, as a high risk of undergoing a psychotic attack. Patients with schizophrenia who used *Cannabis* showed a selective neuropsychological disruption, and similar cognitive deficiencies and brain morphological changes were found among healthy *Cannabis* users and schizophrenia patients.

There is substantial evidence supporting a relationship between *Cannabis* use and schizophrenia [24]. To further investigate the relationship, fMRIs were obtained from patients with schizophrenia and bipolar disorder. One subset of patients had a history of *Cannabis* use, while the other subset contained controls with either schizophrenia or bipolar disorder but without previous *Cannabis* use. When controlling for tobacco use and alcohol use disorders, it was determined that the use of *Cannabis* prior to the onset of illness was associated with cortical thinning in the caudal middle frontal gyrus. No structural brain changes associated with concomitant *Cannabis* use were identified in patients with schizophrenia or bipolar disorder. Interestingly, individuals with psychosis have been shown to have reduced gray

matter density compared to healthy controls. This effect disappeared when the psychosis group was stratified by adolescent *Cannabis* use [25].

Cannabis use and the administration of delta-9-tetrahydrocannabinol have been associated with both acute and long-term deficits in working memory. The effects appear to be related to the disruption of synaptic synchrony within the cortico-basalganglio-thalamic circuits that are part of a broader network subserving WM [34]. The circuitry includes the striatum, globus pallidus, and thalamus, their reciprocal connections to the dorsolateral prefrontal cortex, and densely expresses *Cannabis* type 1 receptors. A study was conducted to determine whether a remote *Cannabis* use disorder was associated with morphological differences in the basalganglio-thalamic circuit and whether such differences were associated with WM deficits and a history of *Cannabis* use [33].

Large-deformation high-dimensional brain mapping with magnetic resonance imaging was used to obtain surface-based representations of the striatum, globus pallidus, and thalamus, compared across groups and correlated with WM and CUD history. Groups were matched on demographics that include 44 healthy controls, 10 subjects with a CUD history, 28 schizophrenia subjects with no history of substance use disorders, and 15 schizophrenia subjects with a CUD history. Surface maps were generated to visualize morphological differences. There were significant *Cannabis*-related parametric decreases in WM across groups.

Similar *Cannabis*-related shape differences were observed in the striatum, globus pallidus, and thalamus in controls and schizophrenia subjects. *Cannabis*-related striatal and thalamic shape differences correlated with poorer WM and younger age of CUD onset in both groups. Schizophrenia subjects demonstrated *Cannabis*-related neuroanatomical differences that were consistent and exaggerated compared with *Cannabis*-related differences found in controls. The cross-sectional results suggest that both CUD groups characterized WM deficits and subcortical neuroanatomical differences.

Over the past several decades, the evidence indicated that exocannabinoids and endocannabinoids play a role in the pathophysiology of schizophrenia. The evidence is that one of the mechanisms whereby cannabinoids induce psychosis is through the alteration in synchronized neural oscillations. Neural oscillations, particularly in the gamma (30–80 Hz) and theta (4–7 Hz) ranges, are disrupted in schizophrenia and are involved in various areas of perceptual and cognitive function [138].

Preclinical evidence from slice and local field potential recordings has shown that central cannabinoid receptor (cannabinoid receptor type 1) agonists decrease the power of neural oscillations, particularly in the gamma and theta bands. Further, the administration of cannabinoids during critical stages of neural development has been shown to disrupt the brain's ability to generate synchronized neural oscillations in adulthood.

In humans, studies examining the effects of chronic *Cannabis* use (utilizing electroencephalography) have shown abnormalities in neural oscillations in a pattern similar to those observed in schizophrenia. Finally, recent human studies have also shown disruptions in neural oscillations after the acute administration of

delta-9-tetrahydrocannabinol. The data suggest that both acute and chronic cannabinoids can disrupt the ability of the brain to generate synchronized oscillations at functionally relevant frequencies. Hence, this may represent one of the primary mechanisms whereby cannabinoids induce disruptions in attention, working memory, sensory-motor integration, and many other psychosis-related behavioral effects.

Cannabidiol exhibits antipsychotic and neuroprotective properties. Cognitive impairment is a major disability in schizophrenia, and antipsychotic drugs (APDs) have minimal efficacy for this symptom domain. The effects of CBD have been reported on cognition in male offspring of a maternal immune activation (polyinosinic-polycytidylic acid (poly I:C)) model relevant to the etiology of schizophrenia. Gender differences are observed in the onset of schizophrenia symptoms and response to APD treatment. Furthermore, the endogenous cannabinoid system is sexually dimorphic in humans and rodents.

A study assesses the therapeutic impact of CBD treatment on behavior and neurochemical signaling markers in female poly I:C offspring [143]. Time-mated pregnant Sprague-Dawley rats ($n = 16$) were administered poly I:C (4 mg/kg; i.v.) or saline (control) on gestational day 15. From postnatal day 56, female offspring received CBD (10 mg/kg, i.p.) or vehicle treatment for approximately 3 weeks. After 2 weeks of CBD treatment, the offspring underwent behavioral testing, including novel object recognition, rewarded alternation T-maze, and social interaction tests to assess recognition memory, working memory, and sociability.

After 3 weeks of CBD treatment, the prefrontal cortex (PFC) and hippocampus (HPC) were collected to assess the effects on endocannabinoid, glutamatergic and gamma-aminobutyric acid (GABA) signaling markers. CBD attenuated poly I:C-induced deficits in recognition memory, social interaction, and glutamatergic N-methyl-d-aspartate receptor binding in the PFC of poly I:C offspring. Working memory performance was similar between treatment groups. CBD also increased glutamate decarboxylase, the rate-limiting enzyme that converts glutamate to GABA, and parvalbumin protein levels in the HPC [63].

In contrast to the CBD treatment effects observed in poly I:C offspring, CBD administration to control rats reduced social interaction, the cannabinoid CB1 receptor, and NMDAR binding density in the PFC, suggesting that CBD administration to healthy rats may have negative consequences on social behavior and brain maturation in adulthood. Overall, the study's findings support the therapeutic benefits of CBD on recognition memory and sociability in female poly I:C offspring and provide insight into the neurochemical changes that may underlie the therapeutic benefits of CBD in the poly I:C model.

Cannabis use and the dopamine receptor (DRD2) gene have been associated with schizophrenia, psychosis-like experiences, and cognition. A case-control study of 272 patients with their first episode of psychosis and 234 controls and a sample of 252 healthy participants were genotyped for functional variation in DRD2, rs1076560. Data on the history of *Cannabis* use were collected on all the participants by administering the *Cannabis* Experience Questionnaire [148]. Data on schizotypy and cognitive performance in the healthy subjects' sample using the

Schizotypal Personality Questionnaire and the N-back working memory task was examined.

A significant interaction was found between the rs1076560 DRD2 genotype and *Cannabis* use in influencing the likelihood of a psychotic disorder. Among *Cannabis* users, carriers of the DRD2, rs1076560, T allele showed a three-fold increased probability of suffering a psychotic disorder compared with GG carriers (OR = 3.07; 95% confidence interval [CI]: 1.22–7.63). Among daily users, T-carrying subjects showed a five-fold increase in the odds of psychosis compared to GG carriers (OR = 4.82; 95% CI: 1.39–16.71). Among the healthy subjects, T-carrying *Cannabis* users had increased schizotypy compared with T-carrying *Cannabis*-naïve subjects, GG *Cannabis* users, and GG *Cannabis*-naïve subjects ($p \leq 0.025$). T-carrying *Cannabis* users had reduced working memory accuracy compared with the other groups ($p \leq 0.008$). Thus, variation of the DRD2, rs1076560 genotype may modulate the psychosis-inducing effect of *Cannabis* use.

Neuropsychiatric disorders such as schizophrenia are associated with cognitive impairment, including learning, memory, and attention deficits. Cannabidiol has anti-inflammatory, neuroprotective, and antipsychotic-like properties. However, its ability to improve the cognitive deficits of schizophrenia remains unclear. A prenatal infection model was used to examine the effect of chronic CBD treatment on cognition and social interaction.

Time-mated pregnant Sprague-Dawley rats ($n = 16$) were administered polyinosinic-polycytidylic acid (poly I:C) (POLY; 4 mg/kg) or saline (CONT) at gestation day 15. Male offspring (PN56) were injected twice daily with 10 mg/kg CBD (CONT+CBD, POLY+CBD; $n = 12$ per group) or vehicle (VEH; CONT+VEH, POLY+VEH; $n = 12$ per group) for 3 weeks. Body weight, food, and water intake were measured weekly [145]. The Novel Object Recognition and rewarded T-maze alternation tests assessed recognition and working memory, respectively, and the social interaction test assessed sociability.

POLY+VEH offspring exhibited impaired recognition and working memory and reduced social interaction compared to CONT+VEH offspring ($p < 0.01$). CBD treatment significantly improved recognition, working memory, and social interaction deficits in the poly I:C model ($p < 0.01$ vs. POLY+VEH), did not affect total body weight gain, food or water intake, and had no effect in control animals ($p > 0.05$). Chronic CBD administration can attenuate the social interaction and cognitive deficits induced by prenatal poly I:C infection. The findings present implications for the potential use of CBD in treating cognitive deficits and social withdrawal in schizophrenia.

Studies have shown that *Cannabis* may disrupt glutamate (Glu), signaling a depressing Glu tone in frequent users. Evidence also consistently revealed lower Glu levels in various brain regions, particularly in the medial prefrontal cortex (mPFC) of chronic schizophrenia patients. Since *Cannabis* may alter Glu synaptic plasticity and its use is a known risk factor for psychosis, studies focusing on Glu signaling in EP with or without a concomitant *Cannabis*-usage seem crucial.

Magnetic resonance spectroscopy was used to measure the effect of *Cannabis* use on prefrontal Glu-levels in EP users vs. both EP nonusers and healthy controls

(HC); [GlumPFC] of 35 EP subjects (18 of whom were *Cannabis* users) and 33 HC [146]. For correlative analysis, neuropsychological performances were scored by the MATRICS-consensus cognitive battery. [GlumPFC] was lower in EP users compared to both HC and EP nonusers ($p < 0.001$ and $p = 0.01$, respectively), while no differences were observed between EP nonusers and HC. A greater [GlumPFC]-decline with age was observed in EP users ($r = -0.46$; $p = 0.04$) but not in EP nonusers or HC.

Among neuropsychological outcomes, working memory was the only domain differentiating patients depending on their *Cannabis* use, with users having poorer performances. *Cannabis* use is associated with reduced prefrontal [GlumPFC] and a stronger Glu-levels decline with age. Glutamatergic abnormalities might influence the cognitive impairment observed in users and have some relevance to the progression of the disease.

Cannabis abuse in adolescence is associated with an increased risk of psychotic disorders. Disrupted-In-Schizophrenia-1 (DISC1) protein is a driver for major mental illness by influencing neurodevelopmental processes. Utilizing a unique mouse model based on host (DISC1) X environment (delta-9-THC administration) interaction, the pathobiological basis for delta-9-THC exposure eliciting psychiatric manifestations was studied.

Wild-type and dominant-negative-DISC1 (DN-DISC1) mice were injected with delta-9-THC (10 mg/kg) or vehicle for 10 days during the mid-adolescence-equivalent period. Behavioral tests were conducted to assess exploratory activity (open field test, light-dark box test) and cognitive function (novel object recognition test). The electrophysiological effect of delta-9-THC was evaluated using acute hippocampal slices, and hippocampal cannabinoid receptor type 1 and brain-derived neurotrophic factor protein levels were measured.

The results indicated that delta-9-THC exposure elicits deficits in exploratory activity and recognition memory, with reduced short-term synaptic facilitation and loss of BDNF surge in the hippocampus of DN-DISC mice but not in wild-type mice. Over-expression of BDNF in the hippocampus of delta-9-THC-treated DN-DISC1 mice prevented the impairment in recognition memory.

The results indicated that induction of BDNF following adolescent delta-9-THC exposure may serve as a homeostatic response to maintain proper cognitive function against exogenous insult. The BDNF surge in response to delta-9-THC is perturbed in the presence of mutant DISC1, suggesting DISC1 may be a useful probe to identify biological cascades involved in the neurochemical, electrophysiological, and behavioral effects of *Cannabis*-related psychiatric manifestations.

Cannabis use is prevalent among people with schizophrenia and, coupled with impaired cognition, is thought to heighten the risk. However, while heavy *Cannabis* use has been associated with cognitive deficits in long-term users, studies among patients with schizophrenia have been contradictory. A meta-analysis of 10 studies comprising 572 patients with established schizophrenia (with and without comorbid *Cannabis* use) was conducted [161]. Patients with a history of *Cannabis* use were found to have superior neuropsychological functioning. This finding was largely

driven by studies that included patients with a lifetime history of *Cannabis* use rather than current or recent use.

A second study examined the neuropsychological performance of 85 patients with first-episode psychosis (FEP) and 43 healthy nonusing controls. Relative to controls, FEP patients with a history of *Cannabis* use (FEP + CANN; $n = 59$) displayed only selective neuropsychological impairments, while those without a history (FEP–CANN; $n = 26$) displayed generalized deficits. When directly compared, FEP + CANN patients performed better on tests of visual memory, working memory, and executive functioning.

Patients with early-onset *Cannabis* use had less neuropsychological impairment than patients with later onset. Together, the findings suggest that patients with schizophrenia or FEP with a history of *Cannabis* use have superior neuropsychological functioning compared with nonusing patients. This association between better cognitive performance and *Cannabis* use in schizophrenia may be driven by a subgroup of “neurocognitively less impaired” patients who only developed psychosis after a relatively early initiation into *Cannabis* use.

***Cannabis* and Bipolar Disorder**

This view is not surprising since the brain, once thought to be fully developed by puberty, actually undergoes critical neurodevelopment throughout adolescence and into at least the mid-to-late twenties, rendering youth more vulnerable to the negative neural effects associated with *Cannabis* use [109]. Accordingly, the age of onset of *Cannabis* use is a critical variable that must be included in *Cannabis*-related research. The factor may help explain some of the inconsistencies observed across investigations. Some research suggests that increased *Cannabis* use may be a trait characteristic of early-onset users, rendering the individuals even more vulnerable to the negative effects of *Cannabis* use [73].

Working memory is an executive function that develops largely during adolescence. It has been shown that executive function is more impaired in adolescent frequent *Cannabis* users than in adult frequent *Cannabis* users. Analyses examining the age of onset of use revealed that while early onset users (those who initiated regular use prior to age 16) displayed more focal patterns of activation relative to later onset users (regular use after age 16), they tended to make more errors on the task, suggestive of possible neural adaptation despite difficulty with behavioral inhibition [78]. Similar behavioral performance and magnitude of brain activation during the MSIT were observed among *Cannabis* users and healthy controls; however, there was greater functional connectivity between the PFC and occipitoparietal cortex in *Cannabis* users and found that the magnitude of connectivity was positively correlated with age of onset of use.

Some investigations have also utilized go/no-go paradigms to examine response inhibition among *Cannabis*-using adolescents [79]. Similar performance was observed between abstinent *Cannabis* users and healthy controls; however, brain

activation patterns were notably different. The *Cannabis* group demonstrated increased activity in frontal, parietal, and occipital areas during inhibition (no-go) trials and poorer task performance among *Cannabis* users and healthy controls, but no differences were detected using ROI analyses [80].

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Convergent findings indicate that *Cannabis* use and variation in the cannabinoid CB1 receptor coding gene (CNR1) modulate prefrontal function during working memory. Other results also suggest that *Cannabis* modifies the physiological relationship between genetically-induced expression of CNR1 and prefrontal WM processing. *Cannabis* may exert its modifying effect on prefrontal physiology by interacting with complex molecular ensembles coregulated with CB1. Since coregulated genes are likely coexpressed, genetically predicted coexpression of a molecular network, including CNR1 interaction with *Cannabis* use in modulating WM processing in humans, was investigated [30].

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The results revealed an interaction between *Cannabis* use and CNR1-PCI in the dorsolateral prefrontal cortex, with a positive relationship between CNR1-PCI and DLPFC activity in *Cannabis* users and a negative relationship in nonusers. DLPFC activity in *Cannabis* users was positively correlated with the frequency of *Cannabis* use. The results suggest that coexpression of a CNR1-related network predicts WM-related prefrontal activation as a function of *Cannabis* use.

Cannabis affects cognitive performance by activating the endocannabinoid system. Using the novel object-recognition memory test in mice, it was revealed that the main psychoactive component of *Cannabis*, delta-9-tetrahydrocannabinol, alters short-term object-recognition memory specifically involving protein kinase C (PKC)-dependent signaling [32]. The systemic or intrahippocampal pretreatment term memory did not modify such a short-term cognitive deficit. In contrast, systemic pretreatment with mammalian target of rapamycin complex 1 inhibitors, known to block the amnesic-like effects of delta-9-THC on long-term memory, did not modify such a short-term cognitive deficit.

Immunoblot analysis revealed a transient increase in PKC signaling activity in the hippocampus after delta-9-THC treatment. Thus, delta-9-THC administration induced the phosphorylation of a specific Ser residue in the hydrophobic motif at the C-terminal tail of several PKC isoforms. The significant immunoreactive band paralleled cognitive performance and did not match size with the major PKC isoforms expressed in the hippocampus except for PKC. Moreover, delta-9-THC transiently enhanced the phosphorylation of the postsynaptic calmodulin-binding protein neurogranin in a PKC-dependent manner. The data demonstrate that delta-9-THC alters short-term object-recognition memory through hippocampal PKC/neurogranin signaling.

The use of *Cannabis* can impair cognitive function, especially short-term memory. A controversial question is whether long-term *Cannabis* use during the late-adolescence period can cause irreversible deficits in higher brain function that persist after drug use stops. To examine the short- and long-term effects of chronic exposure to cannabinoids, rats were administered chronic treatment with the CB1/CB2 receptor agonist WIN55212-2 (WIN; 1.2 mg/kg) for 2 weeks during the late adolescence period (postnatal days 45–60) and tested for behavioral and electrophysiological measures of cognitive performance 24 h, 10 and 30 days after the last drug injection [31].

The impairing effects of chronic WIN on short-term memory in the water maze and the object recognition tasks, as well as long-term potentiation (LTP) in the ventral subiculum (vSub)-nucleus accumbens pathway, were temporary as they lasted only 24 h or 10 days after withdrawal. However, chronic WIN significantly impaired hippocampal-dependent short-term memory measured in the object location task 24 h, 10, 30, and 75 days after the last drug injection. The findings suggest that some forms of hippocampal-dependent short-term memory are sensitive to chronic cannabinoid administration. However, other cognitive impairments are temporary and

probably result from a residue of cannabinoids in the brain or acute withdrawal effects from cannabinoids.

Early-onset *Cannabis* use has been associated with later use/abuse, mental health problems, abnormal cognition, and brain function development. During adolescence, ongoing neurodevelopmental maturation and experience shape the neural circuitry underlying complex cognitive functions such as memory and executive control. Prefrontal and temporal regions are critically involved in those functions. Maturation processes leave those brain areas prone to the potentially harmful effects of *Cannabis* use.

A study compared clinical and neurocognitive measures in individuals with bipolar disorder with a history of *Cannabis* use disorder versus those without CUD. The retrospective analysis of a large cohort ($n = 200$) of bipolar I subjects, either with (CUD+, $n = 50$) or without (CUD-, $n = 150$) history of CUD. The groups were compared on clinical and demographic variables and performance on neurocognitive tests.

Patient groups did not differ regarding age, age of onset, or global assessment of functioning. Compared to the CUD+ group, the CUD- group had a higher proportion of men and a higher proportion of patients with a history of psychosis. CUD+ subjects demonstrated significantly better performance on attention, processing speed, and working memory measures. The history of CUD is associated with a history of psychosis, suggestive of a poorer clinical prognosis. Bipolar patients with a history of CUD had better neurocognitive performance than patients without CUD.

Cannabis use is associated with several psychiatric disorders; however, the causal nature of the associations has been difficult to establish. Mendelian randomization (MR) offers a way to infer causality between exposures with known genetic predictors (genome-wide significant single-nucleotide polymorphisms [SNPs]) and outcomes of interest [197]. MR has previously been applied to investigate the relationship between lifetime *Cannabis* use (having ever used *Cannabis*) and schizophrenia, depression, and attention deficit hyperactivity disorder (ADHD), but not bipolar disorder, representing a gap in the literature. A two-sample bidirectional MR study was conducted on the relationship between bipolar disorder and lifetime *Cannabis* use. Genetic instruments (SNPs) were obtained from recent large genome-wide association studies (GWAS) summary statistics.

We conducted a two-sample bidirectional MR study on the relationship between bipolar disorder and lifetime *Cannabis* use using inverse variance-weighted regression, weighted median regression, and Egger regression. Genetic liability to bipolar disorder was significantly associated with an increased risk of lifetime *Cannabis* use; however, genetic liability to lifetime *Cannabis* use showed no association with the risk of bipolar disorder. The sensitivity analyses showed no evidence of pleiotropic effects. The present findings support a causal effect of liability to bipolar disorder on the risk of using *Cannabis* at least once. No evidence was found for a causal effect of liability to *Cannabis* use on the risk of bipolar disorder. The findings add important new knowledge to understanding the complex relationship between *Cannabis* use and psychiatric disorders.

***Cannabis* in Alzheimer's and Parkinson's Diseases**

Legalization of the medical use of *Cannabis* for Parkinson's disease has bypassed the traditional drug-approval process, leaving physicians with little evidence to guide patients. A study was conducted to obtain data on *Cannabis*-related prescribing practices and views regarding the potential risks and benefits of *Cannabis* among experts caring for patients with PD. [137] An anonymous 73-item survey was conducted online and included neurologists at all National Parkinson Foundation Centers of Excellence.

Fifty-six responders represented centers across five countries and 14 states of America. Twenty-three percent reported some formal education on *Cannabis*. Eighty percent of responders had patients with PD who used *Cannabis*, and 95% were asked to prescribe it. Fifty-two percent took a neutral position on *Cannabis* use with their patients, 9% discouraged use, and 39% encouraged it. Most believed that the literature supported use of *Cannabis* for nausea (87%, $n = 48$), anxiety (60%, $n = 33$), and pain (86%, $n = 47$), but responses were divided concerning motor symptoms. Most respondents expected that *Cannabis* would worsen motivation (59%, $n = 32$), sleepiness (60%, $n = 31$), and hallucinations (69%, $n = 37$). In addition, the most feared negative effects on short-term memory (75%, $n = 42$), long-term memory (55%, $n = 31$), executive functioning (79%, $n = 44$), and driving (96%, $n = 54$). Although many did not believe that *Cannabis* should be recreational (50%, $n = 28$), most believed it should be legalized for medicinal purposes (69.6%, $n = 39$). The study provides data on expert PD physicians' *Cannabis*-related practices, beliefs, and attitudes. A lack of consensus likely reflects a general knowledge gap and a paucity of data to guide clinical practice.

A generally undesired effect of *Cannabis* smoking is a reversible disruption of short-term memory induced by delta-9-tetrahydrocannabinol. The paradigm has been challenged by scientists showing that delta-9-THC can improve neurological function in older animals when chronically administered at low concentrations. Moreover, studies demonstrated that delta-9-THC paradoxically promotes hippocampal neurogenesis, prevents neurodegenerative processes in animal models of Alzheimer's, protects from inflammation-induced cognitive damage, and restores memory and cognitive function in older mice [4].

In Alzheimer's, β -amyloid accumulation reduces hippocampal LTP and prevents eCB-mediated disinhibition and EPSP-to-spike potentiation in vitro. The phenomenon could contribute to memory deficits. Knocking out CB1R in an Alzheimer's mouse model worsens learning impairments, while treatments with an eCB-reuptake inhibitor or exogenous cannabinoids improve memory. In Parkinsonian rodents, striatal homosynaptic eCB-LTP is prevented ex vivo and can be rescued by Levodopa. The globus pallidus exhibits a reduced GABAergic transmission, which is reversed by the coactivation of D2R and CB1R.

At the goldfish Mauthner cell, sustained activity at excitatory synapses triggers 2-AG release, activates CB1R on nearby dopaminergic fibers, and promotes an increased dopamine release [196]. In turn, dopamine acts back via a

D1/5R-mediated pkA signaling, which induces LTP at electrical and glutamatergic chemical synapses.

The relationship between dopamine and eCB signaling has been extensively documented for eCB-LTD. The evidence reveals a close link between dopamine and eCB-LTP. In the globus pallidus, eCB-mediated i-LTD is switched to i-LTP upon D2R activation [183]. Striatal homosynaptic eCB-LTP is prevented when STDP pairings are applied simultaneously to opto-inhibition of nigrostriatal dopaminergic neurons and depends on presynaptic D2R located on cortical afferents, whose activity level shapes the expression domain of eCB-LTP and eCB-LTD. [184]

Cannabis and Alcohol Consumption

Cannabis and alcohol use is prevalent in adolescence, but the long-term behavioral effects of this use remain largely unexplored. A study investigated the effects of adolescent alcohol and delta-9-tetrahydrocannabinol vapor coexposure on cognitive- and reward-related behaviors [136]. Male Sprague-Dawley rats received vaporized delta-9-THC (10 mg vaporized delta-9-THC/four adolescent rats) or vehicle every other day (from the postnatal day (PND) 28–42) and continuous voluntary access to ethanol (10% volume/volume) in adolescence. Alcohol intake was measured during the exposure period to assess the acute effects of delta-9-THC on alcohol consumption. In adulthood (PND 56+), the rats underwent behavioral testing. Adolescent rats showed higher alcohol preference, assessed using the two-bottle choice test on days when they were not exposed to delta-9-THC vapor.

In adulthood, rats that drank alcohol as adolescents exhibited short-term memory deficits and decreased alcohol preference; on the other hand, rats exposed to delta-9-THC vapor showed learning impairments in the delay-discounting task. Vaporized delta-9-THC, alcohol, or their combination did not affect anxiety-like behaviors in adulthood. The results revealed that although adolescent delta-9-THC exposure acutely affects alcohol drinking, adolescent alcohol and *Cannabis* use may not produce long-term additive effects.

The study of the neuropsychological deficits associated with substance abuse has become highly relevant in recent years due to the serious impact they have on the physical and mental health of users. The possible memory deficits and deterioration of executive functions were studied in a sample of 44 participants undergoing drug detoxification and rehabilitation [140]. Neuropsychological tests were applied (Wechsler Memory Scale, Wisconsin Card Sorting Test, Stroop Test, Verbal Fluency Test, and the Trail-Making Test) to participants with a more prolonged history of alcohol and *Cannabis* use had a greater deficit in working memory. Those with prolonged *Cannabis* use also showed greater deficiencies in immediate or short-term memory and better conserved long-term memory, as well as less interference capacity, i.e., less inhibition of automatic responses. They also had impaired alternating attention and needed more time to execute activities that required logical and sequential thought. The study reflected the importance of duration of use as a

significant variable in the increase in memory deficits. The duration and type of substance abuse are determinants of drug-induced cerebral deterioration.

Oleylethanolamide (OEA) is an endogenous NAE that modulates ethanol-seeking behavior and ethanol-induced neuroinflammation. A study analyzed the role of OEA in hippocampal neurogenesis, BDNF-ERK signaling, and spatial memory affected by alcohol. The effects of OEA on the association of alcohol and *Cannabis*, a frequent combination in human alcohol addicts, and whose long-term effects are far from being understood were also examined [155]. OEA (10 mg/kg/day, i.p.) was administered for 5 days/week in a preclinical model of adolescent rats with binge-like consumption (1 day/week) of ethanol (3 g/kg, i.g.) combined or not with acute administrations of delta-9-THC (5 mg/kg, i.p.) for 5 weeks.

OEA restored ethanol/delta-9-THC-related decreases in both short-term spatial memory (spontaneous alternation by Y-maze) and circulating levels of BDNF, reduced cell proliferation (*Mki67* and IdU+ cells) and maturation (*Dcx*, *Calb1*), and improved cell survival (*Casp3* and BrdU+ cells) in the dorsal hippocampus. Oleylethanolamide alone or combined with delta-9-THC also decreased the mRNA levels of neurotrophic factors (*Bdnf*, *Ntf3*) and the NT3 receptor *TrkC* but increased the BDNF receptor *TrkB* in the hippocampus of ethanol-exposed rats. The effects were likely associated with OEA-specific phosphorylation of AKT and ERK1, key cell proliferation, and survival signaling regulators. The results indicated a regulatory role of OEA in short-term spatial memory and hippocampal neurogenesis through BDNF/AKT/ERK1 signaling in response to acute delta-9-THC in an alcoholic context during adolescence.

Evidence of a link between alcohol use and memory impairments in adolescents and young adults is largely correlational. A study examined the associations between drinking and episodic memory and if they were consistent with the causal effect of drinking or accounted for by familial factors confounding such associations [45]. Because *Cannabis* use is associated with a similar performance pattern on episodic memory measures, it was assessed to determine if any association might be attributable to concurrent *Cannabis* use.

An observational study of individuals aged 20–29, comprising two independent population-based cohorts of twins, participated in both cohorts and assessed from 11 ($n = 1251$) under the auspices of the Minnesota Center for Twin and Family Research. A cotwin–control design permitted an estimate of alcohol exposure effects free of shared genetic and environmental confounding influences. Significant associations were followed-up with twin-difference analyses. Propensity scores derived from measures collected at age 11 were used to adjust for unshared confounders. Regression analyses with cumulative alcohol use as the predictor of interest. Multiple measures of attention, learning, and memory from a widely used episodic memory task constituted dependent variables.

Drinking was associated with poorer attention ($p \leq 0.003$) and learning ($p \leq 0.008$). The results were similar across the two cohorts. The within-pair effect in twin-difference analyses was significant only for measures of learning ($p \leq 0.004$). The results were not due to measured unshared confounders or *Cannabis* use. Drinking in adolescence (to age 20) and early adulthood (between 20 and 29)

exerted independent effects on learning. There is a robust and specific association between alcohol consumption and learning that can be reproduced across cohorts. It is not easily accounted for by confounding factors or concurrent *Cannabis* use and is consistent with the causal influence of drinking.

***Cannabis* and Tobacco Coadministration**

The effects of *Cannabis* on cognition have also been compared with those of tobacco, as the two substances are frequently coadministered during recreational use. It was determined that tobacco alone enhances working memory and may have the potential to offset the effects of *Cannabis* on delayed though not immediate verbal recall. In a specific test of memory impairment, the role of *Cannabis* in relation to false memory production was tested using the Deese/Roediger-McDermott (DRM) paradigm [12]. The performance on the DRM of intoxicated regular *Cannabis* users was compared to that of sober regular *Cannabis* users and *Cannabis*-naïve controls. False memory rates did not vary significantly across groups. However, both intoxicated and sober regular *Cannabis* users were more likely to recognize unrelated items, indicating that their memory association with previously learned words were low compared to controls; controls also demonstrated overall greater memory accuracy than both groups of *Cannabis* users.

The literature has noted that frequent *Cannabis* users (four or more days per week) exert significantly poorer effort on tests of learning and memory than controls [13]. Thus, effort performance should be controlled for studies assessing cognition in frequent *Cannabis* users. Similarly, family and background factors must be controlled for in studies of the effects of *Cannabis* on IQ and executive function, especially in adolescents [14].

Cannabis intoxication adversely affects health, yet persistent effects following short-term abstinence in long-term *Cannabis* users are unclear. A matched-subjects, cross-sectional study compared health outcomes of long-term *Cannabis* and long-term tobacco-only users relative to population norms. Nineteen long-term (mean 32.3 years of use, mean age 55.7 years), abstinent (mean 15 h) *Cannabis* users and 16 long-term tobacco users (mean 37.1 years of use, mean age 52.9 years), matched for age, educational attainment, and lifetime tobacco consumption, were compared on measures of learning and memory, response inhibition, information-processing, sustained attention, executive control, and mental and physical health.

Cannabis users exhibited poorer overall learning, delayed recall, and greater interference and forgetting than tobacco users and showed more imperfect memory than norms. Inhibition and executive control were similar between groups, but *Cannabis* users had slower reaction times during information processing and sustained attention tasks [16]. *Cannabis* users had superior health satisfaction and psychological, somatic, and general health than tobacco users. They had similar mental and physical health to norms, while tobacco users had greater stress, role limitations from emotional problems, and poorer health satisfaction. Long-term *Cannabis* users

may exhibit deficits in some cognitive domains despite short-term abstinence and may benefit from interventions to improve cognitive performance. Tobacco alone may contribute to adverse mental and physical health outcomes.

Cannabis and tobacco have contrasting cognitive effects. Smoking *Cannabis* with tobacco is prevalent and may influence cognitive and mental health outcomes. The individual and interactive effects of *Cannabis* and tobacco were evaluated in 24 nondependent *Cannabis* and tobacco smokers in a randomized, placebo-controlled, double-blind, 2 (*Cannabis*, placebo) $\times$ 2 (tobacco, placebo) crossover design [11]. Verbal memory and working memory (WM) performance, including maintenance, manipulation, attention (N-back), psychotomimetic, subjective, and cardiovascular measures, were recorded in each of the four sessions.

Cannabis alone impaired verbal memory. A priori contrasts indicated that tobacco offset the effects of *Cannabis* on delayed recall. However, this was not supported by linear mixed model analysis. *Cannabis* load-dependently impaired WM. By contrast, tobacco improved WM across all load levels. The acute psychotomimetic effects and ratings of “stoned” and “dizzy” induced by *Cannabis* were not altered by smoking. *Cannabis* and tobacco had an independent impact on increasing heart rate and interacting effects on increasing diastolic blood pressure.

Relative to placebo, acute *Cannabis* impaired verbal memory and WM. Tobacco enhanced performance on WM, independently of *Cannabis*. Moreover, it was found that smoking may offset the effects of *Cannabis* on delayed, but not immediate, verbal recall. In contrast, the psychotomimetic and subjective impact of *Cannabis* were unaffected by tobacco coadministration. By reducing the cognitive impairment from *Cannabis*, tobacco coadministration may perpetuate use despite adverse health consequences.

Medical vs. Recreational *Cannabis* Use

Smoking *Cannabis* causes working and short-term memory deficits, which are mediated by cannabinoid receptor activation in the brain. While this may be due to the main psychoactive constituent delta-9-tetrahydrocannabinol, plant extracts also contain other cannabinoid and terpenoid compounds with unknown properties. Toward this end, it has been shown that high concentrations of plant extract rich in cannabidiol can reverse working memory deficits induced by delta-9-THC, a remaining extract contaminant, since the effect was dose-dependent and indicative of memory-enhancing qualities of the CBD-rich extract.

A wider investigation was conducted into the effects of CBD on other forms of amnesia to determine the mechanism of action and reveal its potency against anticholinergic and antiglutamatergic agents. A spatial delayed matching to position task in the open-field water maze was employed. Both scopolamine (0.2 mg/kg i.p.) and dizocilpine (MK801, 0.1 mg/kg i.p.) impaired working memory at 30 s and 4 h. Two doses of CBD-rich extract (5 and 10 mg/kg), which did not affect working memory when given alone, could not reverse the deficits when coadministered with

scopolamine or MK801 [120]. The data suggest that the reversal of working memory deficits by CBD-rich extracts is specific to the cannabinoid system and does not compensate for acutely induced cholinergic or glutamatergic receptor hypoactivity.

Theoretical models of addiction suggest that a substance use disorder represents an imbalance between hypersensitive motivational processes and impaired regulatory executive functions. Working memory may be a powerful predictor of drug use and drug-related problems. Working-memory network function may be a biomarker for predicting course and treatment outcomes in *Cannabis* users.

A functional magnetic resonance imaging study was conducted to assess the predictive power of working-memory network function for future *Cannabis* use and *Cannabis*-related problem severity in heavy *Cannabis* users. Tensor-independent component analysis was used to investigate differences in working-memory network function between 32 heavy *Cannabis* users and 41 nonusing controls during an N-back working memory task. The associations between working-memory network function and *Cannabis* use and problem severity at baseline and 6-month follow-up were examined.

Behavioral performance and working-memory network function did not significantly differ between heavy *Cannabis* users and controls. However, among heavy *Cannabis* users, individual differences in working-memory network response had an independent effect on change in weekly *Cannabis* use 6 months later ($\Delta R(2) = 0.11$, $p = 0.006$, $f(2) = 0.37$) beyond baseline *Cannabis* use ($\Delta R(2) = 0.41$) and a behavioral measure of approach bias ($\Delta R(2) = 0.18$): a stronger network response during the N-back task was related to an increase in weekly *Cannabis* use [121]. The findings revealed that heavy *Cannabis* users requiring greater effort to complete an N-back working-memory task accurately have a higher probability of escalating *Cannabis* use.

Cannabinoid signaling is involved in different brain functions, and it is mediated by the cannabinoid receptor 1 (CNR1), encoded by the CNR1 gene. The evidence suggests an association between cognition and *Cannabis* use. The logical interaction between genetically determined cannabinoid signaling and *Cannabis* use has not been determined. Therefore, a study examined whether CNR1 variation predicts CNR1 prefrontal mRNA expression in postmortem prefrontal human tissue. Then, whether functional variation in CNR1 and *Cannabis* exposure interact in modulating prefrontal function and related behavior during working memory processing was explored.

Two hundred and eight healthy subjects (113 males) were genotyped for the relevant functional SNP and were evaluated for *Cannabis* use by the *Cannabis* Experience Questionnaire. All individuals performed the 2-back working memory task during functional magnetic resonance imaging. CNR1 rs1406977 was associated with prefrontal mRNA, and individuals carrying a G allele had reduced CNR1 prefrontal mRNA levels compared with AA subjects. Functional connectivity MRI demonstrated that G carriers who were also *Cannabis* users had greater functional connectivity in the left ventrolateral prefrontal cortex and reduced working memory behavioral accuracy during the 2-back task compared with the other groups [122].

The results indicate that the deleterious effects of *Cannabis* use are more evident on a specific genetic background related to its receptor expression.

Deficient executive functions play an important role in the development of addiction. Working memory may therefore be a predictor of the course of drug use, but chronic substance use may also impair working memory. A 3-year longitudinal neuroimaging study investigates the relationship between substance use (e.g., alcohol, *Cannabis*, nicotine, illegal psychotropic drugs) and working-memory network function over time in heavy *Cannabis* users and controls [90].

Tensor-independent component analysis (Tensor-ICA) was used to investigate individual differences in working-memory network functionality over time. Forty-nine participants performed an n-back working-memory task at baseline and three-year follow-up. At follow-up, there were 22 current heavy *Cannabis* users, four abstinent heavy *Cannabis* users, and 23 non-*Cannabis*-using controls. Within the group of *Cannabis* users, *Cannabis*-related problems remained stable, whereas alcohol-related problems, nicotine dependence, and illegal psychotropic substance use increased over time.

Behavioral performance and network functionality during the n-back task did not differ between heavy *Cannabis* users and controls. Although n-back accuracy improved, the working-memory network function remained stable over time. Within the group of *Cannabis* users, working-memory network functionality was not associated with substance use. Moreover, baseline network functionality did not predict *Cannabis* use and related problems 3 years later. The results suggest that sustained moderate to heavy levels of *Cannabis*, nicotine, alcohol, and illegal psychotropic substance use does not change working-memory network functionality.

Preclinical and human studies suggest that adolescent *Cannabis* use may be associated with worse cognitive outcomes than adult *Cannabis* use. It has been hypothesized that user status would be negatively associated with cognitive function, and the relationship would be stronger in adolescents than adults. The associations between chronic *Cannabis* use and cognitive function in adolescent and adult *Cannabis* users and controls were investigated [123].

As part of the “CannTeen” project, the cross-sectional study assessed cognitive performance in adolescent *Cannabis* users ($n = 76$; 16–17-year-olds), adolescent controls ($n = 63$), adult *Cannabis* users ($n = 71$; 26–29-year-olds) and adult controls ($n = 64$). Users used *Cannabis* one to 7 days a week. Adolescent and adult *Cannabis* users were matched on *Cannabis* use frequency (4 days/week) and time since last use (2.5 days). Verbal episodic memory (VEM) was assessed using the prose recall task, spatial working memory (SWM) was assessed using the spatial n-back task, and response inhibition was assessed with the stop-signal task. Primary outcome variables were: delayed recall, 3-back discriminability, and stop signal reaction time, respectively. Users had worse VEM than controls ($F(1,268) = 7.423, p = 0.007$).

There were no significant differences between user groups on SWM or response inhibition. Bayesian analyses supported null differences. No significant interactions between the age and user groups were found for VEM, SWM, or response inhibition. Consistent with previous research, there was an association between chronic *Cannabis* use and poorer VEM, but chronic *Cannabis* use was not associated with

SWM or response inhibition. There was no evidence of heightened adolescent vulnerability to *Cannabis*-related cognitive impairment.

Cannabis negatively affects health by inducing respiratory problems and mental and cognitive alterations. Memory and learning disorders and executive dysfunctions are neuropsychological disorders associated with *Cannabis* use. Research reveals that *Cannabis* use during adolescence may disrupt the brain's normal development. In a study, a task based on a card game of four decks and different programs of gains/losses was used with 72 participants, 19 women, and 53 men [124]. The participants were selected through purposive sampling and divided into three groups: early-onset consumers, late-onset consumers, and control (nonconsumers). The task used was the "Cartas" program, a computerized version based on the Iowa Gambling Task (IGT), with two versions: direct and inverse. The computational model "Prospect Valence Learning" was applied to describe the decision according to four characteristics: utility, loss aversion, recency, and consistency.

The results revealed the worst performance of the IGT in the early-onset consumers compared to late-onset consumers and control. The magnitude of the gains-losses influenced the decision-making process of the early-onset consumers and was more determined by short-term results without loss aversion. Differences between groups were also found in the PVL computational model parameters. Early onset *Cannabis* use may involve decision-making problems. Therefore, intervention programs are necessary to reduce the prevalence and delay the onset of *Cannabis* use among teenagers.

Retrospective neuroimaging studies have suggested an association between early *Cannabis* onset and later neurocognitive impairment. The studies have limited their ability to distinguish substance use risk factors from *Cannabis*-induced effects on neurocognition. A prospective cohort study was designed to test whether neurocognitive differences preceded *Cannabis* onset (substance use risk model) and if early *Cannabis* use was associated with poorer neurocognitive development (*Cannabis* exposure model) [125]. The Participants ($n = 85$) completed a visuospatial working memory task during functional magnetic resonance imaging and multiple cognitive assessments (Wechsler Intelligence Scale for Children-IV, Cambridge Neuropsychological Test Automated Battery) at 12 years of age before any reported *Cannabis* use (baseline), and at 15 years of age (follow-up: $n = 85$ cognitive assessments, $n = 67$ neuroimaging). By follow-up, 22 participants reported using *Cannabis* and failed a delta-9-tetrahydrocannabinol urine screen (users).

At baseline, group differences supported a risk model. Those who would initiate *Cannabis* use by 15 years of age had activation differences in frontoparietal (increased) and visual association (decreased) regions and poorer executive planning scores (Stockings of Cambridge) compared with noninitiators. Limited support was found for a *Cannabis* exposure model. At follow-up, activation in the cuneus displayed a significant *Cannabis* dose-response relationship; although neither *Cannabis* dose nor cuneus activation was associated with cognitive performance.

The purported neurocognitive effects of early *Cannabis* onset may not be due to *Cannabis* initiation alone but also driven by limitations or late development of neurocognitive systems predictive of substance use. In addition, more prolonged

Cannabis exposure may be required to observe the cognitive effects of early *Cannabis* onset.

Delta-9-Tetrahydrocannabinol, a major component in *Cannabis*, causes short-term effects on the visual system, but little is known about persisting visual disturbances. A study investigated the effects of delta-9-THC's systemic administration on the retina and explored its underlying mechanisms [126].

BALB/c mice were treated with 1 or 2 mg/kg delta-9-THC intraperitoneally daily for 2 months and with a vehicle as the negative control. The retinal function was tested by electroretinography after delta-9-THC treatment. Morphological and pathological changes in the retina were detected by hematoxylin and eosin staining. Terminal deoxynucleotidyl transferase dUTP nick end labeling assay was used to detect apoptosis in photoreceptor cells. Enzyme-linked immunosorbent assay was used to show the inflammatory responses and oxidative stress. Messenger RNA and protein changes were measured by real-time polymerase chain reaction and Western blot to explore the underlying mechanisms.

The results indicated that a 2-month treatment with delta-9-THC caused retinal damage, evidenced by its functional loss and increased apoptosis in photoreceptor cells through inducing inflammatory responses and oxidative stress. The study demonstrated that systemic administration with delta-9-THC caused toxic effects on the retinas of BALB/c mice, indicating the potential mechanisms of retinal damage caused by *Cannabis* abuse.

***Cannabis* Flowers vs. Concentrate**

A cohort study with a between-groups design was conducted in a community and university setting [42]. The participants completed a baseline and an experimental mobile laboratory assessment that included three points: before, immediately after, and 1 h after ad libitum legal market flower and concentrate use. *Cannabis* flower and concentrate users were randomly assigned to higher-delta-9-THC vs. lower-delta-9-THC products within user groups. Of the 133 individuals enrolled and assessed, 55 were regular flower *Cannabis* users (41.4%), and 66 were regular concentrate *Cannabis* users (49.6%) who complied with the study's *Cannabis* use instructions and had complete data across primary outcomes.

Flower users were randomly assigned to use either 16% or 24% delta-9-THC flower, and concentrate users were randomly assigned to use either 70% or 90% delta-9-THC concentrate that they purchased from a dispensary. Primary outcome measures included plasma cannabinoids, subjective drug intoxication, and neurobehavioral tasks testing attention, memory, inhibitory control, and balance. A total of 121 participants completed the study for analysis: 55 flower users (mean [SD] age, 28.8 [8.1] years; 25 women [46%]) and 66 concentrate users (mean [SD] age, 28.3 [10.4] years; 30 women [45%]).

Compared with flower users, Concentrate users exhibited higher plasma delta-9-THC levels and 11-hydroxy-delta-9-THC across all points. After ad libitum

Cannabis administration, mean plasma delta-9-THC levels were 0.32 (SE = 0.43) $\mu\text{g/mL}$ in concentrate users and 0.14 (SE = 0.16) $\mu\text{g/mL}$ in flower users. Most neurobehavioral measures were not altered by short-term *Cannabis* consumption. However, delayed verbal memory ($F_{1,203} = 32.31$; $p < 0.001$) and balance function ($F_{1,203} = 18.88$; $p < 0.001$) were impaired after use. Differing outcomes for the product type (flower vs. concentrate) or product potency were not observed. Short-term use of concentrates was associated with higher levels of delta-9-THC exposure. Across forms of *Cannabis* and potencies, users' domains of verbal memory and proprioception-focused postural stability were primarily associated with delta-9-THC administration.

Acute Administration of Delta-9-THC vs. CBD

Given increased access to products containing CBD, particularly among medical consumers, researchers have begun to examine the effect of CBD in the presence of delta-9-THC. Overall, several investigations focused on the effects of delta-9-THC and CBD on cognition and clinical state have noted that CBD appears to have opposite effects from delta-9-THC and may even mitigate some of the adverse consequences typically associated with delta-9-THC exposure. For example, in one of the earliest studies to examine whether CBD modulates the effects of delta-9-THC, the acute administration of CBD blocked anxiety produced by the administration of THC [55]. *Cannabis* users with CBD in hair samples reported lower scores on a measure of psychosis-like symptoms [56]. Although one study did not observe the effects of CBD on psychomimetic symptoms in *Cannabis* users who provided samples of their “real world” *Cannabis* products, the authors posit this may be related to some factors, including differing *Cannabis* use patterns across studies and doses or ratios of cannabinoids.

However, in that study, the potential mitigating effects of CBD on memory were also assessed; the results indicated that during intoxication, those using low CBD *Cannabis* products (< 0.14% CBD) exhibited significant declines in memory performance, while those using high CBD *Cannabis* products (> 0.75% CBD) did not demonstrate significant performance decrements [57]. The findings suggest that higher levels of CBD may be protective against memory impairment. Another study revealed that while higher delta-9-THC levels (measured in hair) were associated with recall memory deficits and elevated levels of anxiety and depression, recognition memory was better in those with detectable levels of CBD [58]. Similarly, delta-9-THC-related psychotic symptoms, paranoia, and memory impairment were less common among those who received CBD as a pretreatment than those who received placebo pretreatment [59]. Additionally, hippocampal volumes in *Cannabis* users revealed that while those with no CBD exposure had smaller volumes than healthy controls, no differences were observed between *Cannabis* users with CBD exposure and control participants [60].

Regular *Cannabis* users treated with 200 mg of CBD daily exhibited significant volume increases in specific hippocampal subfields [61]. While some studies support mitigating the negative effects of delta-9-THC, some studies have not observed those effects. In a lab-based study that pretreated individuals with CBD and then administered whole-plant *Cannabis*, no mitigating effect of CBD was observed on subjective ratings (e.g., ratings of the “high” produced, the potency of the *Cannabis* cigarette, liking of the *Cannabis* cigarette), behavioral performance (digit symbol substitution task), or cardiovascular effects (heart rate and blood pressure) [62]. Similarly, no mitigating effects of CBD were observed on heart rate or subjective reports of feeling high in a study in which the participants smoked delta-9-THC-containing *Cannabis*, which had either high or low CBD percentages; mitigating effects were not observed on behavioral (working memory and word recognition tasks) and neurological measures (EEG and ERP) [63].

The unique effects of delta-9-THC and CBD have been explored with fMRI techniques. Using a go/no-go task to assess response inhibition, the acute impact of orally administered delta-9-THC, CBD, and placebo in healthy control subjects was examined. The task requires participants to execute a motor response for all stimuli except those displaying a specific target. It has been shown to activate prefrontal regions, including the inferior frontal gyrus, middle frontal gyrus, and anterior cingulate, in healthy controls [65].

Although performance differences were not observed between the cannabinoid or placebo conditions, fMRI results demonstrated that administration of delta-9-THC reduced activation in frontal regions [66]. In contrast, CBD reduced activation in temporal and insular regions relative to placebo, areas not typically associated with response inhibition [67].

In another study of healthy individuals with minimal previous exposure to *Cannabis*, 10 mg of oral delta-9-THC, 600 mg of CBD, or placebo were administered to participants before completing a verbal paired associate learning task [68]. During the encoding condition, the participants viewed pairs of words, while during the recall condition, they were shown one word and had to say the word previously paired with it. Although the behavioral performance was not significantly affected by delta-9-THC or CBD, fMRI results generated interesting findings.

Under placebo conditions, completion of repeated encoding trials produced a linear reduction in cortical activation in the mediotemporal cortex, specifically the parahippocampal gyrus, a region implicated in verbal information encoding. Following delta-9-THC exposure, however, completion of the task resulted in increased activation in the parahippocampal gyrus across learning trials. In addition, delta-9-THC attenuated striatal and cingulate activation patterns, normally observed during retrieval conditions in nonintoxicated individuals.

In a series of studies, delta-9-THC and CBD were administered to participants who completed various tasks. The results revealed opposite effects of delta-9-THC and CBD on the brain activation patterns in regions typically associated with each task. Delta-9-THC and CBD demonstrated opposite effects in the superior temporal cortex when the participants were presented with auditory stimuli (spoken words) and in the occipital cortex when viewing complex visual stimuli (radial

checkerboard). In the amygdala during affective processing (viewing fearful faces) [69], delta-9-THC exposure resulted in increased psychotic-like symptoms (higher Positive and Negative Syndrome Scale [PANNS] scores), elevated levels of anxiety, feelings of intoxication, and sedation; the effects were not observed after CBD administration. None of the effects were observed after acute CBD administration [70].

The functional correlates of delta-9-THC versus CBD administration were examined in healthy non-*Cannabis* users [71]. After ingesting 10 mg delta-9-THC, 600 mg CBD, or a placebo, the participants viewed fearful and neutral faces while undergoing fMRI. During placebo conditions, the processing of fearful faces was related to activation in visual, limbic, and paralimbic regions typically implicated in facial affect processing.

The results indicated that delta-9-THC and CBD impact the brain quite differently, given their distinct mechanisms of action. The results revealed that the administration of delta-9-THC increased anxiety and modulated activation in the frontal and parietal regions [72]. In contrast, the administration of CBD reduces anxiety and modulates activity in a separate group of brain regions, namely the limbic (amygdala) and paralimbic (anterior and posterior cingulate cortex) regions. CBD has demonstrated the potential to mitigate the adverse consequences of recreational *Cannabis* smokers.

Delta-9-THC vs. Delta-9-Tetrahydrocannabivarin

Delta-9-tetrahydrocannabivarin (THCV) is a cannabinoid that is claimed to be a cannabinoid receptor-neutral antagonist and may inhibit the effects of delta-9-THC. To explore the safety and tolerability of repeated THCV administration and its effects on symptoms normally induced by delta-9-THC, 10 male *Cannabis* users (< 25 use occasions) were recruited for a within-subjects, placebo-controlled, double-blind, crossover pilot study [165]. Ten mg of pure THCV or placebo were administered orally for 5 days, followed by 1 mg intravenous delta-9-THC on the 5th day.

THCV was well tolerated and subjectively indistinguishable from placebo. Delta-9-THC did not significantly increase psychotic symptoms or paranoia or impair short-term memory while producing significant intoxicating effects. The delayed verbal recall was impaired by delta-9-THC and only occurred under a placebo ($Z = -2.201$, $p = 0.028$), suggesting a protective effect of THCV. Delta-9-tetrahydrocannabivarin also inhibited delta-9-THC-induced increased heart rate ($Z = -2.193$, $p = 0.028$). Nine out of 10 participants reported delta-9-THC under THCV condition (compared to placebo) to be subjectively weaker or less intense ($\chi^2(2) = 6.4$, $p = 0.011$). In combination with delta-9-THC, THCV significantly increased memory intrusions ($Z = -2.155$, $p = 0.031$). THCV inhibited some of the well-known effects of delta-9-THC while potentiating others.

Findings on the implication of CB1 cannabinoid receptors in the behavioral effects of METH led to the hypothesis that METH-induced neurotoxicity may alter the brain expression of CB1, thereby affecting its role in behavioral functions. The theory was tested in rats subjected to a model of METH neurotoxicity (4 mg/kg, subcutaneous $\times$ four injections, at 2 h apart). Their CB1 receptor was analyzed for brain expression 3 weeks later [167].

METH exposure significantly enhanced CB1 receptor expression across several brain regions, including the prefrontal cortex, caudate-putamen, basolateral amygdala, CA1 hippocampal region, and perirhinal cortex. In parallel, a different group of METH-exposed rats was used to explore the responsiveness to the potent cannabinoid agonist WIN-55212-2 (WIN) (0.5–1 mg/kg, intraperitoneal), within several paradigms for the assessment of emotional and cognitive functions, such as open field, object explorations, and recognition, and startle reflex. WIN induced anxiolytic-like effects in METH-exposed rats and anxiogenic-like effects in saline-treated controls.

The results revealed that METH neurotoxicity might alter the vulnerability to select behavioral effects of *Cannabis* by inducing distinct regional variations in the expression of CB1 receptors. Furthermore, METH-exposed animals exhibited a significantly lower impact of WIN on the attenuation of exploratory behaviors and short-term (90 min) recognition memory. Conversely, METH neurotoxicity did not significantly affect WIN-induced locomotor activity reduction, exploration time, and acoustic startle.

Delta-8-THC vs. Delta-9-THC

An online survey was developed for delta-8-THC consumers, including qualities of delta-8-THC experiences, comparisons with delta-9-THC, and open-ended feedback [132]. The survey included quantitative and qualitative aspects to provide a detailed description and content for future hypothesis testing. A manufacturer of delta-8-THC products distributed invitations to participate via social media accounts, email contact lists, and discussion boards. The participants ($n = 521$) were mostly identified as White/European American (90%) and male (57%). Pairwise t-tests compared delta-8-THC effect rating items; one-sample t-tests examined responses to delta-9-THC comparison items.

The participants compared delta-8-THC favorably with delta-9-THC and pharmaceutical drugs, with most participants reporting substitution for delta-9-THC (57%) and pharmaceutical drugs (59%). Most delta-8-THC users experienced relaxation (71%), euphoria (68%), and pain relief (55%); a moderate amount of cognitive distortions such as difficulty concentrating (81%), difficulties with short-term memory (80%), and alerted sense of time (74%); and did not experience anxiety (74%) or paranoia (83%).

Drug Addiction

The facilitation of LTP in dopaminergic neurons is reported after exposure to cocaine, ethanol, or nicotine [195]. For prolonged cocaine exposure, such facilitation, accompanied by increased bursting of dopaminergic neurons, is likely mediated by an eCB-dependent disinhibitory feedback loop. Cocaine intake occludes ex vivo eCB-mediated i-LTD while manipulating eCBs signaling (by local application of CB1R or mGluR5 antagonists or by blocking 2-AG synthesis) and alleviates cocaine-induced reduction of inhibitory transmission. Chronic nicotine administration facilitates the induction of CB1R-mediated LTP in the bed nucleus of the stria terminalis, and this facilitation resists a long period of forced abstinence. As this area is involved in cue-induced drug-seeking, the persistent changes could be responsible for increased vulnerability to relapse.

Cannabinoids and Pain

In the spinal cord, eCBs have both anti- and pronociceptive effects through inversed plasticity mechanisms, respectively, by depressing nociceptive and disinhibiting nonnociceptive afferents. The underlying mechanism was evidenced in the semi-intact preparation of the nervous ganglia of the medicinal leech, in which eCB-mediated heterosynaptic potentiation of nonnociceptive synapses is critical to producing behavioral sensitization in response to noxious stimuli. *Cannabinoids work through the endocannabinoids system and inhibit the release of GABA and glutamate in the brain, impacting neuromodulation, dopamine, acetylcholine, and norepinephrine release and affecting reward, learning, and pain.*

Cannabis and its derivatives modulate nociceptive signals in the brain. CB2 receptors in the DRG likely affect pain integration in the afferent pathways, and peripherally CB2 also affects noradrenergic pathways influencing pain [26]. Many users may see more than 50% chronic pain alleviation compared with a placebo. *Cannabis* affects cognition, most notably executive function, memory, and attention, and may deteriorate the boundaries between emotional and executive processing.

Cannabis and Gender Differences

Preclinical and clinical studies suggest that males and females may be differentially affected by *Cannabis* use. A study evaluated the interaction of *Cannabis* use and biological gender on cognition, and the association between observed cognitive deficits and features of *Cannabis* use [37]. Cognitive measures were assessed in those with regular, ongoing, *Cannabis* use ($n = 40$; 22 female) and nonusing peers

($n = 40$; 23 female). Intelligence, psychomotor speed, and verbal working memory were measured with the Wechsler Abbreviated Scale of Intelligence, Digit Symbol Test, and Digit Span and Hopkins Verbal Learning Test, respectively. Associations between cognitive measures and *Cannabis* use features: lifetime *Cannabis* use, age of initiation, time since last use of *Cannabis*, and recent high-concentration of tetrahydrocannabinoid exposure were evaluated.

Female and male *Cannabis* use groups did not differ in features of *Cannabis* use. No significant effects of the group were observed across measures. Significant interactions between group and biological gender were observed in intelligence, psychomotor speed, and verbal learning measures. The greatest group differences were observed between males with and without regular *Cannabis* use. Psychomotor performance was negatively correlated with lifetime *Cannabis* exposure. The findings indicate that biological gender influences the relationship between *Cannabis* and cognition, with males potentially being more vulnerable to the neurocognitive deficits related to *Cannabis* use.

Adolescence into young adulthood represents a sensitive period in which brain development significantly diverges by gender, and regular *Cannabis* use by young people is associated with neuropsychological vulnerabilities. In a cross-sectional study, gender differences in multidomain neuropsychological functioning were examined using the Cambridge Neuropsychological Test Automated Battery (CANTAB) and tested whether gender moderated the relationship between cognitive performance and age of initiation, frequency of *Cannabis* use, amount of *Cannabis* use, and withdrawal symptoms in at least weekly adolescent and young adult *Cannabis* users ($n = 171$; aged 13–25 years; 46.2% female) [127].

Male *Cannabis* users had poorer visual recognition memory, and female *Cannabis* users showed worse attention and executive functions, with medium to large effect on sizes. The gender effects persisted when controlling for age, IQ, amount of alcohol and nicotine use, mood and anxiety symptoms, emotional stability, and impulsive behavior. Earlier age of initiated use and more use were associated with worse attentional functions in females but not males. More use was more strongly associated with worse episodic memory in males than in females. More use was associated with poorer learning in males only. Domain-specific patterns of neuropsychological performance were found by gender, such that males showed poorer visual memory and females showed worse performance on measures of attention (sustained visual, multitasking) and executive functioning (spatial planning/working memory subdomains).

Regions undergoing maturation with CB1 receptors may be at increased risk for *Cannabis*-induced alterations. The relationships between *Cannabis* use and prefrontal, inferior parietal gyrification, and surface area (SA) were examined in youths [128]. The participants were 33 *Cannabis* users and 35 controls (ages 18–25). Multiple regressions and Pearson correlations examined the effects of *Cannabis* use on gyrification, SA, and cognition. Exclusions included comorbid psychiatric/neurological disorders and heavy drug users.

Cannabis use was associated with decreased gyrification in: ventral-medial PFC (RH: [FDR corrected $p = 0.02$], LH: [FDR corrected $p = 0.02$]); medial PFC (RH:

[FDR corrected $p = 0.02$], LH: [FDR corrected $p = 0.02$]); and frontal poles (RH: [FDR corrected $p = 0.02$], LH: [FDR corrected $p = 0.02$]). No differences were observed in bilateral hemispheres, PFC, dorsolateral, ventrolateral, or inferior parietal ROIs. *Cannabis* use was associated with marginally decreased SA in left: medial PFC [FDR corrected $p = 0.09$] and ventral lateral PFC: [FDR corrected $p = 0.09$]. In *Cannabis* users, increased gyrification was associated with improved working-memory performance in right medial ($p = 0.003$), ventral-medial ($p = 0.03$), and frontal pole ROIs ($p = 0.007$). *Cannabis* use was associated with reduced gyrification in PFC regions implicated in self-referential thought and social cognition. The results indicated that the gyrification characteristics might have cognitive implications.

***Cannabis* and HIV**

A study examined the independent and combined effects of HIV and *Cannabis* use (no use, light use, and moderate-to-heavy use) on neurocognitive functioning among a convenience sample of HIV-positive (HIV+) and HIV-negative (HIV-) individuals recruited from HIV community care clinics in the Los Angeles, California. The participants included 89 HIV+ ($n = 55$) and HIV- ($n = 34$) individuals who were grouped into nonusers, light users, and moderate-to-heavy users based on self-reported *Cannabis* use [163]. *Cannabis* users were individuals who reported regular use of *Cannabis* for at least 12 months, with the last reported use within the past month.

The participants were administered a brief cognitive test battery and underwent laboratory testing for CD4 count and viral load. HIV+ individuals demonstrated lower performance on neurocognitive testing than controls, and moderate-to-heavy *Cannabis* users performed more poorly on neurocognitive testing than light users or nonusers. Moderate-to-heavy HIV+ users performed significantly lower on learning/memory than HIV- moderate-to-heavy users (MD = -8.34 ; 95% CI: -16.11 to -0.56) as well as all other comparison groups. In verbal fluency, HIV+ light users outperformed HIV- light users (MD = 7.28 ; 95% CI: 1.62 – 12.39), but no HIV group differences were observed at other *Cannabis* use levels. HIV+ MJ users demonstrated lower viral load (MD = -0.58 ; 95% CI: -1.30 to 0.14) and higher CD4 count than nonusers (MD = 137.67 ; 95% CI: 9.48 – 265.85).

***Cannabis* on Heart Rate and EEG**

A study examines changes in heart rate (HR) and EEG under different doses of *Cannabis* and delta-9-THC. The design differs from an earlier investigation in that (1) the participants were constantly kept alert by being involved in problem-solving tasks; (2) HR and EEG recordings were analyzed by time-locked evoked averaging

techniques; and (3) the presentation of each problem task was under computer control and depended on the participants return to the baseline level of physiological functioning.

EEG and HR patterns were significantly related to dose levels and the tasks performed. In addition, the results suggest that *Cannabis* interferes very little with cognitive functions except for impairing short-term memory at all dose levels of *Cannabis* [169]. The procedure described for measuring the participants' HR and EEG changes. At the same time, they are alert and respond to various perceptual stimuli for the analyses of the changes, offering a particularly powerful technique for looking at subtle drug treatment effects.

The Residual Impact of *Cannabis*

While numerous studies have investigated the residual effects of *Cannabis* use on human brain function, the results have been inconsistent. The effects of prolonged *Cannabis* exposure on human brain function were measured using a meta-analytic, task-based functional MRI (fMRI) across studies employing a range of cognitive activation tasks comparing regular *Cannabis* users with nonusers. Separate meta-analyses were conducted for studies investigating adult and adolescent *Cannabis* users [22]. A systematic literature search identified 20 manuscripts (13 adult and seven adolescent studies) meeting study inclusion criteria.

An adult analysis compared 530 *Cannabis* users to 580 healthy controls, while an adolescent analysis compared 219 *Cannabis* users to 224 healthy controls. In adult *Cannabis* users, brain activation was increased in the superior and posterior transverse temporal and inferior frontal gyri and decreased in the striate area, insula, and middle temporal gyrus. In adolescent *Cannabis* users, activation was increased in the inferior parietal gyrus and putamen compared to healthy controls. Functional alteration in the areas may reflect compensatory neuroadaptive changes in *Cannabis* users.

Most fMRI studies of nonintoxicated *Cannabis* users report altered brain activation patterns relative to nonusers. The alterations appear to be present across numerous brain regions and affect various cognitive domains. Although executive function and memory are considered among the most affected in *Cannabis* users, studies have also revealed alterations in attention tasks, error monitoring/awareness, and reward and emotion paradigms. Several neuroimaging studies have also assessed the residual impact of *Cannabis* by examining users who are not acutely intoxicated or high and who have had at least a brief period of abstinence from *Cannabis*, typically 12–24 h.

Resting-state fMRI has been employed to identify alterations in functional connectivity within or between brain regions following acute and chronic exposure to delta-9-tetrahydrocannabinol. Most studies focused a priori on a limited number of local brain areas or circuits without considering the impact of *Cannabis* on whole-brain network organization. A study attempted to identify changes in the

whole-brain human functional connectome as assessed with ultra-high field (7 T) resting state scans of *Cannabis* users ($n = 26$) during placebo and following vaporization of *Cannabis*.

Whole-brain network approaches identified spatial patterns in functional brain connectomes that distinguished acute from chronic *Cannabis* use and offer an important utility for probing the interplay between short and long-term alterations in functional brain dynamics when progressing from occasional to chronic use of *Cannabis* [198]. Two distinct data-driven methodologies, i.e., network-based Statistics (NBS) and connICA, were used to identify changes in functional connectomes associated with acute *Cannabis* intoxication and history of *Cannabis* use.

Both methodologies revealed a broad hyperconnectivity within the entire range of major brain networks in chronic *Cannabis* users compared to occasional *Cannabis* users, which might be reflective of an adaptive network reorganization following prolonged *Cannabis* exposure. The connICA methodology also extracted a distinct spatial connectivity pattern of hypoconnectivity involving the dorsal, limbic, subcortical, and cerebellum networks and hyperconnectivity between the default mode and ventral attention network that was associated with the feeling of subjective high during delta-9-THC intoxication.

Therapeutic Perspectives

Endocannabinoids have long been involved in several brain disorders, particularly drug addiction and pain. Most dysregulation of eCB-mediated LTP involves heterosynaptic facilitation of LTP through disinhibitory mechanisms. Acute and chronic stress reduced anandamide levels and modified 2-AG signaling and CB1R expression, leading to persistent changes in eCB-mediated plasticity expression and polarity *ex vivo* and *in vivo* [191].

Mimicking anandamide reduction by selectively overexpressing Fatty acid amide hydrolase at hippocampal CA3-CA1 synapses led to increased anxiety and an enhancement of LTP expression *in vitro*, while i-LTD and DSI remained unchanged [192]. Fatty acid amide hydrolase overexpression in BLA pyramidal neurons can also attenuate stress and anxiety-like behaviors; it could dry out tonic anandamide signaling at GABAergic synapses and shift the excitation/inhibition balance toward inhibition of BLA output neurons.

Evidence of elevated anandamide during extinction training corroborates the persistent facilitation of fear extinction induced by pharmacologically increasing anandamide levels in BLA, hippocampus, or mPFC and with its impairment by CB1R antagonists or FAAH overexpression [193]. While increased FAAH activity is observed in BLA and hippocampus following shock exposure, local application of FAAH inhibitor renormalizes stress-induced plasticity changes, reallowing CA1-CA3 LTP expression while causing a decrease of BLA LTP *in vivo*.

The manipulations operated immediately or 24 h after a situational reminder of fear-conditioning persistently attenuated fear expression in mice. Enhanced BLA

i-LTD was also reported after local administration of FAAH inhibitor under stressed conditions and could selectively enhance neuronal excitability in specific BLA glutamatergic networks. Activation of hippocampal TRPV1, which was shown to enhance CA1 LTP via the GABAergic system in vitro, could prevent the stress-induced switch from LTP to LTD and stress-induced impairment of spatial memory retrieval [194].

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Chapter 6

Psychotropic Effects of *Cannabis*



Brain development is a complex phenomenon from fetal life to adolescence. During this period, the brain's maturation proceeds through a series of ordered events, including critical periods of plasticity. The brain is particularly sensitive to the environment during the changes. The endocannabinoid system participates directly and indirectly in the plasticity and maturation processes. The main psychoactive component of *Cannabis*, delta-9-tetrahydrocannabinol, can cross the placental barrier; it is present in breast milk and diffuses in the brain; it interacts with endocannabinoid signaling, especially through activating cannabinoid receptors which can lead to abnormal neurodevelopmental processes and the functions of neuronal circuits [1].

Exposure to *Cannabis* in utero, in the perinatal phase, and during adolescence disrupts brain maturation and can cause disturbances in the cognitive, psychotic, and addictive levels that persist far beyond exposure [1]. Several factors modulate the risk of such complications; studies performed in animal models and human cohorts have shown that exposure during both the critical perinatal and adolescence phases is a risk factor per se. Current knowledge encourages the dissemination of objective information to young people to prevent and limit early exposure and its consequences.

The *Cannabis* plant can synthesize more than 500 compounds, including terpenes, flavonoids, and more than 100 cannabinoids. Delta-9-tetrahydrocannabinol acts as a partial agonist at cannabinoid 1 and 2 receptors. It is responsible for the characteristic effects of *Cannabis*, such as euphoria, relaxation, and changes in perceptions. Delta-9-THC can also produce dysphoria, anxiety, and psychotic symptoms. It is used therapeutically in nausea and vomiting due to chemotherapy, as an appetite stimulant, and in chronic pain. Cannabidiol acts as a noncompetitive negative allosteric modulator of the CB1 receptor, an inverse agonist of the CB2 receptor, and an inhibitor of the reuptake of the endocannabinoid anandamide. Cannabidiol also activates 5-HT1A serotonergic receptors and vanilloid receptors. Its use in treatment-resistant epilepsy syndromes is approved in some countries. Cannabidiol does not produce the typical effects associated with delta-9-THC and has anxiolytic

and antipsychotic effects. Cannabidiol's most common adverse effects are diarrhea, somnolence, nausea, and transaminase elevations when used with antiepileptics [3].

Structural and Functional Effects of *Cannabis* on the Brain

Cannabidiol and delta-9-tetrahydrocannabinol have been the most investigated cannabinoids at the human and preclinical levels, and the neurobiological mechanisms underlying their effects remain unclear. Human experimental evidence complemented by observational studies suggests that delta-9-THC may have psychotogenic effects while CBD may have antipsychotic effects [30]. However, it remains to be seen whether their effects on brain function are consistent with their opposing behavioral effects. Neuroimaging evidence investigating the acute effects of delta-9-THC and CBD on human brain function using a range of neuroimaging techniques was conducted to identify the key brain substrates where delta-9-THC and CBD have opposing effects.

Delta-9-tetrahydrocannabinol increases brain activation and blood flow, whereas CBD decreases brain activation and blood flow. A pattern of opposite effect profiles of the two cannabinoids was evident with some degree of consistency, primarily attributed to the head-to-head challenge studies of delta-9-THC and CBD. The evidence suggests that opposite effects of delta-9-THC and CBD may be present in the striatum, parahippocampus, anterior cingulate/medial prefrontal cortex, and amygdala, with opposite effects less consistently identified in other regions. There is more clarity on the mechanisms underlying their opposite behavioral effects because it may offer insights into the underlying pathophysiological mechanisms of psychotic disorders and suggest novel targets and biomarkers for drug discovery.

Regular *Cannabis* use has been associated with various acute and chronic mental health problems, such as anxiety, depression, psychotic symptoms, and neurocognitive impairments. A review summarizes and critically evaluates brain-imaging studies of *Cannabis* in recreational and regular *Cannabis* users. Regular use of *Cannabis* results in volumetric gray matter and white matter structural changes in the brain, particularly in the hippocampus and the amygdala. Regular use of *Cannabis* affects cognitive processes such as attention, memory, inhibitory control, decision-making, emotional processing, social cognition, and their associated brain areas.

There is evidence that regular *Cannabis* use leads to altered neural function during attention and working memory and that recruitment of activity in additional brain regions can compensate for it. Similar to other drugs of abuse, *Cannabis* cues activated areas in the reward pathway. Pharmacological studies indicated a modest increase in human striatal dopamine transmission after administering delta-9-THC in healthy volunteers. Regular *Cannabis* use resulted in reduced dopamine transporter occupancy and reduced dopamine synthesis but not in reduced striatal D2/D3 receptor occupancy compared with healthy control participants.

Studies also indicated different effects of delta-9-tetrahydrocannabinol, and cannabidiol on emotion, cognition, and associated brain regions in healthy volunteers,

whereby CBD protects against the psychoactive effects of delta-9-THC. Brain-imaging studies using selective high-affinity radio ligands for the imaging of cannabinoid CB1 receptor availability in Positron Emission Tomography (PET) showed downregulation of CB1 in regular users of *Cannabis*. Regular use of cannabinoids exerts structural and functional changes in the human brain. The changes have profound implications for our understanding of the neuropharmacology of *Cannabis* and its effects on cognition, mental health, and the brain [32].

Acute and chronic exposures to cannabinoids exert pharmacological alterations in the mammalian brain that have profound implications for our understanding of the neuropharmacology of cannabinoid-based drugs and their effects on mental health and the brain. Recreational and chronic *Cannabis* use has been associated with various acute and chronic effects, including antinociceptive actions, anxiety, depression, psychotic symptoms, and neurocognitive impairments. The mechanisms underlying cannabinoid-based drug effects are not fully known. However, given the neuromodulatory functions of the endocannabinoid system, it seems likely that agonistic activity at the cannabinoid type-1 receptors might modulate the functions of other neurotransmitter systems.

A functional polymorphism in the catechol-o-methyltransferase gene (COMT Val(158)Met) may moderate the psychosis-inducing effects of *Cannabis*. In order to extend the finding to dynamic effects in the flow of daily life, a momentary assessment study of psychotic symptoms in response to *Cannabis* use was conducted [107]. The experience sampling technique was used to collect data on *Cannabis* use and the occurrence of symptoms in daily life in patients with a psychotic disorder ($n = 31$) and healthy controls ($n = 25$).

Carriers of the COMT Val(158)Met Val allele, but not subjects with the Met/Met genotype, showed an increase in hallucinations after *Cannabis* exposure, conditional on prior evidence of psychometric psychosis liability. The findings confirm that in people with psychometric evidence of psychosis liability, COMT Val(158)Met genotype moderates the association between *Cannabis* and psychotic phenomena in the flow of daily life.

Behavioral and functional magnetic resonance imaging measures were recorded while they performed a go/no-go task. The sample was subdivided based on the Positive and Negative Syndrome Scale positive score following the administration of delta-9-THC into transiently psychotic ($n = 11$) and nonpsychotic ($n = 10$) groups. During the delta-9-THC condition, transiently psychotic subjects made more frequent inhibition errors than nonpsychotic subjects. They showed differential activation relative to the nonpsychotic group in the left parahippocampal gyrus, the left and right middle temporal gyri, and the right cerebellum. In those regions, delta-9-THC had opposite effects on activation relative to placebo in the two groups.

The transiently psychotic group also indicated less activation than the nonpsychotic group in the right middle temporal gyrus and cerebellum, independent of the effects of delta-9-THC. In the first demonstration of intersubject variability in sensitivity to the psychotogenic effects of delta-9-THC, it was found that the presence of acute psychotic symptoms was associated with a differential effect of

delta-9-THC on activation in the ventral and medial temporal cortex and cerebellum, suggesting that those regions mediate the effects of the drug on psychotic symptoms.

Schizophrenia is a psychiatric disorder with a high prevalence. In the temporal domain, cannabinoid receptor gene knockout mice exhibit behavioral alterations and similar symptoms in schizophrenia, *Cannabis* intoxication, and dopamine D2 activation. While a specific nucleotide homology between CB1 and D2 accounts for the pathophysiology, preinserted spirochetal DNA on the polyadenylation signal of CB1 reveals the etiology of schizophrenia.

If mutations occur within this 3' regulatory domain in analogy to thalassemia, the genetic expression of CB1 is disrupted, and sequential information is lost in time. CB1, previously unrecognized as a candidate gene, unifies the different aspects of schizophrenic psychosis: *Cannabis*-induced model psychosis, disrupted information processing, Spatiotemporal distortions and other psychotic symptoms, disturbed neuronal migration, schizophrenic brain disorder, familial transmission, and prenatal infection by *Borrelia burgdorferi* [111].

Chronic *Cannabis* use can induce psychotic states that resemble schizophrenia. Nevertheless, schizophrenic patients often smoke *Cannabis* as a form of self-medication to counter the aversive symptoms of schizophrenia. An ameliorating effect of cannabinoid self-administration (SA) has an ameliorating effect on negative and cognitive schizophrenia-like symptoms induced experimentally by the non-competitive N-methyl-D-aspartate receptor antagonist phencyclidine (PCP) been demonstrated. Whether cannabinoid SA alleviates or exacerbates schizophrenia-like positive symptoms is still unclear.

A follow-up study was conducted to evaluate the effect of self-administered cannabinoids on PCP-induced schizotypal positive symptoms in adult rats [113]. Male rats were trained to self-administer either the cannabinoid CB1 receptor agonist WIN 55,212-2 (WIN; 12.5 µg/kg/infusion) or its vehicle (Veh) intravenously. The effects of acute and chronic intermittent intraperitoneal administration of PCP (2.5 mg/kg) on motor parameters were then tested in Veh-SA and WIN-SA. Cannabinoid SA significantly attenuated the psychotomimetic effects of PCP exposure observed in control rats.

Following acute PCP administration, WIN-SA animals displayed more frequent rearing and lower anxiety-like profile than Veh-SA rats. WIN-SA rats also exhibited lower behavioral sensitization to chronic PCP treatment, as demonstrated by reduced hyperlocomotion in response to an acute PCP challenge. Additionally, parallel experiments performed in experimenter-administered rats that received WIN at comparable SA doses confirmed the ameliorating effects of cannabinoid exposure on PCP-induced schizotypal behaviors, indicating that motivational effects were not responsible for the ameliorative effects of cannabinoids. The results indicate that *Cannabis* may exert protective effects on positive schizotypal symptoms in adult animals, such as hyper-motility and anxiety.

In an examination of the alterations in dopaminergic function in schizophrenia, surreptitious case scenarios were identified that provided insights into the subjective and neurochemical effects of *Cannabis* [115]. A 38-year-old drug-free

schizophrenic patient took part in a single photon emission computerized tomographic (SPECT) study of the brain and smoked *Cannabis* secretly during a pause during an imaging session. *Cannabis* had an immediate calming effect, followed by worsening psychotic symptoms a few hours later.

Comparing the two sets of images obtained before and immediately after smoking *Cannabis* indicated a 20% decrease in the striatal dopamine D2 receptor binding ratio, suggestive of increased synaptic dopaminergic activity. This observation offers a plausible biological explanation for the psychotogenic effects of *Cannabis* in vulnerable individuals. It raises speculations about an interaction between cannabinoid and dopaminergic systems in the brain reward pathways.

Cannabinoids on Physical and Mental Health

Preclinical and clinical data on the interactions of cannabinoid types 1 and 2 receptors with the central neurotransmitters; dopamine, serotonin, noradrenaline, GABA, glutamate, and opioids raised a growing concern over the effects of cannabinoids and their interaction with other neurotransmitters on physical and mental health [33]. The emergence of *Cannabis* for medical purposes and the legalization and decriminalization of *Cannabis* increased the use of highly potent synthetic cannabinoids.

Epidemiological evidence demonstrates that *Cannabis* use is associated with an increased risk of psychotic outcomes and confirms a dose-response relationship between the level of use and the risk of later psychosis [34]. High-potency *Cannabis* and synthetic cannabinoids carry the greatest risk. Experimental administration of tetrahydrocannabinol, an active ingredient of *Cannabis*, induces transient psychosis in normal subjects, but the effect can be ameliorated by coadministration of cannabidiol. Cannabidiol is a constituent of traditional hashish but is largely absent from modern high-potency forms of *Cannabis*.

The argument continues over the extent to which genetic predisposition is correlated to, or interacts with, *Cannabis* use and what proportion of psychosis could be prevented by minimizing heavy use. There is no convincing evidence that *Cannabis* use increases the risk of other psychiatric disorders, but there are no such doubts concerning its detrimental effect on cognitive function. The negative aspects are magnified if use starts in early adolescence. Irrespective of whether *Cannabis* is decriminalized or legalized, the evidence that it is a component cause of psychosis is now sufficient for public health messages outlining the risk, especially of regular use of high-potency *Cannabis* and synthetic cannabinoids.

Cannabis, or delta-9-tetrahydrocannabinol, is associated with psychotic symptoms or disorders. The neurochemical mechanism that underlies the psychotomimetic effect is poorly understood. Although dopaminergic dysfunction is generally recognized as the final common pathway in psychosis, evidence of the effects of delta-9-tetrahydrocannabinol or *Cannabis* use on dopaminergic measures in the brain is equivocal. It is thought that *Cannabis* or delta-9-tetrahydrocannabinol may

not act on dopamine firing directly but indirectly by altering glutamate neurotransmission.

Studies examining the acute and chronic effects of *Cannabis* or delta-9-tetrahydrocannabinol on glutamate signaling in research animals and men consistently suggests that delta-9-tetrahydrocannabinol depresses glutamate synaptic transmission via CB1 receptor activation, affecting glutamate release, inhibiting receptors and transporters' function, reducing enzyme activity, and disrupting glutamate synaptic plasticity after prolonged exposure [35]. Research on humans tends to support the evidence that chronic *Cannabis* use reduces levels of glutamate-derived metabolites in both cortical and subcortical brain areas.

Cannabinoids in the Pathophysiology of Psychosis

One of the risk factors for increasing psychotic disorders is the use of *Cannabis*. It has been shown that the inactivation of dopamine and other catecholamines causes a common polymorphism generating substantial variations in COMT enzyme activity. To understand the role of *Cannabis* in the etiology of schizophrenia, with and without premorbid usage, a study was conducted with 80 male patients [75]. Genotyping of COMT enzyme Val158Met gene polymorphisms was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The Val/Val genotype was significantly higher in patients with premorbid *Cannabis* use (88.9%) than in patients without premorbid *Cannabis* use (68.4%). Also the mean total positive and negative syndrome scale (PANSS) score in the Val/Val genotype group was significantly higher than the patients with the Met allele scores. The findings confirm the association between COMT Val158Met polymorphism and premorbid *Cannabis* use in causing schizophrenia.

The endocannabinoid system is a lipid-based endogenous signaling system. Its relevance to psychosis is through the association between *Cannabis* use and the onset and course of illness and the antipsychotic properties of cannabidiol, a potential endocannabinoid system enhancer. A quantitative synthesis of the differences in blood and cerebrospinal fluid markers of the endocannabinoid system between patients and healthy controls and qualitative synthesis of the association of those markers with symptom severity, stage of illness, and responsiveness to treatment investigated the clinical relevance of the endocannabinoid system modifications in psychotic disorders.

Three individual meta-analyses were performed to identify the differences in endocannabinoid markers between people with schizophrenia and healthy controls [76]. Five studies, including 226 patients and 385 controls, reported significantly higher concentrations of anandamide in the cerebrospinal fluid of patients than in controls (standardized mean difference [SMD], 0.97; 95% CI, 0.67–1.26; $p < .001$; $I^2 = 54.8\%$). In nine studies, with 344 patients and 411 controls, significantly higher anandamide levels in blood were found in patients compared with controls (SMD, 0.55; 95% CI, 0.05–1.04; $p = .03$; $I^2 = 89.6\%$). In three studies involving 88 patients

and 179 controls, a significantly higher expression of type 1 cannabinoid receptors on peripheral immune cells was reported in patients compared with controls (SMD, 0.57; 95% CI, 0.31–0.84; $p < .001$; $I^2 = 0\%$). Higher endocannabinoid system tone was found at an early stage of illness in antipsychotic naïve or free individuals. It had an inverse association with symptom severity and was normalized after successful treatment. Moderate to a high level of heterogeneity in methods was found between studies.

Based on the contribution of the endocannabinoid system to the pathophysiology of schizophrenia, the primary propsychotic ingredient of *Cannabis*, delta-9-tetrahydrocannabinol, is used in preclinical as well as clinical research to mimic schizophrenia-like symptoms. While it is common to administer lipid-based formulations of delta-9-tetrahydrocannabinol in human studies orally, intraperitoneal injections of water-based solutions are used in animal models. Because of the poor water solubility of delta-9-tetrahydrocannabinol, a vehicle such as ethanol and emulsifiers are needed for the preparations. In order to test whether a lipid-based solvent would be superior to a water-based vehicle in rats, we compared the effects on locomotor activity and prepulse inhibition of the acoustic startle reaction, as well as pharmacokinetic data obtained from rats' serum and brain tissue samples. Up to 50 mg/kg of delta-9-tetrahydrocannabinol in the lipid-based formulation could not induce any behavioral alterations.

Analysis of serum and brain delta-9-tetrahydrocannabinol levels revealed that although the final concentration reached in the brain was comparable for both pharmaceutical preparations, the water-based formulation achieved a faster kinetic [77]. In contrast, 5 mg/kg of the water-based delta-9-tetrahydrocannabinol preparation significantly reduced locomotor activity and induced a small but significant PPI reduction, prepulse intensity dependent. The reflexive motor response to the startle stimulus was not affected by the water-based delta-9-tetrahydrocannabinol solution.

Preclinical and clinical data indicated that the cannabinoid and glutamatergic systems are implicated in the pathophysiology of schizophrenia (SZ), the prototypical psychotic disorder. This has led to distinct *Cannabis* and ketamine models of SZ. The models need not be mutually exclusive. In several brain regions implicated in the putative neural circuitry of SZ (e.g., hippocampus, frontal cortex, cerebellum), cannabinoid receptor type 1 and glutamate N-methyl-D-aspartate receptors (NMDARs) have direct and indirect interactions [45]. CB1R agonists and NMDAR antagonists act upon gamma-aminobutyric acid (GABA) interneurons to reduce GABAergic neurotransmission resulting in the unsynchronized activity of pyramidal neurons, disrupting neural network oscillations involved in information processing, thus leading to psychotomimetic effects.

Treatment studies targeting substance use among people with psychosis have reported mixed results. Substance users with psychosis in no treatment or minimal treatment control groups achieve similar reductions in substance use compared to those in more active substance use treatment, indicating a role for natural recovery from substance use. A meta-analysis quantified the amount of natural recovery from substance use within control groups of treatment containing samples of psychotic substance users, focusing on changes in *Cannabis* use [46].

The reductions in the frequency of substance use within 30 days revealed significant but modest reductions (mean reduction of 0.3–0.4 SD across the time points) in the frequency of substance use were found at 6–24 months of follow-up. The findings highlight the potential role of natural recovery from substance use among individuals with psychosis, although they do not rule out the effects of regression on the mean. Additionally, the results provide a baseline for estimating likely changes or needed effect sizes in intervention studies. Identifying the processes that underpin the changes to identify strategies would better support the self-management of substance use in people with psychosis.

Despite extensive study, available treatments for schizophrenia are only modestly effective and have serious metabolic and neurological side effects. A promising pharmacological target in the context of schizophrenia is the endocannabinoid system. Modulation of the endocannabinoid system by delta-9-tetrahydrocannabinol induces acute psychotic effects and cognitive impairment. Cannabidiol may have antipsychotic properties and thus may be a promising new agent in treating schizophrenia.

The antipsychotic properties of CBD in human subjects also indicated the ability of CBD to counteract psychotic symptoms and cognitive impairment associated with *Cannabis* use and acute delta-9-THC administration [47]. The cannabinoid may lower the risk of developing psychosis that is related to *Cannabis* use. The effects are possibly mediated by opposite effects of CBD and delta-9-THC on brain activity patterns in key regions implicated in the pathophysiology of schizophrenia, such as the striatum, hippocampus, and prefrontal cortex. The first small-scale clinical studies with CBD treatment of patients with psychotic symptoms further confirm the potential of CBD as an effective, safe, and well-tolerated antipsychotic compound.

Among the phytocannabinoids without psychotropic effects, the prevalent one in *Cannabis* is cannabidiol. Although CBD was initially considered a type 2 cannabinoid receptor antagonist, it does not produce robust cannabinoidergic activity [48]. Heterogeneous results were obtained in experimental animal models of anxiety disorders, psychotic stages, and neurodegenerative diseases. Cannabidiol has been authorized by the United States of America, Food and Drug Administration to treat some rare forms of epilepsy, and many trials have begun to treat autism spectrum disorders.

Cannabis use has been found to increase the risk of psychosis. It is unclear whether, after a first psychotic episode has occurred, continued *Cannabis* use is associated with the poor functional outcome of psychosis. As part of a randomized, open-label, controlled trial, the association of *Cannabis* use and measures for psychopathology and social role functioning after 2 years of follow-up and for the recently proposed outcome measures of symptomatic remission, functional remission, and clinical recovery was explored in a group of 124 patients who have nonaffective first-episode psychosis diagnosed according to DSM-IV and included from a catchment area in the Netherlands of 3.1 million inhabitants [49].

Continued *Cannabis* use was not associated with symptomatic or functional remission or clinical recovery. After 2 years, *Cannabis* use was related to certain

aspects of social role functioning (economic and social activities; explained variance 5.6% and 8.4%, respectively) but not to psychopathology (Positive and Negative Syndrome Scale Positive, Negative, or General Symptoms). The findings support the notion that continued *Cannabis* use after the onset of first-episode psychosis is correlated with worse social outcomes and should be discouraged whenever possible. However, its role in outcome is modest in comparison to other factors.

Twenty cases of *Cannabis* psychosis were compared with a control group of 20 patients with acute schizophrenic episode on some demographic, clinical, illness-related, and outcome variables in a case-control study designed using a retrospective chart review [70]. The two groups were comparable in demographic, past, and family histories of mental illness, premorbid personality, psychomotor activity, Schneiderian first-rank symptoms, and mild cognitive deficits. In contrast to the control group, the cases had a psychosis of shorter duration characterized by reactive and congruent affect, the relative absence of schizophrenic formal thought disorder, and a predominantly polymorphic clinical picture. Relapse was always preceded by *Cannabis* use.

The study indicated that, despite certain overlaps, “*Cannabis* psychosis” may still be considered nosologically distinct from schizophrenia. The study implies that the role of *Cannabis* in any acute psychosis should be investigated carefully to prevent overdiagnosis of schizophrenia.

Cannabis use is an important risk factor for the development of psychosis and other transition to schizophrenia. The prevalence of patients with psychosis and comorbid *Cannabis* use (dual diagnosis) is rising with no approved specialized pharmacological treatment option. Cannabidiol has potential both as an antipsychotic and as a *Cannabis* substituting agent.

Cannabis use disorders are prevalent among people with schizophrenia spectrum disorders with a range of detrimental effects, e.g., reduced compliance to medication and psychosocial interventions and increased levels of psychotic-dimension symptoms. The treatments of cannabis use disorders (CUD) in schizophrenia spectrum disorders (SSD) patients appeared effective in pharmacological interventions but lacked randomized controlled trials. Contingency management was only effective while active [83].

Psychosocial interventions, e.g., motivational interviewing and cognitive behavior therapy, were ineffective in most studies with *Cannabis* as a particular outcome but effective in studies that grouped *Cannabis* with other substance use disorders. Insufficient evidence exists on treating this form of dual-diagnosis patients. Grouping several substances as a single outcome may overlook differential effects. Randomized controlled trials should investigate psychosocial, pharmacological, and contingency management combinations.

Through the cannabinoid receptor 1, the endocannabinoid system plays a physiological role in maintaining the activity of glutamate N-methyl-D-aspartate (NMDA) receptor within harmless limits. The influence of cannabinoids must be proportional to the stimulus to prevent NMDAR over-activation or exaggerated hypofunction that may precipitate symptoms of psychosis [85]. The association of CB1s with NMDARs, which mediates the reduction of cannabinoid analgesia promoted by NMDAR antagonism, could also support the precipitation of

schizophrenia brought about by smoking *Cannabis*, mostly among vulnerable individuals. This possibility was investigated using neuroprotection and analgesia as reporters of the CB1-NMDAR connection.

It was found that the Sigma 1 receptor (σ 1R) acts as a safety switch, releasing NMDARs from the influence of CB1s and thereby avoiding glutamate hypofunction. In wild-type mice, ligands of the σ 1R did not affect the CB1-NMDAR regulatory association. In σ 1R(-/-) mice, the activity of NMDARs increases and cannot be regulated by cannabinoids, and NMDAR antagonism produces no effect on cannabinoid analgesia.

The σ 1R controls the extent of CB1-NMDAR interaction, and its failure might constitute a vulnerability factor for *Cannabis* abuse, potentially precipitating schizophrenia that might otherwise be induced later by the endogenous system. However, experimental NMDAR hypofunction enabled σ 1R antagonists to release NMDARs from the negative control of CB1s. Of the σ 1R antagonists tested, their order of activity was: S1RA > BD1047 “NE100 = BD1063”, although SKF10047, PRE-084, and (+)pentazocine were inactive yet able to abolish the effect of S1RA in this paradigm.

Clinical and preclinical evidence suggests a potential role for cannabidiol as a pharmacotherapy for various neuropsychiatric disorders. Cannabinoid type 1 receptor stimulation in the ventral hippocampus (VHipp) potentiates affective memory formation through inputs to the mesolimbic dopamine system, thereby altering emotional salience attribution. In contrast, delta-9-tetrahydrocannabinol is associated with acute and neurodevelopmental propsychotic side effects through its interaction with the central cannabinoid type 1 receptor.

Data has linked schizophrenia with neuroinflammatory conditions, and glial cells, such as microglia and astrocytes, have been related to the pathogenesis of schizophrenia. A study evaluated repeated treatment with cannabidiol (30 and 60 mg/kg) and if the treatment would attenuate the behavioral and glial changes observed in an animal model of schizophrenia based on the NMDA receptor hypofunction (chronic administration of MK-801, an NMDA receptor antagonist, for 28 days) [93]. The behavioral alterations were evaluated in the social interaction and novel object recognition (NOR) tests.

The tests have been widely used to study changes related to the negative symptoms and cognitive deficits of schizophrenia. Changes in NeuN (a neuronal marker), Iba-1 (a microglia marker), and GFAP (an astrocyte marker) expression in the medial prefrontal cortex (mPFC), dorsal striatum, nucleus accumbens core and shell, and dorsal hippocampus by immunohistochemistry were evaluated.

Cannabidiol effects were compared to those induced by the atypical antipsychotic clozapine. Repeated MK-801 administration impaired performance in the social interaction and NOR tests. It also increased the number of GFAP-positive astrocytes in the mPFC and the percentage of Iba-1-positive microglia cells with a reactive phenotype in the mPFC and dorsal hippocampus without changing the number of Iba-1-positive cells. No change in the number of NeuN-positive cells was observed.

The behavioral disruptions and the changes in expression of glial markers induced by MK-801 treatment were attenuated by repeated treatment with cannabidiol or clozapine. The data reinforces the proposal that cannabidiol may induce antipsychotic-like effects. Although the effects' possible mechanism of action is still unknown, it may involve cannabidiol's anti-inflammatory and neuroprotective properties. The data support the view that inhibiting microglial activation may improve schizophrenia symptoms.

Clinical and preclinical evidence demonstrate that distinct phytocannabinoids act within the VHipp and associated corticolimbic structures to modulate emotional memory processing through changes in mesolimbic DA activity states, salience attribution, and signal transduction pathways associated with schizophrenia-related pathology. The changes in DA activity and salience attribution, evoked by dysfunctional VHipp regulatory actions and delta-9-THC exposure, could predispose susceptible individuals to psychotic symptoms. Although delta-9-THC can accelerate the onset of schizophrenia, CBD displays antipsychotic properties that can prevent the acquisition of emotionally irrelevant memories and reverses amphetamine-induced neuronal sensitization through selective phosphorylation of the mechanistic target of the rapamycin (mTOR) molecular signaling pathway [86].

Cannabis use can induce acute psychotic symptoms and increase the risk of schizophrenia. Impairments in inhibitory control and processing are known to occur both under the influence of *Cannabis* and in schizophrenia. *Cannabis*-induced impairment in inhibitory processing is related to the acute induction of psychotic symptoms under its influence is unclear. The effects of acute oral administration of 10 mg of delta-9-THC on the inhibitory control and regional brain activation during inhibitory processing in humans were examined, and whether the effects are related to the induction of psychotic symptoms under its influence using a repeated-measures, placebo-controlled, double-blind, within-subject design [88].

Thirty-six healthy, English-speaking, right-handed men with minimal previous exposure to *Cannabis* and other illicit drugs were examined twice using functional magnetic resonance imaging. At the same time, they performed a response inhibition (Go/No-Go) task. Relative to placebo, delta-9-THC caused transient psychotic symptoms, anxiety, intoxication and sedation, inhibition errors, and impaired inhibition efficiency.

The severity of psychotic symptoms was directly correlated with inhibition error frequency and inversely with inhibition efficiency under delta-9-THC. Delta-9-THC attenuated left inferior frontal activation, which was inversely correlated with the inhibition errors and severity of psychotic symptoms and positively with inhibition efficiency under its influence. The results provide evidence that impairments in cognitive processes involved in the inhibitory control of thoughts and actions and inferior frontal function under the influence of *Cannabis* may have a role in the emergence of transient psychotic symptoms under its influence.

The medical use of cannabinoids is limited mainly by their undesirable effects. A study compared an oral *Cannabis* extract and low-dose diazepam in a crossover experiment in drug-naïve healthy women [95]. Sixteen healthy females participated in the randomized, double-blind, active comparator-controlled, single-dose,

balanced two-way crossover study. *Cannabis* extract with standardized delta-9-tetrahydrocannabinol content (20 mg) or active placebo (5 mg diazepam) was administered orally. The subjects were assessed by self- and observer-rated visual analog scales (VAS), the Brief Psychiatric Rating Scale (BPRS), and three psychomotor tests for 6 h after administration of the test agents.

Visual analog scales indicated significantly elevated fatigue, drowsiness, dizziness, and feeling high after *Cannabis* compared to baseline and diazepam. BPRS scores were significantly higher after *Cannabis* intake. A decrease in psychomotor activity after *Cannabis* administration was evident in only one psychomotor test. One participant in the *Cannabis* condition experienced severe transient psychotic symptoms. The orally administered *Cannabis* produced significant central depressant side-effects compared to diazepam, mostly subjective effects (VAS) but marginal effects on psychomotor performance in 15 healthy females. Regarding the medical use of *Cannabis*, rigorous benefit-risk analysis and a detailed psychiatric assessment before and during treatment are necessary.

The evidence supports the hypothesis that psychotic symptoms result from abnormal salience attribution and that salience attribution is largely mediated through the prefrontal cortex, the striatum, and the hippocampus [96]. Although the areas show differential activation under the influence of delta-9-tetrahydrocannabinol and cannabidiol, little is known about the effects of the cannabinoids on the functional connectivity between those regions. Healthy occasional *Cannabis* users were evaluated by employing event-related functional magnetic resonance imaging following oral administration of delta-9-THC, CBD, or a placebo capsule.

Employing a seed cluster-based functional connectivity analysis using the average time series from each seed cluster for a whole-brain correlational analysis, the effect of drug conditions on functional connectivity between the seed clusters and the rest of the brain during an oddball salience processing task was investigated. Relative to the placebo condition, delta-9-THC and CBD had opposite effects on the functional connectivity between the dorsal striatum, the prefrontal cortex, and the hippocampus.

Delta-9-THC reduced frontostriatal connectivity, related to its effect on task performance, whereas CBD enhanced the connection. Conversely, mediotemporal-prefrontal connectivity was enhanced by delta-9-THC and reduced by CBD. The results suggest that single doses of delta-9-THC and CBD modulate the functional integration of brain regions involved in salience processing and relate to salient stimuli processing.

The Endocannabinoid System and HPA Axis Regulation

Approximately one-third of patients diagnosed with schizophrenia do not achieve adequate symptom control with standard antipsychotic drugs. Some of those may prove responsive to clozapine but not responsive to Aapps, which remained an important clinical problem and increased health care costs. In many patients,

schizophrenia is associated with natural and iatrogenic metabolic abnormalities (obesity, dyslipidemia, impaired glucose tolerance, or type 2 diabetes mellitus), hyperadrenalism, and an exaggerated HPA response to stress chronic systemic inflammation.

The endocannabinoid system plays an important role in maintaining normal mental health; it modulates emotion, reward processing, sleep regulation, aversive memory extinction, and HPA axis regulation. The endocannabinoid system over activity contributes to visceral fat accumulation, insulin resistance, and impaired energy expenditure. The *Cannabis* plant synthesizes a large number of pharmacologically active compounds. In contrast to the euphoric and propsychotic effects of delta-9-tetrahydrocannabinol, nonintoxicating phytocannabinoids have emerged in preclinical and clinical models as potential APs.

The anti-inflammatory and immunomodulatory effects of the nonintoxicating cannabidiol are well established. Cannabidiol combined with a CB1 receptor-neutral antagonist augments the effects of standard APs and targets the metabolic, inflammatory, and stress-related components of the schizophrenia phenotype [51]. Since the likely mechanism of action does not rely upon dopamine D2 receptor antagonism, synergistic combinations with existing APs are plausible. Research on associations between symptoms and substance use in first-episode psychosis was conducted to test the hypotheses that the presence of positive symptoms is associated with alcohol use prior to admission, and the absence of prominent negative symptoms associated with *Cannabis* use prior to admission [52].

The participants included those admitted for a first episode of psychosis in a public-sector University-affiliated hospital that serves a predominantly socially disadvantaged, urban population. Discharge summaries of consecutively admitted patients were reviewed to extract data on basic demographic and clinical characteristics, 11 symptoms, and alcohol and *Cannabis* use within 6 months prior to hospitalization. The 72 patients included in the analysis were 18–40.

Alcohol use 6 months prior to hospitalization was associated with a higher frequency of positive psychotic symptoms among first-episode patients. *Cannabis* use was associated with a lower likelihood of having prominent negative symptoms. The associations remained even after controlling for relevant covariates in logistic regression models. Although the direction of causality cannot be established, the association between positive psychotic symptoms and alcohol use represents a self-medication effect. In contrast, the association between lesser negative symptoms and *Cannabis* use results from the fact that interpersonal deficits and reduced hedonic capacity minimize drug-seeking activities.

***Cannabis* and Auditory Evoked Mismatch Negativity**

The close relationship between *Cannabis*, the endocannabinoid and dopaminergic systems, and the onset of schizophrenic psychosis indicated that *Cannabis*-induced psychotic states might also resemble schizophrenia. Reduced amplitudes of

auditory evoked mismatch negativity (MMN) have often been found in schizophrenic patients, indicating deficient auditory information processing and working memory.

A study on the effects of *Cannabis* on MMN amplitude in 22 healthy volunteers (age 28 $\pm$ 6 years, 11 male) compared delta-9-tetrahydrocannabinol and standardized *Cannabis* extract containing delta-9-THC and cannabidiol in a prospective, double-blind, placebo-controlled crossover design [53]. The MMNs resulting from 1000 auditory stimuli were recorded by 32 channel EEG. The standard stimuli were 1000 Hz, 80 dB SPL, and 100 ms duration. The deviant stimuli differed in frequency (1500 Hz). Significantly greater MMN amplitude values at central electrodes were found under *Cannabis* extract but not under delta-9-THC.

MMN amplitudes at central electrodes were significantly correlated with 11-OH-delta-9-THC concentration, the most important psychoactive metabolite of delta-9-THC. There were no significant differences between MMN amplitudes at frontal electrodes. Since the main difference between delta-9-THC and standardized *Cannabis* extract is CBD, which seems to have neuroprotective and antipsychotic properties, it can be speculated whether the greater MMN amplitude might imply higher cortical activation and cognitive performance is related to the positive effects of CBD. The effect may be relevant for auditory cortex activity because only MMN amplitudes at the central but not at the frontal electrodes were enhanced under *Cannabis*.

Delta-9-tetrahydrocannabinol and other agonists at the central cannabinoid receptor may induce characteristic psychomotor effects, psychotic reactions, and cognitive impairment resembling schizophrenia. The effects of delta-9-THC can be reduced in animal and human models of psychopathology by two exogenous cannabinoids, cannabidiol, and SR141716. Cannabidiol is the second most abundant constituent of *Cannabis* and has weak partial antagonistic properties at the CB1 receptor.

Cannabidiol inhibits the reuptake and hydrolysis of anandamide, the most important endogenous CB1 receptor agonist, and exhibits neuroprotective antioxidant activity. SR141716 is a potent and selective CB1 receptor antagonist. Since both cannabidiol and SR141716 can reverse many of the biochemical, physiological, and behavioral effects of CB1 receptor agonists, it has been proposed that both cannabidiol and SR141716 have antipsychotic properties. Studies in animals, healthy human volunteers, and schizophrenic patients support the notion. Studies also indicated that cannabinoids such as cannabidiol and SR141716 have a pharmacological profile similar to that of atypical antipsychotic drugs [54].

***Cannabis* and Cognitive Performance**

The effects of *Cannabis* on cognitive performance in patients with SCZ have been mixed. Nearly 50% of patients with schizophrenia have co-occurring *Cannabis* use disorder, which has been associated with decreased treatment efficacy, increased

risk of psychotic relapse, and poor global functioning. A study of brain networks related to executive function may clarify the relationship between *Cannabis* use and cognition in dual-diagnosis patients.

Patients with SCZ and *Cannabis* use disorder ($n = 12$) and healthy controls ($n = 12$) completed two functional magnetic resonance imaging resting scans [55]. Before the second scan, the participants smoked a 3.6% tetrahydrocannabinol *Cannabis* cigarette or ingested a 15 mg delta-9-tetrahydrocannabinol pill. Resting-state functional connectivity was used to examine the default mode network during both scans, as connectivity/activity within this network is negatively correlated with the network involved in executive control and shows reduced activity during task performance in normal individuals. At baseline, relative to controls, the participants exhibited default mode network hyperconnectivity that correlated with positive symptom severity and reduced anticorrelation between the default mode network and the executive control network.

Cannabinoid administration reduced default mode network hyperconnectivity and increased default mode network/executive control network anticorrelation. The magnitude of anticorrelation in the controls and the participants after cannabinoid administration positively correlated with WM performance. The finding that default mode network brain connectivity is plastic may have implications for pharmacotherapeutic development, as treatment efficacy could be assessed through the ability of therapies to normalize underlying circuit-level dysfunction.

Cannabis derivatives produce their CNS effect by activating the endocannabinoid system, a signaling system comprising specific receptors, intrinsic lipid ligands, and the associated enzymatic machinery (transporters, biosynthetic and degradative enzymes). A review provided preclinical and clinical information on the endocannabinoid system's role in psychotic disorders such as schizophrenia.

The data indicate the presence of dysregulation in the endocannabinoid system (in terms of cannabinoid receptors and endocannabinoid ligands) in animal models of psychosis and schizophrenic patients [56]. The observations indicated that the pharmacological modulation of the endocannabinoid system had been considered a new therapeutic possibility for psychotic disorders. Preclinical studies have not provided detailed results, with agonists and antagonists exhibiting positive, negative, or no effect. Only cannabidiol and the antagonist/inverse agonist rimonabant were tested at the human level. However, additional controlled trials are required to confirm the therapeutic exploitation of those compounds.

An important aspect in the relationship between the endocannabinoid system and schizophrenia is the impact of *Cannabis* consumption on psychotic disorders, especially when they occur at vulnerable ages such as adolescence. Studies from animal models support adolescence as a highly vulnerable age for the consequences of *Cannabis* exposure on different domains (such as cognition and social behavior) that are altered in psychotic disorders.

Cannabis use can impair verbal learning, provoke acute psychosis, and increase the risk of schizophrenia. It is unclear where *Cannabis* acts in the human brain to modulate verbal learning and induce psychotic symptoms. The effects of delta-9-tetrahydrocannabinol and cannabidiol were examined on regional brain function

during verbal paired associate learning [57]. The subjects were studied using a block design functional magnetic resonance imaging paradigm on three separate occasions while performing a verbal paired associate learning task. Each imaging session was preceded by the ingestion of delta-9-THC (10 mg), cannabidiol (600 mg), or placebo in a double-blind, randomized, placebo-controlled, repeated-measures, within-subject design.

Fifteen healthy, native, English-speaking, right-handed men who had used *Cannabis* 15 times or less and had minimal exposure to other illicit drugs in their lifetime were studied. Delta-9-tetrahydrocannabinol increased psychotic symptoms and levels of anxiety, intoxication, and sedation, whereas no significant effect was noted on the parameters following the administration of cannabidiol. Performance in the verbal learning task was not significantly modulated by either drug.

Administration of delta-9-tetrahydrocannabinol augmented activation in the parahippocampal gyrus during blocks 2 and 3 such that the normal linear decrement in activation across repeated encoding blocks was no longer evident. Delta-9-tetrahydrocannabinol also attenuated the normal time-dependent change in ventrostriatal activation during retrieval of word pairs, which was directly correlated with concurrently induced psychotic symptoms. In contrast, the administration of cannabidiol had no such effect. The modulation of mediotemporal and ventrostriatal function by delta-9-tetrahydrocannabinol may underlie the effects of *Cannabis* on verbal learning and psychotic symptoms, respectively.

Cannabinoids are proposed in a wide array of medical indications. The consequences of the recreational use of *Cannabis* and experimental studies bring some insights into the potential long-term consequences of cannabinoid use [62]. Epidemiological studies have consistently demonstrated that *Cannabis* use is associated with a risk of persistent cognitive deficits and an increased risk of schizophrenia-like psychoses. The risks are modulated by the dose and duration of use, age, and genetic factors, including a partially shared genetic predisposition with schizophrenia. Studies of healthy humans indicate that *Cannabis* and its principal psychoactive component, delta-9-tetrahydrocannabinol, could produce transient, dose-dependent, psychotic symptoms and cognitive effects, which can be attenuated by cannabidiol.

Dopaminergic activity and cannabinoid receptor expression were measured in distinct brain regions. The hippocampal neurogenic activity of neural stem cells was determined, and protein levels of neuroplasticity-related biomarkers were quantified. Adolescent low-dose delta-9-THC exposure increased spontaneous open-field activity without affecting prepulse inhibition and attentional set-shifting performance. Region-specific dopaminergic alterations and CB1 receptor up-regulation in the prefrontal cortex were observed. Impaired spatial memory, as assessed with the object location task and Morris water maze test, was associated with significantly decreased proliferative activity (SOX2-positive cells), neurogenic potential (decreased doublecortin-positive cells) in the adult hippocampus, and defective neuroplasticity, including reduced BDNF expression in the hippocampus and prefrontal cortex.

The finding reveals the adverse impact of adolescent low-dose delta-9-THC on the psychomotor profile, dopaminergic neurotransmission, compensatory cannabinoid receptor response, and cognition-related neurobiological and behavioral functions. The adolescent low-dose delta-9-THC animal model does not induce tangible psychotic-like effects, such as those reported in high-dose delta-9-THC studies. However, it impairs cognitive functions and points to hippocampal vulnerability and disrupted neurogenesis.

Delta-9-tetrahydrocannabinol has been known to impair cognitive abilities in several domains, particularly memory and attention. Reduced amplitudes of auditory evoked P300 are a robust finding in schizophrenic patients, indicating deficient attentional resource allocation and active working memory [82]. Given the psychotic-like effects of delta-9-tetrahydrocannabinol, a cannabinoid hypothesis of schizophrenia has been proposed.

A prospective, double-blind, placebo-controlled crossover study investigated the acute effects of cannabinoids on P300 amplitude in 20 healthy volunteers (age 28.2 $\pm$ 3.1 years, ten male) by comparing delta-9-tetrahydrocannabinol and standardized *Cannabis* extract containing delta-9-tetrahydrocannabinol and cannabidiol. P300 waves were recorded during a choice reaction task. Delta-9-tetrahydrocannabinol revealed a significant reduction of P300 amplitude at midline frontal, central, and parietal electrodes.

Cannabidiol has been known to abolish many of the psychotropic effects of delta-9-tetrahydrocannabinol but, unexpectedly, failed to demonstrate a reversal of delta-9-tetrahydrocannabinol-induced P300 reduction. Moreover, there were no correlations between cannabinoid plasma concentrations and P300 parameters. The data indicated that delta-9-tetrahydrocannabinol might lead to acute impairment of attentional functioning and working memory. It can be speculated whether the lack of effect of cannabidiol may be due to an insufficient dose used or to an involvement of neurotransmitter systems in the P300 generation, which are not influenced by cannabidiol.

Delta-9-Tetrahydrocannabinol and Psychosis

Delta-9-tetrahydrocannabinol can induce transient psychotic symptoms in healthy individuals and exacerbate them in those with established psychosis. However, not everyone experiences those effects, suggesting that certain individuals are susceptible. An investigation was conducted to determine if individuals who are sensitive to the psychotomimetic effects of delta-9-tetrahydrocannabinol (TP) under experimental conditions would show differential hippocampal activation compared with those who are not (NP) [92].

Thirty-six healthy males under identical conditions under the influence of placebo or delta-9-tetrahydrocannabinol (10 mg) were administered orally, on two separate occasions, in a pseudo-randomized, double-blind, repeated measures within-subject, crossover design using psychopathological assessments and

functional MRI. They were classified into those who experienced transient psychotic symptoms (TP; $n = 14$) following delta-9-tetrahydrocannabinol administration and those who did not (NP, $n = 22$). At the same time, they performed a verbal learning task.

Under placebo conditions, there was a significantly greater engagement of the left hippocampus ($p < 0.001$) in the TP group compared with the NP group during verbal encoding, which survived the leave-one-out analysis. The level of hippocampal activation was directly correlated (Spearman's $\rho = 0.44$, $p = 0.008$) with the severity of transient psychotic symptoms induced by delta-9-tetrahydrocannabinol. The difference was not present when the two subgroups from the same sample defined by sensitivity to the anxiogenic effects of delta-9-tetrahydrocannabinol were compared. The results indicated that altered hippocampal activation during verbal encoding might serve as a marker of sensitivity to the acute psychotomimetic effects of delta-9-THC.

The Antipsychotic Properties of Cannabidiol

Clinical and preclinical evidence points to a critical role for cannabidiol as a potential pharmacotherapy for various neuropsychiatric disorders. In contrast to delta-9-tetrahydrocannabinol, associated with acute and neurodevelopmental propsychotic side effects, cannabidiol possesses no known psychoactive or dependence-producing properties. However, evidence has demonstrated that cannabidiol strongly modulates the mesolimbic dopamine system and may possess promising antipsychotic properties. Despite the psychotropic differences between cannabidiol and delta-9-THC, little is known regarding their molecular and neuronal effects on the mesolimbic dopamine system, nor how those differential effects may relate to their potential pro vs. antipsychotic properties.

The evidence demonstrates cannabidiol's modulatory effects on dopamine activity states within the mesolimbic pathway, functional interactions with the serotonin 5-HT_{1A} receptor system, and their downstream molecular signaling effects [69]. With clinical evidence showing that cannabidiol may normalize affective and cognitive deficits associated with schizophrenia, cannabidiol may represent a promising treatment for schizophrenia, acting through novel molecular and neuronal mesolimbic substrates.

A study evaluated the efficacy of cannabidiol versus a first-choice second-generation antipsychotic (risperidone) in patients with early psychosis and comorbid *Cannabis* use [71]. The study was a phase II randomized, double-blinded, parallel-group, active-comparator clinical trial that includes 130 patients aged 18–64 with a recent diagnosis of psychosis, comorbid *Cannabis* use, and not treated with antipsychotics. The participants were randomized to 7 weeks of treatment with either cannabidiol 600 mg (300 mg BID) or risperidone 4 mg (2 mg BID).

The participants underwent clinical assessment after 1, 3, 5, and 7 weeks, telephone assessment the weeks in between, and a safety visit 2 weeks after the end of

the treatment. The primary outcomes were cessation of *Cannabis* use (self-reported) and psychotic symptom severity. The secondary outcomes include frequency and quantity of *Cannabis* use, global illness severity, psychosocial functioning, subjective well-being, cognition, sleep, circadian rhythmicity, and metabolomics. The result is a potential treatment paradigm for patients suffering from dual diagnosis.

In a population-based association study, the hypothesis was tested that a micro-satellite polymorphism in the promoter region of the cannabinoid CB1 receptor gene confers susceptibility to mood disorders and psychotic features [78]. The cannabinoid CB1 receptor gene was genotyped in 154 mood disorder patients and 165 normal controls. The results indicated that the triplet repeat polymorphism in the promoter region of the cannabinoid CB1 receptor gene was not likely to be involved in the pathogenesis or the psychotic symptoms of mood disorders.

Laboratory rodents and human studies have revealed that the cannabinoid can prevent psychotic-like symptoms induced by high doses of delta-9-tetrahydrocannabinol. Studies have demonstrated that cannabidiol has antipsychotic effects, as observed in animal models and healthy volunteers. Cannabidiol displayed a pharmacological profile similar to that of atypical antipsychotic drugs, as seen in behavioral and neurochemical techniques in animal models. Cannabidiol prevented human experimental psychosis and was effective in open case reports and clinical trials in patients with schizophrenia with a remarkable safety profile.

The results support the idea that cannabidiol may be a future therapeutic option in psychosis, in general, and schizophrenia, in particular [79]. Moreover, fMRI results strongly indicate that the antipsychotic effects of cannabidiol in relation to the psychotomimetic effects of delta-9-tetrahydrocannabinol involve the striatum and temporal cortex that have been traditionally associated with psychosis. Although the mechanisms of the antipsychotic properties are still not fully understood, a hypothesis that could have a heuristic value to inspire new studies has been revealed.

First Episode Psychosis

A major factor associated with poor prognostic outcomes after a first psychotic break is *Cannabis* misuse, which is prevalent in schizophrenia and particularly common in individuals with recent-onset psychosis [58]. Behavioral interventions aimed at reducing *Cannabis* use have been unsuccessful in that population. Cannabidiol provides antipsychotic, anxiolytic, and neuroprotective effects. Cannabidiol has a broad pharmacological profile, but contrary to delta-9-THC, CBD does not activate CB1 or CB2 receptors and has at most subtle subjective effects.

Moreover, cannabidiol attenuates delta-9-THC's detrimental effects, both acutely and chronically, including psychotogenic, anxiogenic, and deleterious cognitive effects. Cannabidiol, may, therefore, improve the disease trajectory of individuals with early psychosis and comorbid *Cannabis* misuse, in particular, a population with the currently poor prognostic outcome and no effective specialized intervention.

A study of patients admitted with psychotic symptoms evaluates the impact of *Cannabis* use on psychiatric hospital outcomes [59]. The participants were administered a urine toxicology screening test for the presence of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (delta-9-THC-COOH). The participants with positive tests were compared to those with negative tests on several measures, including length of stay, presence or lack of thirty-day readmission, Brief Psychotic Rating Scale (BPRS) score, and antipsychotics and sedatives/anxiolytics.

The study includes 120 patients; 29 were women and 91 were men. The patients testing positive for delta-9-THC-COOH had a shorter length of stay than those testing negative for delta-9-THC-COOH after adjusting for age, prior psychiatric admissions, history of a psychotic-spectrum disorder, and comorbid additional substance use ($p = 0.02$). There were no significant differences in thirty-day readmissions, thirty-day postdischarge presentation to the psychiatric emergency department, BPRS scores, and medication administration. Patients with psychotic symptoms and *Cannabis* use require shorter inpatient psychiatric hospitalizations.

Cannabinoids are the most commonly used illegal substances. Spice and K2 are synthetic cannabinoid products that contain a mixture of herbs and plant matter combined with synthetic compounds similar to tetrahydrocannabinol, the psychoactive component of *Cannabis*. Because the effects of Spice and K2 are similar to *Cannabis*, many users are smoking the products as legal substitutes despite package labeling that they are not designed for human consumption [68]. Synthetic cannabinoid products appeal to users because they are easily accessible and not readily detected in standard urine drug screens. The active components in synthetic cannabinoid products are highly potent and poorly characterized. The agents have been associated with serious psychological and physiological side effects.

A study evaluated resting-state cerebral blood flow activities found in first-episode psychosis and the acute effects of cannabinoids and opioids; mental states that could be profoundly differ from normal functioning [87]. The study examined the cannabinoids and opioids on resting state activity patterns as investigated by different neuroimaging techniques. Twenty-two studies encompassing different neuroimaging techniques were selected. Cerebral perfusion and resting blood flow was measured by single-photon emission computed tomography (SPECT), positron emission tomography (PET), perfusion-weighted imaging (PWI), arterial spin labeling (ASL), and resting-state functional magnetic resonance imaging (resting-state fMRI) during acute cannabinoids or opioids challenges were compared to findings in patients with first-episode psychosis.

The number of subjects included in the review encompassed 279 first-episode psychosis/controls (mean age = 28 ± 8.6 years, 40.1% females), 315 participants with cannabinoids (mean age = 29 ± 7.1 years, 31.8% females), and 113 participants with opioids (mean age = 30 ± 3.9 years, 17.3% females). It was found that effects on regional activity were highly conflicting within the same condition group. Comparing baseline activity patterns between first-episode psychosis and acute cannabinoid or opioids effects indicate consistent evidence suggesting positive symptoms of first-episode psychosis and depersonalization experiences after *Cannabis* administration both resulted in an increased anterior cingulate activity.

Nonadherence and comorbid *Cannabis* abuse are associated with poor clinical outcomes in first-episode psychosis. A study was conducted to examine variation in medication adherence associated with cessation or continuation of *Cannabis* use and to determine the impact of the interaction between *Cannabis* use and adherence to medication on symptom outcome at 12 months [94]. From a consecutive patient cohort ($n = 192$) with a DSM-IV diagnosis of the first episode of psychosis, 62 patients who met DSM-IV criteria for a currently active *Cannabis* abuse disorder were followed up for 1 year. Complete data on repeated measures of medication adherence, symptoms, and *Cannabis* use were available for 48 of the 62 patients.

Twenty-eight patients (58.7%) continued and 20 (41.2%) discontinued *Cannabis* use after entering treatment. While both groups were relatively nonadherent at 6 months, 25 out of 28 (92%) of the former group became adherent compared to eight of 20 (40%) of those who stopped *Cannabis* use ($p < .01$). There was no overall effect of continued *Cannabis* use on symptom levels at 12 months, after controlling for medication adherence patients with continuous *Cannabis* use had a significantly higher level of symptoms ($F(1, 30) = 2.74, p = 0.03$).

First-episode psychosis patients with an active *Cannabis* use disorder may choose to stop *Cannabis* and not take medications or continue *Cannabis* but become adherent to medications; medication adherence appears to help both groups. However, continuous users remain at higher risk of poor symptom outcomes while on medication.

Cannabidiol on Anxiety, Depression, and Psychosis

Cannabis use is associated with a spectrum of effects including, euphoria, relaxation, anxiety, perceptual alterations, paranoia, and impairments in attention and memory [80]. *Cannabis* contains several cannabinoid compounds with synergistic or antagonistic effects on the popular active ingredient delta-9-tetrahydrocannabinol. The overall effect of *Cannabis* is thought to be related to the ratio of its composite constituents, in particular, the ratio of delta-9-tetrahydrocannabinol to cannabidiol. Since cannabinoids induce subjective and cognitive changes that share qualitative similarities with schizophrenia, cannabinoids have been used to model psychosis.

Some limitations of cannabinoid models of psychosis include the relatively high variability in experiences between different individuals, the potential for inducing unwanted effects, such as toxic psychosis in study subjects, and the lack of data showing that effective antipsychotic treatments can reverse the behavioral and cognitive/motor effects of cannabinoids. Nabiximols (Sativex) is an oromucosal spray containing delta-9-tetrahydrocannabinol and cannabidiol in an approximate 1:1 ratio.

While not extensively studied, most studies confirm that nabiximols, despite the different routes of administration and the presence of cannabidiol, have similar or slightly reduced subjective/cognitive effects compared to similar doses of oral delta-9-tetrahydrocannabinol. While the presence of cannabidiol may have utility in

some models, likely, the concentrations are not high enough to meaningfully affect those aspects important for psychosis research. While it may present an alternative to oral delta-9-tetrahydrocannabinol, oromucosal nabiximols may not present substantial advantages for psychosis research.

Cannabidiol has therapeutic potential for many nonpsychiatric and psychiatric disorders, such as anxiety, depression, and psychosis [81]. Cannabidiol lacks the psychotomimetic and other psychotropic effects of delta-9-tetrahydrocannabinol. The pharmacological effects of cannabidiol in different biological systems have been extensively investigated by in vitro studies. In vivo studies indicate that the mechanisms are not unitary but depend on the measured behavioral response. Acute anxiolytic and antidepressant-like effects seem to rely mainly on the facilitation of 5-HT_{1A}-mediated neurotransmission in key brain areas related to defensive responses, including the dorsal periaqueductal grey, bed nucleus of the stria terminalis and medial prefrontal cortex.

Other effects, such as anticomulsive, increased extinction, impaired reconsolidation of aversive memories, and facilitation of adult hippocampal neurogenesis, could depend on the potentiation of anandamide-mediated neurotransmission. Activation of TRPV1 channels may help explain the antipsychotic effect and the bell-shaped dose-response curves commonly observed with cannabidiol.

Bipolar Disorder

Cannabis can elicit an acute psychotic reaction, and its long-term use is a risk factor for schizophrenia [72]. The endogenous cannabinoids are involved in information processing and plasticity at synapses in the hippocampus, basal ganglia, and cerebral cortex. Delta-9-tetrahydrocannabinol activates cannabinoid 1 receptors, which are localized to the terminals of glutamate and GABA neurons in the brain.

Exogenously applied CB1 receptor agonists disrupt neuronal dynamics and synaptic plasticity resulting in cognitive deficits and impairment of the highest psychological functions. Other propsychotic drugs, such as ketamine and methamphetamine, exert their effects in the same microdomain of synaptic spines as delta-9-tetrahydrocannabinol. Additionally, many of the most robust findings in psychiatric genetics include components that localize to dendritic spines and have important roles in information processing and plasticity.

A study revealed the distinct clusters of subtypes among patients with bipolar disorder and the relationship between the clinical features of bipolar disorder patients, particularly substance use disorders, and the clusters [74]. The study assessed 96 patients hospitalized in a psychiatric clinic for a bipolar disorder manic episode. The patients were evaluated during the initial 3 days of their admission using the Young Mania Rating Scale (YMRS), Montgomery–Asberg Depression Rating Scale (MADRS), Scale for the Assessment of Positive Symptoms (SAPS), Michigan Alcoholism Screening Test (MAST), and a sociodemographic questionnaire. The factor structures of the psychopathological scale items were determined

with factor analyses and based on the factor loadings, and cluster analyses were performed. The relationships among the clusters and the clinical variables were evaluated.

The analyses generated three factors, increased psychomotor activity, dysphoria, and psychosis. A hierarchical cluster analysis was applied to the three-factor loadings and revealed that factor 1 (increased psychomotor activity) was high in cluster 1 and that the effects of factors 2 (dysphoria) and 3 (psychosis) were high in cluster two. Within cluster 1 (Psychomotor elevation), 39% of the patients were diagnosed with an alcohol use disorder.

In comparison, 31.6% of patients in cluster two (dysphoric-psychotic) were diagnosed with alcohol and *Cannabis* use disorders. In cluster two, 47.4% of the patients had one suicide attempt and 21.1% had two or more suicide attempts. There was a significant difference in the presence of substance use disorders between patients with psychomotor elevation and patients in the dysphoric-psychotic cluster. Pure manic patients with bipolar disorder self-medicate using the sedative effects of alcohol and the causal relationship between *Cannabis* and psychosis. Using a dimensional approach to study bipolar disorder may enhance the detection of the biological correlates of bipolar disorder and improve the treatment and outcomes of the disorder.

Cannabis can induce mood change and sometimes psychotic symptoms in normal persons. In the brain, the main active ingredient of *Cannabis* acts via the cannabinoid CB1 receptor located on chromosome 6q14-15. Linkage studies have indicated the presence of a bipolar disorder susceptibility locus on chromosome 6q.

Addictive and Psychiatric Comorbidity

A study was conducted to assess the addictive and psychiatric comorbidity in *Cannabis* users seen in the specific setting at the Lariboisière hospital [89]. Two hundred and seven *Cannabis* users were included in the investigation. Twelve-month and lifetime diagnoses of abuse and dependence (*Cannabis*, alcohol, cocaine/crack) (DSM-IV), current and lifetime mood disorders, anxiety disorders, eating disorders, and psychotic disorders were assessed (Mini-International Neuropsychiatric Interview).

Logistic regression analyses identified adjusted odds ratios associated with gender and healthcare seeking ($p = 0.01$). Of 147 men (71%) and 60 women (29%), 29.3 ± 8.6 years (15.2–51.6 years), most of the outpatients asked for health care themselves (59.7%), whereas 19.4% were asked to seek health care by relatives (19.4%) or because of an academic, health, or justice injunction (20.4%). In total, 49.3% of the outpatients are single, 35.7% cohabitate, 9.3% are married, and 6.3% are separated or divorced. About 20.4% of the outpatients are students, 35.7% have a professional activity, 19% are jobless, 2.4% are impaired, 0.5% is retired at home, and 12.1% do not have an official income.

Twelve-month and lifetime prevalence of abuse/dependence were: *Cannabis* (10.1/82.1% and 8.7/88.4%), alcohol (9.7/8.7% and 19.3/18.8%), cocaine/crack (2.4/3.4% and 4.8/11.6%). The mean duration of *Cannabis* dependence for the current dependent users is 8.4 ± 5.8 years. The mean number of “joints” during the last 6 months is 6 ± 4.3 , and the mean amount of *Cannabis* per week is 12.5 ± 11.3 g. About 51.3% of the dependent users report externalized and internalized disorders at school during childhood and adolescence.

In total, 19.4% of the dependent users have a suicide attempt history, and 18.9% have a psychiatric hospitalization history, more frequently women ($p < 0.01$ and $p = 0.02$). About 73.8% have a psychologist or psychiatrist care history. In total, 38.1% of users have at least one current mood disorder, females more frequently than males ($p < 0.001$). Current and lifetime prevalence of mood disorders are major depressive disorder (MDD) (29.1% and 57.1%); current dysthymia (20.3%); hypomania (1.9 and 6.7%); mania (2.9 and 12.8%). Females are more frequent than males with current and lifetime MDD ($p < 0.001$). About 53.2% of users have at least one current anxiety disorder, females more frequently than males ($p < 0.001$).

Current and lifetime prevalence of anxiety disorders was: panic disorder (10% and 16.4%); agoraphobia (13.9% and 17.4%); social phobia (26.9% and 32.8%); obsessive-compulsive disorder (9.5% and 12.9%); posttraumatic stress disorder (PTSD) (6.5% and 16.4%); current generalized anxiety disorder (26.8%). Females have more frequent current and lifetime: agoraphobia ($p = 0.01$ and $p < 0.001$), PTSD ($p < 0.001$), and current social phobia ($p = 0.049$). Current and lifetime eating disorders prevalence were: anorexia (0% and 1.5%); bulimia (4% and 8%); females more frequently have bulimia ($p = 0.02$ and $p < 0.001$). In total, 4.8% have a psychotic disorder. Adjusted odds ratios of associated variables to gender (women/men) are lifetime MDD OR = 4.71 [2.1–10.61] ($p < 0.001$) and later age of onset of *Cannabis* abuse OR = 1.1 [1.04–1.17] ($p = 0.002$). Adjusted odds ratios associated with personal health care seeking compared to nonpersonal motivated health care seeking are the numbers of criteria of 12-month *Cannabis* dependence OR = 1.26 [1.06–1.51] ($p = 0.009$) and age OR = 1.07 [1.03–1.12] ($p = 0.002$). The investigation confirms the high mood and anxiety disorders comorbidity in *Cannabis* dependent users seen in a specific setting and underlines the need to evaluate those disorders.

Cannabis use can both increase and reduce anxiety in humans. An investigation examined the effects of delta-9-tetrahydrocannabinol and cannabidiol on regional brain function during emotional processing. The subjects were studied on three separate occasions using an event-related functional magnetic resonance imaging paradigm while viewing faces that implicitly elicited different anxiety levels. Each scanning session was preceded by either ingesting 10 mg of delta-9-THC, 600 mg of CBD, or a placebo in a double-blind, randomized, placebo-controlled design.

The participants were 15 healthy, English-native, right-handed men who had used *Cannabis* 15 times or less. Regional brain activation (blood oxygenation level-dependent response), electrodermal activity (skin conductance response [SCR]), and objective and subjective ratings of anxiety were recorded. Delta-9-tetrahydrocannabinol increased anxiety and levels of intoxication, sedation, and

psychotic symptoms; whereas there was a trend for a reduction in anxiety following the administration of CBD [90]. The number of SCR fluctuations during the processing of intensely fearful faces increased following the administration of delta-9-THC but decreased following the administration of CBD.

Cannabidiol attenuated the blood oxygenation level-dependent signal in the amygdala and the anterior and posterior cingulate cortex while subjects were processing intensely fearful faces, and its suppression of the amygdala and anterior cingulate responses was correlated with the concurrent reduction in SCR fluctuations.

Delta-9-tetrahydrocannabinol and CBD had distinct effects on the neural, electrodermal, and symptomatic response to fearful faces. Delta-9-tetrahydrocannabinol mainly modulated activation in the frontal and parietal areas. The effects of CBD on activation in limbic and paralimbic regions may contribute to its ability to reduce autonomic arousal and subjective anxiety. In contrast, the anxiogenic effects of delta-9-THC may be related to effects in other brain regions.

Treating patients with schizophrenia and substance use disorder poses a challenge for clinicians. Continued use of *Cannabis* and cocaine can exacerbate psychotic symptoms and worsen the course of the disease. No pharmacotherapy is available for patients with *Cannabis* use disorder. Cannabidiol and delta-9-tetrahydrocannabinol are the main active constituents in *Cannabis sativa*, with the latter being linked to an increased risk of psychosis. A male patient was diagnosed with schizophrenia, combined personality disorder, *Cannabis* use disorder, and cocaine use disorder for over 8 years and was hospitalized 30 times due to psychotic relapses and continued substance use.

The patient demonstrates the potential of smoked cannabidiol as a substitute for severe and chronic *Cannabis* use disorder [97]. Consequently, cannabidiol cigarettes with a low delta-9-tetrahydrocannabinol content (<1%) were used as adjunctive therapy. Additionally, off-label treatment with methylphenidate to support abstinence was established. The patient reported feeling significantly less need to consume illegal *Cannabis* with high delta-9-tetrahydrocannabinol content. He stopped the use of *cocaine* for the time being and has not been hospitalized.

In schizophrenia, *Cannabis* abuse/dependence is associated with poor compliance and psychotic relapse. Despite this, the reasons for *Cannabis* abuse remain elusive, but emotions may play a critical role in this comorbidity. Accordingly, a functional magnetic resonance imaging study of emotional memory in schizophrenia patients with *Cannabis* abuse (dual-diagnosis, DD) was performed. The participants comprised 14 DD patients, 14 nonabusing schizophrenia patients (SCZ), and 21 healthy controls (HC) who had to recognize positive and negative pictures while being scanned.

Recognition of positive and negative emotions was prominently impaired in SCZ patients relative to HC, while differences between DD and HC were smaller. For positive and negative stimuli, there were significant activations in frontal, limbic, temporal, and occipital regions in HC; in frontal, limbic, and temporal regions in DD; and in temporal, parietal, limbic, and occipital regions in the SCZ group. The results suggest that emotional memory and prefrontal lobe functioning are

preserved in DD relative to SCZ patients. The results are consistent with previous findings indicating that *Cannabis* abuse is associated with fewer negative symptoms and better cognitive functioning in schizophrenia [102].

Cannabis use has been associated with sex-dependent effects on the brain and behavior. It has been demonstrated that adult female rats exposed to delta-9-tetrahydrocannabinol develop long-term cognitive and emotional reactivity alterations. In contrast, preliminary evidence suggests the presence of a different phenotype in male rats.

To thoroughly depict the behavioral phenotype induced by adolescent delta-9-THC exposure in male rats, adolescent animals were treated with increasing doses of delta-9-THC twice a day (PND 35–45), and, in adulthood, a battery of behavioral tests was performed to measure affective- and psychotic-like symptoms as well as cognition.

Motor and Cognitive Impairments

A study investigated whether cannabidiol administration would attenuate reserpine-induced motor and cognitive impairments in rats. Repeated administration of reserpine in rodents induces motor impairments accompanied by cognitive deficits and has been applied to model both tardive dyskinesia and Parkinson's disease. In Parkinson's patients, cannabidiol can attenuate the psychotic symptoms induced by L-DOPA and improve quality of life [91].

Male Wistar rats received four injections of cannabidiol (0.5 or 5 mg/kg) or vehicle (days 2–5). On days three and five the animals received one injection of 1 mg/kg reserpine or vehicle. Locomotor activity, vacuous chewing movements, and catalepsy were assessed from day one to day seven. On days eight and nine, the animals' performance on the plus-maze discriminative avoidance task for learning/memory assessment was evaluated. Cannabidiol (0.5 and 5 mg/kg) attenuated the increase in catalepsy behavior and oral movements – but not the decrease in locomotion – induced by reserpine. Cannabidiol (0.5 mg/kg) also ameliorated the reserpine-induced memory deficit in the discriminative avoidance task. The data show that cannabidiol can attenuate motor and cognitive impairments induced by reserpine, suggesting the use of this compound in the pharmacotherapy of Parkinson's disease and tardive dyskinesia.

Two retrospective epidemiologic studies have shown that *Cannabis* is the main psychoactive substance detected in the blood of drivers suspected of driving under the influence of psychotropic drugs [50]. An oral administration double-blind cross-over study with eight healthy male *Cannabis* smokers aged 22–30 years was conducted. Three treatments and one placebo were administered to the participants at a 2-week interval: 20 mg dronabinol, 16.5 mg delta-9-THC, and 45.7 mg delta-9-THC as a *Cannabis* milk decoction. The participants reported their subjective drug effects and willingness to drive under various circumstances on a visual analog

scale. Clinical observations, a psychomotor test, and a tracking test on a driving simulator were also carried out.

Compared to *Cannabis* smoking, delta-9-THC, 11-OH-THC, and THC-COOH blood concentrations remained low throughout the study (<13.1 ng delta-9-THC/mL, <24.7 ng 11-OH-THC/mL, and <99.9 ng THC-COOH/mL). Two subjects experienced serious anxiety symptoms indicating that the unwanted side-effect may occur when driving under the influence of *Cannabis* or when driving and smoking a joint. No clear association between the adverse reactions and a susceptibility gene to the propensity to anxiety and psychotic symptoms (genetic polymorphism of the catechol-O-methyltransferase) could be found.

The questionnaires indicated that the willingness to drive was lower when the drivers were assigned a trivial task and higher when the mission was crucial. The subjects were aware of the effects of *Cannabis*, and their performances on the road sign and tracking test were greatly impaired, especially after ingesting the strongest dose. The *Cannabis* Influence Factor (CIF), which relies on the molar ratio of active and inactive cannabinoids in the blood, provides a good estimate of the fitness to drive.

Schizotypal Personality Disorder

Cannabis use predicts psychosis in longitudinal studies, but it is difficult to infer causation. Some precursor variables predict both, including childhood trauma and adversity. Additionally, some of the desired effects of *Cannabis* use resemble the symptoms of psychosis. It would be preferable to assess psychotomimetic or “unusual” experiences that include psychotic symptoms without assuming pathology. Finally, it is possible that similar people are prone to psychosis and drawn to *Cannabis* use, perhaps because they are sensitive or attracted to unusual experiences.

Schizotypy provides a trait measure of proneness to unusual experiences. A study was conducted to examine cross-sectional relationships between *Cannabis* use, schizotypy, and unusual experiences while controlling for current trauma symptoms [41]. A volunteer online sample ($n = 129$, 64% women, predominantly students) who had used *Cannabis* at least once was recruited. People who reported active effects of past trauma were excluded with a brief primary care posttraumatic stress disorder screen. Participants completed the Oxford-Liverpool Inventory of Feelings and Experience, the Cognitive Failures Questionnaire, and substance use and socio-demographics measures. Most respondents recounted unusual experiences after *Cannabis* use, and many of those might have been considered symptoms of psychosis if they had received medical attention.

In regression analysis, the only predictor of the unusual experiences scale of O-LIFE was schizotypy (measured by the remaining subscales; 4% of variance). There were no correlations between *Cannabis* use frequency and schizotypy or unusual experiences. The findings suggest that, after controlling for schizotypy and excluding people actively experiencing the effects of past trauma, the frequency of

Cannabis use does not predict unusual experiences. However, individuals with schizotypal personality traits may have more unusual experiences when using *Cannabis*.

Implicit and Explicit Psychotic Disorders

Cannabis use is common in patients with recent-onset schizophrenia, associated with poor disease outcomes. More insight into the cognitive-motivational processes related to *Cannabis* use in schizophrenia may inform treatment strategies. A study compared implicit and explicit *Cannabis* associations in individuals with and without the psychotic disorder [40]. The participants were 70 patients with recent-onset psychotic disorder and 61 healthy controls with various levels of *Cannabis* use. Three Single-Category Implicit Association Tests (SC-IAT) were used to assess relaxed, active, and negative implicit associations toward *Cannabis* use. Explicit expectancies of *Cannabis* use were assessed with a questionnaire using the same words as the SC-IAT.

There were no differences in implicit associations between patients and controls; however, patients scored significantly higher on explicit negative affect expectancies than controls. Both groups demonstrated strong negative implicit associations toward *Cannabis* use. Explicit relaxed expectancies were the strongest predictors of *Cannabis* use and craving. There was a trend for implicit active associations to predict craving.

The findings indicate that patients with schizophrenia have associations toward *Cannabis* similar to controls, but they have stronger negative explicit *Cannabis* associations. The strong negative implicit associations toward *Cannabis* could imply that users of *Cannabis* engage in behavior they do not implicitly like. Explicit relaxing expectancies of *Cannabis* might be an important mediator in the continuation of *Cannabis* use in patients and controls.

Delta-9-THC and CDB on Cognitive Functions

The nonintoxicating cannabinoid compound, cannabidiol, has antipsychotic and anxiolytic properties and thus may be a promising agent in treating psychotic and anxiety disorders. A systematic review provides an up-to-date systematic literature overview of neuroimaging studies on the acute impact of cannabidiol on human brain function. The study of cannabidiol on brain function during a resting state or in the context of cognitive tasks in healthy volunteers indicated that acute cannabidiol enhanced frontostriatal resting state connectivity compared to placebo and delta-9-THC. Cannabidiol modulates brain activity and had opposite effects when compared to delta-9-THC following task-specific patterns during various cognitive paradigms, such as emotional processing (frontotemporal), verbal memory

(frontostriatal), response inhibition (fronto-limbic-striatal), and auditory/visual processing (temporo-occipital) [2].

Neuroimaging studies indicate that acute cannabidiol use induces significant alterations in brain activity and connectivity patterns during resting state and performance of cognitive tasks in both healthy volunteers and patients with a psychiatric disorder that includes modulation of functional networks relevant for psychiatric disorders, possibly reflecting cannabidiol's therapeutic effects. In individuals at high clinical risk for psychosis and patients with established psychosis, acute cannabidiol produced intermediate brain activity compared to placebo and healthy controls during cognitive task performance. Cannabidiol modulated resting limbic activity in subjects with anxiety and metabolite levels in patients with autism spectrum disorders.

Cannabidiol has potential application in various contexts; however, its effective dose in different disease states remains unclear. An investigation into what doses have been applied in clinical populations revealed a dynamic range of CBD in various medical contexts. A total of 1038 articles were reviewed, of which 35 studies met inclusion criteria covering 13 medical contexts [7]. Twenty-three studies reported a significant improvement in primary outcomes, such as psychotic symptoms, anxiety, and seizures, with doses ranging between 1 and 50 mg/kg/d. Plasma concentrations were not provided in any publication.

The review highlights that CBD has a potentially wide range of activities in several pathologies. Cannabidiol was reported as well tolerated, and epilepsy was the most frequently studied medical condition, with all studies demonstrating positive effects of CBD on reducing seizure frequency or severity (average 15 mg/kg/d within randomized controlled trials). There was no indication of the positive activity of CBD in small randomized controlled trials (range $n = 6-62$) assessing diabetes, Crohn's disease, ocular hypertension, fatty liver disease, or chronic pain. However, low doses (average 2.4 mg/kg/d) were used in the studies.

Delta-9-tetrahydrocannabinol can acutely induce psychotic symptoms and impair episodic and working memory, and cannabidiol may attenuate the effects [5]. A study on the effects of delta-9-THC and CBD, both alone and in combination, on psychotic symptoms and memory function used an experimental medicine approach to predict treatment sensitivity. Forty-eight *Cannabis* users from the community based on schizotypal personality questionnaire scores (low and high) and frequency of *Cannabis* use (light and heavy) were studied in a randomized, double-blind crossover design compared the effects of (i) placebo, (ii) delta-9-THC 8 mg, (iii) CBD 16 mg, and (iv) delta-9-THC 8 mg plus CBD 16 mg administered by inhalation through a vaporizer.

The Brief Psychiatric Rating Scale, Psychotomimetic States Inventory (PSI), immediate and delayed prose recall (episodic memory), and 1- and 2-back (working memory) were assessed daily. The results indicated that delta-9-THC increased overall scores on the PSI, negative symptoms on the Brief Psychiatric Rating Scale, and robustly impaired episodic and working memory. Coadministration of CBD did not attenuate the effects. CBD alone reduced Psychotomimetic States Inventory scores in light users only. At a ratio of 2:1, CBD does not attenuate the acute

psychotic and memory-impairing effects of vaporized delta-9-THC. Frequent *Cannabis* users show a blunted antipsychotic response to CBD, which is of concern due to the high rates of *Cannabis* use disorder in patients with schizophrenia.

Studies have shown that delta-9-THC can impair cognitive functions, especially attention, episodic memory, working memory, and executive functions. The impairments have been related to the duration, frequency, dose, and age at the onset of *Cannabis* use [14]. Cognitive deficits may disappear with abstinence. However, abnormalities may be long-lasting in subjects who began smoking *Cannabis* before age 15. The lifetime prevalence of *Cannabis* use disorders is about 1% in the general population. The main characteristics of *Cannabis* use disorders are craving, persistent desire or unsuccessful efforts to cut down or control *Cannabis* use, and persistent avoidance of familial, social, occupational, or recreational activities because of *Cannabis* use.

Functional magnetic resonance imaging was used to assess the effects of delta-9-tetrahydrocannabinol and cannabidiol during visual and auditory processing in 14 healthy volunteers who were scanned on three occasions. Identical 10 mg delta-9-tetrahydrocannabinol, 600 mg cannabidiol, and placebo capsules were allocated in a balanced double-blinded pseudo-randomized crossover design. Plasma levels of each substance, physiological parameters, and measures of psychopathology were taken at baseline and regular intervals following ingestion of the test substances [100].

The volunteers listened passively to words read and viewed a radial visual checkboard in alternating blocks during fMRI scanning. Administration of delta-9-tetrahydrocannabinol was associated with increased anxiety, intoxication, and positive psychotic symptoms, whereas cannabidiol had no significant symptomatic effects.

Delta-9-tetrahydrocannabinol decreased activation relative to placebo in bilateral temporal cortices during auditory processing and increased and decreased activation in different visual areas during visual processing. Cannabidiol was associated with activation in the right temporal cortex during auditory processing.

Single doses of delta-9-tetrahydrocannabinol and cannabidiol modulate brain function in areas that process auditory and visual stimuli and relate to induced psychotic symptoms. When contrasted, delta-9-tetrahydrocannabinol and cannabidiol had opposite effects in the right posterior superior temporal gyrus, the right-sided homolog to Wernicke's area. Moreover, the attenuation of activation in this area (maximum 61, -15, -2) by delta-9-tetrahydrocannabinol during auditory processing was correlated with its acute effect on psychotic symptoms.

Psychosis and Schizophrenia

The determination of the structure of personality seems fundamental in the understanding of the symptoms. A personality of a psychotic structure increases the risk of traumatization and posttraumatic stress disorder. At the same time, the fragility of this structure causes increased sensitivity to the trauma which takes on a

particular echo. Trauma can test a latent psychotic structure to reveal its existence. The experience of psychosis may be traumatic in itself for patients with, notably, seclusion and sedation during hospitalization. Clinicians often fail to screen routinely for trauma and posttraumatic stress disorder symptoms in patients with severe mental illness because few systematic guidelines exist for identifying and treating this comorbidity. The symptoms of posttraumatic psychosis should be differentiated from neurological confusion caused by a traumatic brain injury.

The links between psychotic and psycho-traumatic symptoms are complex and multidirectional. While paying attention to those symptoms, the clinician has to distinguish the structure of the subject's personality to articulate the psychotherapy and the pharmacological treatment. Further investigational studies may determine whether antipsychotics enhance treatment response in patients with psychotic features of posttraumatic stress disorder.

Advances in the neurobiology of cannabinoids have renewed interest in the association between *Cannabis* and psychotic disorders. In a three-day, double-blind, randomized, placebo-controlled study, the behavioral, cognitive, motor, and endocrine effects of 0 mg, 2.5 mg, and 5 mg intravenous delta-9-tetrahydrocannabinol were characterized in 13 stable, antipsychotic-treated schizophrenia patients. The data were compared with effects in healthy subjects. Delta-9-tetrahydrocannabinol transiently increased learning and recall deficits; positive, negative, and general schizophrenia symptoms; perceptual alterations; akathisia, rigidity, and dyskinesia; deficits in vigilance; and plasma prolactin and cortisol. Schizophrenia patients were more vulnerable to delta-9-THC effects on recall than control subjects. No serious short- or long-term adverse events were associated with study participation.

Longitudinal studies in the general population have shown that *Cannabis* use is associated with a twofold increase in the risk of psychotic disorders, particularly schizophrenia, compared to controls. The risk of psychosis increases in a dose-dependent manner. The onset of *Cannabis* use predicts a higher risk of schizophrenia. The effects are exerted primarily through delta-9-THC interaction with cannabinoid 1 receptors in the brain. *Cannabis* exposure may disrupt the last steps of brain maturation through the endocannabinoid system, thereby increasing the risk of psychosis during adolescence.

The co-occurrence between posttraumatic and psychotic symptoms is described in the immediate suites of trauma but can also be chronic. This symptomatic co-occurrence is often approached under the sole angle of a primary posttraumatic stress disorder or a primary psychosis without the federative will to unify the psychotic and posttraumatic symptoms within the same framework. Individuals with schizophrenia or schizoaffective disorder report higher rates of trauma and assault than the general population. High rates of posttraumatic stress disorder have been noted in severe mental illness cohorts [16]. Psychotic phenomena may commonly manifest in patients with chronic posttraumatic stress disorder. Posttraumatic stress disorder often induces a risk of substance use disorder supplying psychotic symptoms.

Cannabis increases hallucinations, cocaine strengthens an underlying paranoid tone, and alcohol implies withdrawal hallucinosis. Such consumption could be a

risk factor for the development of chronic psychosis. From another point of view, by basing themselves on the plasma dopamine beta-hydroxylase activity, some authors made the analogy between major psychotic depression and posttraumatic stress disorder with psychotic features (also characterized as a distinct psychotic subtype of posttraumatic stress disorder). Other studies found no correlation between posttraumatic stress disorder with psychotic features and family predisposition for schizophrenia and schizoaffective disorder.

Delta-9-tetrahydrocannabinol is associated with transient exacerbation in core psychotic and cognitive deficits in schizophrenia. The data do not provide a reason to explain why schizophrenia patients use or misuse *Cannabis*. Furthermore, delta-9-THC might differentially affect schizophrenia patients relative to control subjects [17]. The enhanced sensitivity to the cognitive effects of delta-9-THC warrants further study into whether brain cannabinoid receptor dysfunction contributes to the pathophysiology of the cognitive deficits associated with schizophrenia.

The influence of *Cannabis* abuse on the severity of existing psychotic and affective symptoms is still unclear. The Hamilton Depression Rating Scale (HAM-D-21), Positive and Negative Syndrome Scale (PANSS), and Young Mania Rating Scale (YMRS) were used to evaluate 470 consecutively admitted psychotic or affective patients. Fifty-four active (in the previous month) *Cannabis* abusers were detected via urine tests and Structured Clinical Interview for DSM-IV (SCID-IV) questionnaire. In 24 cases, substances other than *Cannabis* were abused; 392 patients were nonabusers. The patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria [18].

Cannabis abusers ($n = 54$) were significantly younger and more frequently males than nonuser patients. In this group, there were more schizophrenic patients and fewer affective and anxiety patients (chi-square = 11.76; $p < .01$). The double-diagnosed patients had more prominent psychotic symptoms than the nonusers ($n = 392$)-PANSS positive: 19.056 $\pm$ 8.30 vs. 16.128 $\pm$ 8.031 ($p < .02$; $t(446) = 2.510$). The difference was statistically significant for hallucinatory behavior, excitement, grandiosity, and hostility. The General PANSS scale rate of abusers was lower: 33.012 $\pm$ 9.317 vs. 37.3575 $\pm$ 11.196 ($p < .01$; $t = 2.727$), especially for depression, anxiety, somatic concern, guilt feelings, tension, motor retardation, and volition disturbances. Rates of PANSS-negative scale of abusers and nonusers were not significantly different (13.815 $\pm$ 6.868 vs. 14.983 $\pm$ 6.446) except for lower rates of social withdrawal and stereotyped thinking for abusers.

No significant difference in the general level of manic symptoms (YMRS) between abusers and nonusers was observed (6.778 $\pm$ 10.826 vs. 4.910 $\pm$ 7.754). However, the severity of thought/language disturbances and poor insight was significantly higher in the abusers. *Cannabis* abusers were less depressive (HAM-D): 5.944 $\pm$ 10.291 vs. 12.896 $\pm$ 13.946 ($p < .0005$, $t = 3.535$). Such differences were observed in the high number of subscales. Abusers' rates were higher for paranoid symptoms and general somatic symptoms. *Cannabis* possibly produces some antidepressive and anxiolytic effects on psychotic and affective inpatients.

To investigate the biological mechanisms underlying the higher risk for psychosis in those who use *Cannabis*, a genome-wide environment-interaction study (GWEIS) was conducted. In a sample of individuals without a psychiatric disorder ($n = 1262$), the interactions between regular *Cannabis* use and genotype with psychotic-like experiences (PLE) as the outcome was examined. Psychotic-like experiences were measured using the Community Assessment of Psychic Experiences (CAPE). The sample was enriched for those at the extremes of both *Cannabis* use and PLE to increase power.

Single nucleotide polymorphism in the P2RX7 gene (rs7958311) was associated with a risk for a high level of psychotic experiences in regular *Cannabis* users ($p = 1.10 \times 10^{-7}$) and in those with high levels of lifetime *Cannabis* use ($p = 4.5 \times 10^{-6}$). The functional relevance of P2RX7 in *Cannabis* users was suggested by in vitro experiments on activated monocytes. The interaction was replicated in individuals with high levels of lifetime *Cannabis* use in the IMAGEN cohort ($n = 1217$, $p = 0.020$).

Exposure of the cells to delta-9-tetrahydrocannabinol or cannabidiol reduced the immunological response of the P2X7 receptor, which was dependent on the identified genetic variant. P2RX7 variants have been implicated in psychiatric disorders before, and the P2X7 receptor is involved in pathways relevant to psychosis, such as neurotransmission, synaptic plasticity, and immune regulation [19]. It was concluded that P2RX7 plays a role in vulnerability to developing psychotic symptoms when using *Cannabis* and points to a new pathway that newly developed P2X7 antagonists can potentially target.

Substance use and the first signs of psychosis commonly begin in adolescence as recreational use of *Cannabis* and 3,4-methylenedioxymethamphetamine (MDMA). When attenuated psychotic symptoms (APS) occur during substance use disorder, they are commonly attributed to *Cannabis* use, neglecting the role of other substances abused, such as MDMA, in the risk of psychosis [20].

Retrospective self-reports on APS (Prodromal Questionnaire, PQ-16) and amount of *Cannabis* and MDMA use in ($n = 46$) adolescent psychiatry outpatients with substance use disorder ($n = 17$, 35%) who reported MDMA consumed additionally to *Cannabis* were examined. Also examined were the associations of APS with *Cannabis* and MDMA use in stepwise hierarchical regressions while controlling for trauma history, birth complications, and gender. APS was not related to *Cannabis* ($B = 0.04$, $p = 0.842$) but to MDMA use ($B = 4.88$, $p = 0.001$) and trauma history ($B = 0.72$, $p = 0.001$). Gender ($B = -0.22$, $p = 0.767$) and birth complications ($B = -0.68$, $p = 0.178$) were not associated with APS.

The results indicate that the combination of MDMA and *Cannabis* use is associated with APS among adolescent substance use disorder patients. There was no indication of an association between *Cannabis* use and APS. It is speculated that *Cannabis* increases the risk for psychosis after a longer period of use and in combination with other risk factors, such as trauma history.

Cannabidiol has an antipsychotic effect in people with established psychosis. Patients with established psychosis and on standard antipsychotic treatment were studied on separate days, at least 1 week apart, to investigate the effects of a single

dose of orally administered CBD (600 mg) compared to a matched placebo, using a double-blind, randomized, matched placebo-controlled, repeated-measures, within-subject crossover design [21].

Three hours after administering the study drug, the participants were scanned using a block design functional magnetic resonance imaging paradigm while performing a verbal paired associate learning task. Fifteen psychosis patients completed both study days and 13 completed both scanning sessions. Nineteen healthy controls were also scanned using the same fMRI paradigm under identical conditions but without drug administration. The effects of CBD on brain activation were measured using the blood oxygen level-dependent hemodynamic response, and fMRI signals were studied in the mediotemporal, prefrontal, and striatal regions of interest.

Compared to healthy controls, psychosis patients under matched placebo had altered prefrontal activation during verbal encoding, mediotemporal and prefrontal activation, and greater mediotemporal-striatal functional connectivity during verbal recall. Cannabidiol attenuated dysfunction in those regions such that activation under its influence was intermediate between the matched placebo condition and healthy controls. Cannabidiol also attenuated hippocampal-striatal functional connectivity and caused trend-level symptom reduction in psychosis patients. The results suggest that the normalization of mediotemporal and prefrontal dysfunction and mediotemporal-striatal functional connectivity may underlie the antipsychotic effects of CBD.

While research suggests that active *Cannabis* use is a barrier to the emotional experiences of anticipating pleasure and expressing emotion in early psychosis, the relationship between lifetime *Cannabis* use, emotional experience, and social function over time is not completely understood. The influence of lifetime *Cannabis* use on emotional experience in prolonged psychosis and the influence of the interaction of *Cannabis* use and emotional expression on social function was characterized [24].

Emotional expression, the experience of pleasure at the moment, anticipatory anhedonia, and social functioning were measured concurrently using the Emotional Expressivity Scale, Temporal Experience of Pleasure Scale, and Social Functioning Scale, respectively. Participants were adults with schizophrenia either with ($n = 35$) or without ($n = 36$) a lifetime history of *Cannabis* use. Compared to nonusing participants, individuals with a history of *Cannabis* use expressed lesser abilities to express emotion, were less likely to expect pleasure, and had a poorer social function. *Cannabis* use moderated the relationship between anticipatory pleasure and prosocial activities. Lifetime *Cannabis* use in schizophrenia may be associated with greater deficits in emotional expressivity, the anticipation of pleasure, and social function. *Cannabis* use may also disrupt the relationship between anticipatory pleasure and social functioning.

Many confounding variables potentially contribute to the clinical presentation of hospitalized patients in the psychiatric unit. Illicit drug use, in particular, has been associated with acute agitation. Questions can be raised about what lasting effects drug use prior to admission may have throughout a patient's hospital stay. A study examines the association between *Cannabis* use and the hospital course of patients

admitted to the psychiatric inpatient unit with a diagnosis of schizophrenia, schizoaffective disorder, or bipolar disorder [26].

The study subjects with a discharge diagnosis of bipolar disorder, schizophrenia, schizoaffective disorder, or psychosis not otherwise specified ($n = 201$) were retrospectively identified, and those with positive results of urine drug screen for *Cannabis* on admission were compared to negative counterparts.

Agitation and aggression were measured using an adaptation of the Excited Component of the Positive and Negative Syndrome Scale (PANSS-EC). The markers were also quantified by comparing charted episodes of restraint and seclusion and the administration of as-needed medications, such as benzodiazepines and anti-psychotics. Positive urine drug screen results for *Cannabis* were correlated with young ($p = .001$) males ($p = .003$) with bipolar disorder ($p = .009$) exhibiting active manic symptoms ($p = .003$) at the time of admission. *Cannabis* use was further associated with a shorter length of stay ($p = .008$), agitation-triggering adapted PANSS-EC nursing assessments ($p = .029$), and oral medications as needed ($p = .002$) for agitation.

Cannabis use, as defined by positive urine drug screen results, was more common in patients with bipolar disorder and was accompanied by a higher incidence of inpatient agitation. Although the patients also had short hospital lengths of stay, there was no clear relationship between the level of agitation and length of stay across all patient groups. One possible explanation for patients with bipolar disorder experiencing short lengths of stay is that their source of agitation may be more closely related to a complex effect of *Cannabis* use rather than a sole etiology of mental illness. Inpatient clinicians should be aware of patient *Cannabis* use proximate to admission.

Cannabis consumption is a social and cultural phenomenon. It has a specific place in each society; therefore, psychotropic effects are judged differently. In medieval Islam, *Cannabis* consumption indicated connections with religious and mystic events and was formed through consuming Sufis. The main risk of *Cannabis* use was especially the induction of “madness” or psychotic states in case of high dosage and high frequency of consumption. Nevertheless, there were no social sanctions. Today in western countries, *Cannabis* consumption is connected with everyday activity and is mainly consumed by adolescents. The main risk is seen in the continued consumption of illicit drugs since *Cannabis* consumption is widely held as the first step for such activity. On the other hand, the induction of psychotic states or schizophrenia-like psychosis is rarely named in public discussions.

There is evidence of decreased pleasure and deficits in reward anticipation in both psychotic illness and drug addiction. Individuals with low anticipatory pleasure may preferentially engage in behaviors associated with an immediate reward, such as *Cannabis* use [27]. Ninety-one psychosis patients and 91 controls without a history of psychosis were administered the Temporal Experience of Pleasure Scale, a self-report which measures anticipatory and consummatory pleasure. *Cannabis* use diagnosis was assessed using the Structured Clinical Interview for DSM IV. The subjects reported the frequency of *Cannabis* consumption and time since last use.

The patients did not show a significant deficit in anticipatory or consummatory pleasure compared to controls; however, patients with a functional *Cannabis*-use disorder tended to have lower consummatory pleasure than controls with active *Cannabis* disorder ($p < .05$). Patients who continued to use *Cannabis* during treatment of their first episode of psychosis reported significantly lower anticipatory pleasure compared to those who had a lifetime *Cannabis* diagnosis but were able to maintain abstinence ($F(1, 60) = 5.6, p = .021$). Frequency of *Cannabis* use was negatively correlated to anticipatory and consummatory pleasure (Pearson $R = -.46, -.48$ respectively) in 37 patients currently using *Cannabis* but not in 46 *Cannabis*-using controls (partial $R = -.04, -.07$ respectively). Anticipatory pleasure may not be decreased in early psychosis patients [28]. The lower hedonic response may be associated with persistent, heavy *Cannabis* use in patients in the early phase of psychotic disorders.

Cannabis can induce transient psychotic and anxiety symptoms and long-lasting disorders. The acute psychoactive effects of delta-9-tetrahydrocannabinol may be modulated by previous *Cannabis* exposure. Secondary data analyses tested whether modest previous *Cannabis* exposure modulated the acute effects of delta-9-tetrahydrocannabinol on attentional salience and emotional processing and their neurophysiological substrates. Twenty-four healthy men used fMRI to participate in a double-blind, randomized, placebo-controlled, repeated-measures, within-subject, and delta-9-tetrahydrocannabinol challenge study.

Compared with nonusers (NUs; $n = 12$; < 5 -lifetime *Cannabis* joints smoked), abstinent-modest *Cannabis* users (CUs; $n = 12$; $24.5 \pm$ nine-lifetime *Cannabis* joints smoked) showed less efficient attentional salience processing and recruited different/additional brain areas to process attentional salient and emotional stimuli ($p \leq .01$). The delta-9-tetrahydrocannabinol challenge disrupted attentional salience and emotional-processing-related brain activity and induced transient anxiety and psychotic symptoms ($p \leq .02$). However, delta-9-tetrahydrocannabinol-induced psychotic symptoms and attentional salience behavioral impairment were more pronounced in NUs compared with CUs ($p \leq .04$). Also NUs under delta-9-tetrahydrocannabinol shifted toward recruitment of other brain areas to perform the tasks.

Cannabis users displayed residual effects of *Cannabis* exposure but more blunted responses to the acute symptomatic, behavioral, and neurophysiological effects of delta-9-tetrahydrocannabinol, which were more marked in Nus. Conversely, CUs were less affected by the acute challenge in an exposure-dependent manner, showing a neurophysiological pattern similar to that of NUs under a placebo [29]. Only in NUs, delta-9-tetrahydrocannabinol-induced psychotic symptoms and cognitive impairment severity was associated with a more pronounced neurophysiological alteration ($p \leq .048$).

Experimental and clinical evidence points to a close relationship between *Cannabis*, the endogenous cannabinoid system, and schizophrenia. Animal and human studies found dysregulation of endocannabinoid signaling in psychosis [84]. Elevated anandamide levels in schizophrenia patients negatively correlated with psychotic symptomatology indicate a protective role, whereas

2-arachidonoylglycerol appears to counteract psychosis-related cognitive impairments. Pharmacological manipulation of the endogenous cannabinoid system might be associated with potential antipsychotic properties.

Preclinical studies using different animal models of psychosis and clinical trials investigating the antipsychotic effects of cannabidiol and rimonabant are presented together with the possible underlying mechanisms of action. The results predominantly confirm the hypothesis of an antipsychotic activity of both cannabinoids. In comparison, cannabidiol appears superior to rimonabant with a pharmacological profile similar to atypical antipsychotic drugs.

Psychoses are relatively low-prevalence disorders that disproportionately impact individuals and society negatively. *Cannabis* use is one factor that can exacerbate the negative consequences associated with psychotic disorders. Studies have examined the effects or reasons for using *Cannabis* self-reported by individuals with psychosis to compare such factors directly in individuals with and without psychosis within a single study [36].

At baseline and follow-up, the participants with psychosis most commonly reported using *Cannabis* for positive mood alteration (36% and 42%), coping with negative affect (27% and 29%), and for social activity reasons (38% and 29%). The control group most commonly reported using *Cannabis* for relaxation (34% and 43%) and social activity reasons (49% and 51%). Participants with psychosis were less likely to report relaxation as the most important effect after use (27%) or expect it at follow-up (49%) compared to the control group (53% and 70%). In both groups, addiction and positive affect enhancement were the composite variable scores that correlated most consistently with concurrent amount and frequency of use.

As *Cannabis* use is more frequent in patients with psychosis than in the general population and is known to be a risk factor for psychosis, the question arises whether *Cannabis* contributes to recently detected brain volume reductions in schizophrenic psychoses. A study investigated how *Cannabis* use is related to the cingulum volume, a brain region involved in the pathogenesis of schizophrenia, in a sample of at-risk mental state and first-episode psychosis subjects [37].

Cannabis use was assessed with the Basel Interview for Psychosis. Using repeated measures analyses of covariance, it was investigated whether current *Cannabis* use is associated with the cingulum volume, correcting for age, gender, alcohol consumption, whole brain volume, and antipsychotic medication. A cross-sectional magnetic resonance imaging study of manually traced cingulum in 23 first-episode psychosis subjects and 37 at-risk mental state subjects was performed. There was a significant three-way interaction between region (anterior/posterior cingulum), hemisphere (left/right cingulum), and *Cannabis* use (yes/no).

Post hoc analyses revealed that it was due to a significant negative effect of *Cannabis* use on the volume of the posterior cingulum, independent of the hemisphere and diagnostic group and all other covariates that were controlled for. In the anterior cingulum, a significant negative effect was found only for the left hemisphere, which was again independent of the diagnostic group. The negative associations of current *Cannabis* use with grey matter volume of the cingulate cortex, a region rich in cannabinoid CB1 receptors, were found. As this finding has not been

consistently found in healthy controls, it might suggest that at-risk mental state and first-episode psychosis subjects are particularly sensitive to exogenous activation of the receptors.

Establishment of residual cognitive and psychotic effects (effects present at the time that all active cannabinoids are eliminated from the body) putatively produced by prolonged heavy *Cannabis* use is difficult because of many confounding variables like slow elimination of active cannabinoids, lack of supervision during abstinence, poor use of well-matched control groups and the presence of withdrawal symptoms. Residual cognitive effects were observed in some but not all tests after prolonged heavy *Cannabis* use [39]. The effects were mostly mild and the relationship between *Cannabis* use, psychotic effects, and schizophrenia was unclear. *Cannabis* conceivably gave relief, but it also appeared that *Cannabis* caused schizophrenia in young people and (or) enhanced the symptoms, especially in young people poorly able to cope with stress or in whom the antipsychotic therapy was unsuccessful.

Cannabis use increases rates of psychotic relapse and treatment failure in schizophrenia patients. *Cannabis* use reduces the efficacy of antipsychotic drugs, but there has been no direct demonstration of this in a controlled study. Exposure to delta-9-tetrahydrocannabinol reverses the neurobehavioral effects of the antipsychotic drug risperidone in mice. Delta-9-THC exposure did not influence D2 and 5-HT_{2A} receptor binding, the major targets of antipsychotic action, but it lowered the brain concentrations of risperidone and its active metabolite, 9-hydroxy risperidone.

As risperidone and its active metabolite are excellent substrates of the ABC transporter P-glycoprotein (P-gp), it has been hypothesized that delta-9-THC might increase P-gp expression at the blood-brain barrier and thus enhance efflux of risperidone and its metabolite from brain tissue. It has been confirmed that the brain disposition of risperidone and 9-hydroxy risperidone is strongly influenced by P-gp, as P-gp knockout mice displayed greater brain concentrations of the drugs than wild-type mice.

Furthermore, it has been demonstrated that delta-9-THC exposure increased P-gp expression in various brain regions important to risperidone's antipsychotic action. It was shown that delta-9-THC exposure did not influence the neurobehavioral effects of clozapine [105]. Clozapine shares a very similar antipsychotic mode of action to risperidone but, unlike risperidone, is not a P-gp substrate. The results imply that clozapine or non-P-gp substrate antipsychotic drugs may be better first-line treatments for schizophrenia patients with a history of *Cannabis* use.

Cannabis use has a negative impact on psychosis. A study determines the efficacy of a specific motivational intervention on young *Cannabis* users with psychosis. The participants under 35 years were randomly assigned to Treatment As Usual (TAU) alone or treatment as usual plus Motivational Intervention (MI + TAU). Assessments were completed at baseline and three, six, and 12 months follow-up for 62 participants (32 TAU and 30 MI + TAU). TAU was comprehensive and included case management, early intervention, and a mobile team when needed.

Cannabis use decreased in both groups at follow-up. The participants who received MI in addition to TAU displayed a greater reduction in the number of joints smoked per week and greater confidence to change *Cannabis* use at 3- and 6-month

follow-ups. However, differences between groups were not significant at 12 months. Motivational intervention is well accepted by patients with psychosis and has a short-term impact on *Cannabis* use when added to standard care. However, the differential effect was not maintained at 1-year follow-up. The motivational intervention appears to be a useful active component to reduce *Cannabis* use which should be integrated into routine clinical practice [106].

Delta-9-tetrahydrocannabinol and cannabidiol have distinct symptomatic and behavioral effects. An assessment was conducted on whether pretreatment with CBD can prevent the acute psychotic symptoms induced by delta-9-THC. Functional magnetic resonance imaging (fMRI) was used in healthy volunteers to examine whether delta-9-THC and CBD had opposite effects on regional brain function [108]. Fifteen healthy men with minimal earlier exposure to *Cannabis* were scanned while performing a verbal memory task, a response inhibition task, a sensory processing task, and when viewing fearful faces.

The subjects were scanned on three occasions, each preceded by oral administration of delta-9-THC, CBD, or placebo. BOLD responses were measured using fMRI. In the second experiment, six healthy volunteers were administered delta-9-THC intravenously on two occasions, after placebo or CBD pretreatment, to examine whether CBD could block the psychotic symptoms induced by delta-9-THC.

In the second experiment, pretreatment with CBD prevented the acute induction of psychotic symptoms by delta-9-tetrahydrocannabinol. Delta-9-THC and CBD had opposite effects on activation relative to placebo in the striatum during verbal recall, in the hippocampus during the response inhibition task, in the amygdala when subjects viewed fearful faces, in the superior temporal cortex when subjects listened to the speech, and in the occipital cortex during visual processing. Delta-9-THC and CBD can have opposite effects on regional brain function, which may underlie their different symptomatic and behavioral effects and CBD's ability to block the psychotogenic effects of delta-9-THC.

The aberrant processing of salience is a fundamental factor underlying psychosis. *Cannabis* can induce acute psychotic symptoms, and its chronic use may increase the risk of schizophrenia. Its psychotic effects may be mediated through an influence on attentional salience processing. Volunteers were studied using event-related functional magnetic resonance imaging to examine the effects of delta-9-tetrahydrocannabinol and cannabidiol on regional brain function during salience processing [109]. On three occasions, after the administration of delta-9-THC, CBD, or placebo, the participants performed a visual oddball detection paradigm that involved allocating attention to infrequent (oddball) stimuli within a string of frequent (standard) stimuli.

Fifteen healthy men with minimal previous *Cannabis* use participated in the evaluation. During the processing of oddball stimuli, relative to placebo, delta-9-THC attenuated activation in the right caudate but augmented it in the right prefrontal cortex. Delta-9-tetrahydrocannabinol also reduced the response latency to standard relative to oddball stimuli. The effect of delta-9-THC in the right caudate was negatively correlated with the severity of the psychotic symptoms it induced and its effect on response latency.

Delta-9-Tetrahydrocannabinol and CBD differentially modulate prefrontal, striatal, and hippocampal functions during attentional salience processing. The effects of CBD on task-related activation were in the opposite direction of delta-9-THC; relative to placebo, CBD augmented left caudate and hippocampal activation but attenuated right prefrontal activation. The effects may contribute to the effects of *Cannabis* on psychotic symptoms and the risk of psychotic disorders.

Cannabis can induce transient psychotic symptoms, but not all users experience the adverse effects. In a double-blind, placebo-controlled, pseudo-randomized study, 21 healthy men with minimal experience of *Cannabis* use were given either 10 mg delta-9-THC or placebo orally [110]. The neural response to delta-9-tetrahydrocannabinol in healthy volunteers in whom the drug did or did not induce acute psychotic symptoms was conducted.

Drugs that induce psychosis may increase task-irrelevant random neural activity or neural noise. Increased levels of neural noise have been demonstrated in psychotic disorders. The hypothesis that neural noise could also be involved in the psychotomimetic effects of delta-9-tetrahydrocannabinol was tested. Neural noise was indexed by measuring the level of randomness in the electroencephalogram during the prestimulus baseline period of an oddball task using Lempel-Ziv complexity, a nonlinear measure of signal randomness.

The acute, dose-related effects of delta-9-tetrahydrocannabinol on Lempel-Ziv complexity and signal power were studied in humans ($n = 24$) who completed three test days, during which they received intravenous delta-9-tetrahydrocannabinol (placebo, 0.015 and 0.03 mg/kg) in a double-blind, randomized, crossover and counterbalanced design.

Delta-9-tetrahydrocannabinol increased neural noise in a dose-related manner. There was a strong positive relationship between neural noise and the psychosis-like positive and disorganization symptoms induced by delta-9-tetrahydrocannabinol, independent of total signal power [112]. There was no relationship between noise and negative-like symptoms.

Also delta-9-tetrahydrocannabinol reduced total signal power during both active drug conditions compared to placebo, but no relationship was detected between signal power and psychosis-like symptoms. At doses that produced psychosis-like effects, delta-9-tetrahydrocannabinol increased neural noise in humans in a dose-dependent manner. Furthermore, increases in neural noise were related to delta-9-tetrahydrocannabinol-induced psychosis-like symptoms but not negative-like symptoms. The findings indicated that increases in neural noise might contribute to the psychotomimetic effects of delta-9-tetrahydrocannabinol.

Dependence and Behavioral Disturbances

Although *Cannabis* is a recreational drug, it can potentially lead to dependence and behavioral disturbances, and its heavy use may increase the risk for psychotic disorders. Delta-9-tetrahydrocannabinol produces various pharmacological effects in

animals and humans. Studies to understand the mechanism of action of *Cannabis* concentrate on the pharmacokinetics and pharmacodynamics of cannabinoids in humans. However, there is limited research on the chronic adverse effects and retention of cannabinoids in human subjects.

Cannabis can be detected in body fluids following exposure through active/passive inhalation and exposure through breastfeeding. *Cannabis* detection depends on accurate analytical procedures for detecting metabolites and verifying recent use. In a review, an attempt was made to summarize the properties of *Cannabis* and its derivatives and to discuss the implications of its use with an emphasis on bioavailability, the limit of detection, carry-over period, and passive inhalation, important factors for detection and diagnosis.

In addition to producing dependence, *Cannabis* use is associated with a wide range of psychiatric disorders. While there is a clear relationship between the use of *Cannabis* and psychosis, different hypotheses have been propounded. One such, which describes psychosis occurring exclusively with *Cannabis* use, has limited evidence. There is strong evidence that *Cannabis* use may precipitate schizophrenia or exacerbate its symptoms. There is also reasonable evidence that *Cannabis* use exacerbates the symptoms of psychosis [31].

Heavy *Cannabis* use (30–50 mg oral and 8–30 mg smoked) can specifically cause a mania-like psychosis and, more generally, act as a precipitant for manic relapse in bipolar patients. *Cannabis* exposure may be a contributing factor that interacts with other known and unknown (genetic and environmental) factors culminating in psychiatric illness. In many developed countries, persons with severe mental disorders are more likely to use, abuse, and become dependent on psychoactive substances, especially *Cannabis*, than the general population.

***Cannabis* and Pregnancy**

With the prevalence, potency, and acceptability of *Cannabis* use during pregnancy, it is important to understand the developmental effects of prenatal *Cannabis* exposure. A review discussed the methodological considerations for studies of prenatal *Cannabis* exposure, including the assessment of exposures, covariates, and outcomes, and review findings from prospective longitudinal studies of prenatal *Cannabis* exposure.

There is evidence for associations between prenatal *Cannabis* exposure and regional growth at birth, but not for long-term effects on growth. Prenatal *Cannabis* exposure appears to have subtle yet enduring effects on memory and achievement in children and adolescents. Despite differences in sample demographics and measurement, there are remarkably consistent effects of prenatal *Cannabis* exposure on externalizing behaviors, such as delinquency and substance use, which persist into adulthood.

Animal studies demonstrate direct effects on the development of the brain via the activation of the endogenous endocannabinoid systems [43]. *Cannabis*-induced

activation of the endocannabinoid system causes alterations in the release of neurotransmitters and the modulation of brain plasticity in neural pathways that underlie cognition, motivation, and behavior regulation. Longitudinal analyses demonstrate the importance of early *Cannabis* initiation for pathways between prenatal *Cannabis* exposure and adult functioning, including substance use and abuse, memory deficits, and psychotic symptoms. It is recommended that research consider *Cannabis* use before pregnancy, the timing and route of exposure, polysubstance exposures, and intergenerational effects.

Cannabis use among pregnant women is increasing with permissive sociocultural attitudes toward it. Prenatal *Cannabis* exposure, however, is associated with adverse outcomes among offspring, ranging from reduced birth weight to child psychopathology. It has been shown that male rat offspring prenatally exposed to delta-9-tetrahydrocannabinol exhibit extensive molecular, cellular, and synaptic changes in dopamine neurons of the ventral tegmental area, resulting in a susceptible mesolimbic dopamine system associated with a psychotic-like endophenotype. The phenotype only reveals itself upon a single exposure to delta-9-THC in males but not females. The impact of prenatal *Cannabis* exposure on female behaviors and mesolimbic dopamine system function by combining in vivo single-unit extracellular recordings in anesthetized animals and ex vivo patch clamp recordings, along with neurochemical and behavioral analyses, was characterized.

Prenatal *Cannabis* exposed female offspring do not show any spontaneous or delta-9-THC-induced behavioral disease-relevant phenotypes [66]. The delta-9-THC-induced increase in dopamine levels in the nucleus accumbens was reduced in prenatal *Cannabis* exposed female offspring, even when ventral tegmental area dopamine activity in vivo and ex vivo did not differ compared to control. The findings indicate that prenatal *Cannabis* exposure impacts mesolimbic dopamine function and its related behavioral domains in a sex-dependent manner and warrant further investigations to decipher the mechanisms determining the sex-related protective effect from intrauterine delta-9-THC exposure.

Mortality studies have revealed qualitative and quantitative changes in causes of death among heroin and injecting drug users, probably due to increasing exposure to harm reduction programs, the introduction of highly active antiretroviral therapy, the aging of drug users, and rising concurrent use of illegal drugs and prescription drugs. For morbidity, nonfatal overdose is still one of the most important concerns; likewise, the higher prevalence of hepatitis C among noninjecting drug users and hepatitis A, B, and C coinfection. *Cannabis* use has been consistently associated with the emergence of psychotic symptoms, yet that seems not to be the case for anxiety and depressive disorders.

The use of 3,4-methylenedioxymethamphetamine has been linked with short-term negative effects on cognitive performance (i.e., visual memory). A series of longitudinal studies have shown enduring unfavorable effects of prenatal cocaine and *Cannabis* exposure on children's physical, cognitive, and language development [67].

The growing acceptance and legalization of *Cannabis* have markedly increased the risks of child psychopathology, including psychotic-like experiences, which

lowers the age of onset for a first psychotic episode. As most patients with schizophrenia go through a premorbid condition long before this occurs, understanding the neurobiological underpinnings of the prodromal stage of the disease is critical to improving illness trajectories and therapeutic outcomes. The ventral tegmental area dopamine neuron and sensorimotor gating functions of psychotic-like endophenotype rats exposed to acute stress or a challenge of the D2 receptor agonist apomorphine was characterized by using in vivo single-unit extracellular recordings and Prepulse Inhibition (PPI) analyses.

Male rat offspring prenatally exposed to delta-9-tetrahydrocannabinol exhibit extensive molecular and synaptic changes in dopaminergic neurons of the ventral tegmental area, converging on a hyperdopaminergic state and leading to a silent psychotic-like endophenotype that is unmasked by a single exposure to delta-9-THC [73]. The data extend the knowledge of the multifaceted sequelae imposed by psychotic-like endophenotypes in the mesolimbic dopamine system of male preadolescent rats.

At prepuberty, psychotic-like endophenotype male rat offspring display a reduced population activity of ventral tegmental area dopamine neurons in vivo, most of which are tonically active. Psychotic-like endophenotype male progeny also exhibit enhanced sensitivity to dopamine D2 receptor activation and a vulnerability to acute stress, which is associated with compromised sensorimotor gating functions. This renders a neural substrate susceptible to subsequent challenges that may trigger psychotic-like outcomes.

The ethnic minority position is associated with an increased risk for psychotic outcomes, which experiences of social exclusion, defeat, and discrimination may mediate. Sexual minorities are subject to similar stressors. A study was conducted to examine whether sexual minorities are at increased risk for psychotic symptoms and to explore mediating pathways [44]. A cross-sectional survey assessed the cumulative incidence of psychotic symptoms with the Composite International Diagnostic Interview in two random general population samples (NEMESIS-1 and NEMESIS-2).

The participants were sexually active and aged 18–64 ($n = 5927$, $n = 5308$). Being lesbian, gay, or bisexual (LGB) was defined as having sexual relations with at least one same-sex partner during the past year. Lifetime experience of any psychotic symptom was analyzed using logistic regression, adjusted for gender, educational level, urbanicity, foreign-born parents, living without a partner, *Cannabis* use, and other drug use.

The rate of any psychotic symptom was elevated in the LGB population as compared with the heterosexual population both in NEMESIS-1 [odds ratio (OR) 2.56, 95% confidence interval (CI) 1.71–3.84] and NEMESIS-2 (OR 2.30, 95% CI 1.42–3.71). Childhood trauma, bullying, and experience of discrimination partly mediated the association. The finding that LGB orientation is associated with psychotic symptoms adds to the expanding literature linking minority status with psychosis and other mental health problems. It suggests that exposure to minority stress represents an important mechanism.

***Cannabis* and the Vulnerable Adolescent Group**

As countries continue to legalize recreational *Cannabis* use after its medical approval, India continues to debate whether to decriminalize *Cannabis*. One of the strongest arguments against *Cannabis* decriminalization is the dependence on *Cannabis* and the risk of psychosis, at least in vulnerable groups. Endocannabinoids are involved in neuronal proliferation and differentiation during the patterning of the central nervous system. Aberrant neurostimulation caused by repeated heavy exocannabinoid exposure may increase the probability of propsychotic experiences.

Adolescent *Cannabis* use has been shown in many studies to increase the risk of later psychosis [25]. Childhood trauma is associated with both substance misuse and the risk of psychosis. A study was conducted to investigate whether there is a significant interaction between *Cannabis* use and childhood trauma in increasing the risk of experiencing psychotic symptoms during adolescence. Psychiatric interviews using the Schedule for Affective Disorders and Schizophrenia for School-Age Children semistructured instrument were carried out with 211 adolescents aged between 12 and 15 years and their parents as part of a population-based study. The interview enquired about early traumatic events, *Cannabis* use, and adolescent psychiatric symptoms.

In separate analyses, both *Cannabis* use and childhood trauma were significantly associated with the risk of experiencing psychotic symptoms. However, the presence of childhood trauma and early *Cannabis* use significantly increased the risk of psychotic symptoms beyond the risk posed by either factor alone, indicating a greater than additive interaction between childhood trauma, which may have implications for identifying individuals at high risk of experiencing psychotic symptoms. For example, measures to actively discourage or intensively treat *Cannabis* use in children and adolescents who have experienced abuse may help to prevent the development of psychosis in this vulnerable group.

Cannabis abuse in adolescence is associated with an increased risk of psychotic disorders. The Disrupted-In-Schizophrenia-1 (DISC1) protein is a driver for major mental illness by influencing neurodevelopmental processes [60]. Wild-Type and dominant-negative-DISC1 (DN-DISC1) mice were injected with delta-9-THC (10 mg/kg) or a vehicle for 10 days during the mid-adolescence-equivalent period. Utilizing a unique mouse model based on the host (DISC1) X environment, delta-9-THC administration interaction, the pathobiological basis by which delta-9-THC exposure elicits psychiatric manifestations were examined. Behavioral tests were conducted to assess exploratory activity (open field test, light-dark box test) and cognitive function (novel object recognition test). The electrophysiological effect of delta-9-THC was evaluated using acute hippocampal slices and hippocampal cannabinoid receptor type 1 and brain-derived neurotrophic factor (BDNF) protein levels.

The results indicate that delta-9-THC exposure elicits deficits in exploratory activity and recognition memory with reduced short-term synaptic facilitation and loss of BDNF surge in the hippocampus of DN-DISC mice but not in wild-type

mice. Over-expression of BDNF in the hippocampus of delta-9-THC-treated DN-DISC1 mice prevented the impairment in recognition memory. The results imply that induction of BDNF following adolescence delta-9-THC exposure may serve as a homeostatic response to maintain proper cognitive function against exogenous insult. The BDNF surge in response to delta-9-THC is perturbed in the presence of mutant DISC1, indicating that DISC1 may be a useful probe to identify the biological cascades involved in the neurochemical, electrophysiological, and behavioral effects of *Cannabis* related psychiatric manifestations.

Cannabis consumption can induce psychiatric symptoms in otherwise healthy subjects and unmask a florid psychotic picture in patients with a prior psychotic risk. Chronic and long-term *Cannabis* exposure may exert significant negative effects in brain areas enriched with cannabinoid receptors. It remains unclear if a brain alteration caused by *Cannabis* dependency may lead to a clinically significant phenotype or a psychotic outbreak at some point in an abuser's life. A study investigated the morphological brain differences between chronic *Cannabis* users with *Cannabis*-induced psychosis (CIP) and nonpsychotic *Cannabis* users (NPCU) without any psychiatric conditions and correlated brain deficits with selective socio-demographic, clinical, and psychosocial variables [61].

Magnetic resonance imaging scans of ten CIP patients and 12 NPCUs were acquired and the type of drug, frequency, duration and socio-demographic, clinical, and psychosocial dependency parameters were measured. *Cannabis*-induced psychosis patients had extensive grey matter (GM) decreases in the right superior frontal gyrus, right precentral, right superior temporal gyrus, insula bilaterally, right precuneus, right medial occipital gyrus, right fusiform gyrus, and left hippocampus in comparison to chronic *Cannabis* users without psychosis.

Cannabis-induced psychosis patients negatively correlated with the Brief Psychiatric Rating Scale (BPRS) domain, BPRS-Activity, and selective GM volumes. The results indicate that *Cannabis*-induced psychosis is characterized by selective brain reductions not present in NPCU. Therefore, neuroimaging studies provide a potential ground for identifying putative biomarkers associated with the risk of developing psychosis in *Cannabis* users.

Animal and human studies have demonstrated increased but abnormal cortical and subcortical area activation due to chronic *Cannabis* use [10]. Some areas are involved in the pathogenesis of psychosis or schizophrenia. A literature review hypothesized the possible role of *Cannabis* in causing or precipitating psychosis through repeated aberrant neurostimulation. The aberrant neurostimulation model of *Cannabis* psychosis may explain pathogenesis, individual vulnerabilities, and the developing therapeutic strategies for this debated entity.

A retrospective observational study of patients at least 12 years of age and who had visited a psychiatrist in the emergency unit of the Centre Hospitalier Universitaire de Sherbrooke documents the short-term impact of recreational *Cannabis* on active *Cannabis* use, *Cannabis* use disorder, and various psychotic disorders [11]. The population was divided into an adult (over 18) and a teenager group (12–17). The data were compared to consultations made earlier using a generalized linear mixed

model. The consultations of this type over 5 months immediately following the legalization of recreational *Cannabis* in Canada were included.

Consultations in patients over 18 years of age in prelegalization ($n = 1247$) and postlegalization ($n = 1368$) groups revealed a statistically significant increase in the use of *Cannabis* (28.0–37.1%; odds ratio [OR] = 1.81 [1.34–2.44], $p = 0.00011$), as well as an increase in diagnoses of active *Cannabis* use disorder (17.7–24.3%; OR = 1.53 [1.13–2.08], $p = 0.0064$), was observed. The increase in *Cannabis* use disorder was more prominent among patients between 18 and 24 years of age (17.3–25.9%; OR = 2.27 [1.17–4.40], $p = 0.015$). There was no statistically significant difference in terms of psychotic disorder diagnoses (27.4–29.2%; OR = 1.17 [0.84–1.63], $p = 0.35$). Conversely, a greater proportion of patients with a personality disorder diagnosis in the postlegalization period (39.6–44.9%; OR = 1.35 [1.02–1.80], $p = 0.038$) was observed.

Examination of pediatric (under 18 years of age) consultations revealed no statistically significant differences. Although an affirmative conclusion was tentative, the findings suggest a first link between the legalization of *Cannabis* in Canada and increased diagnoses of *Cannabis* use disorder and *Cannabis* use in general among patients in a university hospital psychiatric emergency department.

Cannabis use is a frequent problem among people with psychotic disorders related to a worse prognosis. Studies described the reasons for and the subjective effects of *Cannabis* use in patients with psychosis in 73 studies, of which 12 met the inclusion criteria. Most studies included heterogeneous patients at different illness stages, and a few included a nonpsychotic comparison group [12]. The most common reasons for *Cannabis* use were those related to social activities, mood disturbance, relaxation, and getting high. The most common reasons for *Cannabis* use in patients with psychosis were social activities, mood disturbances, relaxation, and getting high. It reveals that a more homogeneous methodology is needed to include different illness stages, to facilitate the interventions to reduce *Cannabis* use in all phases of psychotic disorder development.

Psychiatric disorders are consistent with the gene x environment model, and non-specific environmental factors such as childhood trauma, urbanity, and migration have been implicated. The factors dysregulate the biological pathways involved in response to stress. Stress is a well-known precipitating factor implicated in psychiatric disorders such as depression, bipolar disorder, anxiety, and possibly schizophrenia. More precisely, psychosocial stress induces dysregulation of the hypothalamic–pituitary–adrenal axis and could modify neurotransmission, which raises the question of the involvement of stress-related biological changes in psychotic disorders.

The literature reveals dysregulation of the hypothalamic–pituitary–adrenal axis in schizophrenia. The dysregulation seems present in the prodromal phases, subjects for ultra-high risk, early schizophrenia, and first episode psychosis. Following the stress-vulnerability model, stress could act directly on the psychotic onset and precipitate the transition of vulnerable subjects to full-blown psychosis.

Stress has been studied for many years in schizophrenia by subjective methods (questionnaires) or objective methods (standardized experimental protocols) with

biological sampling and brain-imaging methods. The methods have suggested a link between dysregulation of the hypothalamic–pituitary–adrenal axis and psychotic symptoms through abnormal basal cortisol levels and flattened reactivity to social stress. A literature review analyzed the stress and the onset of schizophrenia, considering the causal role vs. the associated role of hypothalamic–pituitary–adrenal axis dysregulation in schizophrenia and the factors that influence it, particularly during prodromal and earlier phases.

Imaging results indicate indirect modifications, including abnormal pituitary or hippocampal volume. Several factors dysregulating the hypothalamic–pituitary–adrenal axis have also been highlighted, such as consumption of drugs (i.e., *Cannabis*), childhood trauma, or genetic factors (such as COMT or MTHFR variants). Psychological stress induces subcortical dopaminergic activation attributable to hypothalamic–pituitary–adrenal axis dysregulation. The dysregulation is present in the prodromal phase in patients who have experienced a first psychotic episode and in siblings of schizophrenic patients. Stress dysregulation is a plausible hypothesis for understanding the psychosis onset.

The dysregulation fits into the gene x environment model. In subjects with genetic predispositions, stressful environmental factors can modify biological pathways implicated in psychiatric disorders, promoting the emergence of symptoms. The effect of stress on brain pathways could participate in the mechanisms underlying the onset of psychotic symptoms, both as a precipitating factor and as a marker of a predisposing vulnerability [13]. However, many confounding factors obscure the literature, and further studies are needed in schizophrenic patients, ultra-high risk and early schizophrenia, and first-episode psychosis patients to clarify the precise role of stress in psychotic transition. Identification of stress biomarkers could help diagnosis and prognosis and pave the way for specific care strategies based on stress-targeted therapies.

Studies in rodents have confirmed the effects and revealed that adolescent exposure results in structural changes and impaired synaptic plasticity, impacting the front-limbic systems critically involved in higher brain functions. The endocannabinoid system plays an important role in brain maturation. Its over-activation by cannabinoid receptor type 1 agonists (e.g., delta-9-THC) during adolescence and the resulting changes in neuroplasticity could alter brain maturation and cause long-lasting changes that persist in the adult brain. Exposure to cannabinoids can have a long-term impact on the brain, with an interindividual variability that could be conveyed by personal and family history of psychiatric disorders and genetic background. Adolescence and early adulthood are critical periods of vulnerability.

The associations of first-episode psychosis, metabolic abnormalities, childhood trauma, and risk of developing psychosis are well established; however, the relationship between childhood trauma and metabolic dysregulation in abnormal glucose metabolism is very limited. Tests to determine whether, already at illness onset, first-episode psychosis patients with a history of childhood trauma show dysregulation of a broad range of glucose metabolism markers were conducted [63]. In particular, in 148 first-episode psychosis patients' serum concentrations of c-peptide, insulin, plasminogen-activator-inhibitor-1, resistin, visfatin, glucagon,

glucagon-like peptide-1, gastric-inhibitor-peptide, leptin, and ghrelin were evaluated. The Childhood Experience of Care and Abuse Questionnaire and stressful life events with a semistructured interview assessed childhood trauma. Psychopathology, *Cannabis* and tobacco habits, and Body Mass Index were recorded.

Serum concentrations of markers were analyzed from peripheral blood. Ninety-five patients (56% males, mean age 29.5) reported childhood trauma. Multivariate models showed that childhood trauma is associated only with the concentrations of c-peptide and insulin after adjusting for age, sex, BMI, and stressful life events. First-episode psychosis patients who had experienced childhood trauma showed higher c-peptide and insulin serum concentrations.

Childhood trauma might be associated with metabolic abnormalities in the first stage of psychosis, indicating that a thorough anamnestic evaluation at psychosis onset that would include the history of childhood trauma could be helpful for clinicians in order to implement early programs of healthy lifestyle education and to guide the choice of therapeutic interventions for trauma.

Adolescent *Cannabis* use is associated with adult psychopathology. When delta-9-tetrahydrocannabinol is administered to adolescent rats, there are long-lasting effects in adults. A study to determine the specific adult bio-behavioral profile after adolescent low-dose delta-9-THC, which mirrors adolescent recreational *Cannabis* use, was conducted in adolescent male Sprague-Dawley rats treated with escalating low-dose of delta-9-THC. They were evaluated for their spontaneous locomotion, sensorimotor gating, higher order, and spatial cognitive functions in adulthood [64].

A study investigated the neural correlates of psychotic-like experiences in youths during tasks involving inhibitory control, reward anticipation, and emotion processing; it also tested whether the neurofunctional correlates of risk were predictive of psychotic symptoms 2 years later [65]. Functional imaging responses to three paradigms – the stop-signal, monetary incentive delay, and faces tasks – were collected in youths aged 14 years as part of the IMAGEN study. At baseline, the youths were assessed on psychotic-like experiences, and those reporting significant experiences were compared with matched control subjects.

The prediction analyses were conducted with a subsample ($n = 246$) and the full sample ($n = 1196$). Significant brain activity differences between the groups were used to predict, with cross-validation, the presence of psychotic symptoms in the context of mood fluctuation at age 16, assessed in the full sample. Relative to control subjects, youths reporting psychotic-like experiences showed increased hippocampus/amygdala activity while processing neutral faces and reduced dorsolateral prefrontal activity during failed inhibition.

The most notable regional difference for classifying 16-year-olds with mood fluctuation and psychotic symptoms relative to the control groups (those with mood fluctuations but no psychotic symptoms and those with no mood symptoms) was the hyperactivation of the hippocampus/amygdala when controlling for baseline psychotic-like experiences and *Cannabis* use. The results indicate the importance of the limbic network's increased response to neutral facial stimuli as a marker of the

extended psychosis phenotype. The findings might enable early intervention strategies for at-risk youths.

In rodent models, chronic exposure to delta-9-tetrahydrocannabinol, during adolescence leads to abnormal behavior in adulthood. In female rats, this maladaptive behavior is characterized by endophenotypes for depressive-like and psychotic-like disorders and cognitive deficits. Most depressive-like behaviors triggered by adolescent delta-9-tetrahydrocannabinol exposure can be rescued by manipulating endocannabinoid signaling in adulthood with the anandamide-inactivating enzyme FAAH inhibitor, URB597.

The molecular mechanisms underlying URB597's antidepressant-like properties remain established. The impact of adult URB597 treatment on the cellular and functional neuroadaptations that occurred in the prefrontal cortex and dentate gyrus of the hippocampus upon delta-9-tetrahydrocannabinol during adolescence through biochemical, morphofunctional, and electrophysiological studies was examined. It was found that the positive action of URB597 is associated with the rescue of delta-9-tetrahydrocannabinol-induced deficits in endocannabinoid-mediated signaling and synaptic plasticity in the prefrontal cortex and the recovery of functional neurogenesis in the dentate gyrus of the hippocampus [101].

Moreover, the rescue property of URB597 on depressive-like behavior requires the activity of the CB1 cannabinoid receptor. By providing novel insights into the cellular and molecular mechanisms of URB597 at defined cortical and hippocampal circuits, the results highlight that positive modulation of endocannabinoid signaling could be a strategy for treating mood alterations secondary to adolescent Cannabis use.

Poorer memory performance and psychotic-like behaviors were present after adolescent delta-9-THC treatment in male rats, without alterations in the emotional component. At the cellular level, the expression of the NMDA receptor subunit, GluN2B, and the levels of the AMPA subunits, GluA1 and GluA2, were significantly increased in hippocampal postsynaptic fractions from delta-9-THC-exposed rats compared to controls. Increases in the levels of the presynaptic marker, synaptophysin, and the postsynaptic marker, PSD95, were also present.

Interestingly, KCl-induced [(3)H]D-ASP release from hippocampal synaptosomes, but not gliosomes, was significantly enhanced in delta-9-THC-treated rats compared to controls. Moreover, in the same brain region, the adolescent delta-9-THC treatment also resulted in a persistent neuroinflammatory state, characterized by increased expression of the astrocyte marker, GFAP, increased levels of the pro-inflammatory markers, TNF- α , iNOS, and COX-2, as well as a concomitant reduction of the anti-inflammatory cytokine, IL-10. None of the alterations was observed in the prefrontal cortex [103].

The phenotype observed in males is mainly associated with marked dysregulations in the hippocampus. In contrast, the prevalence of alterations in the emotional sphere in females is associated with profound changes in the prefrontal cortex. Together with previous findings in females, the data suggest that the sex-dependent detrimental effects induced by adolescent delta-9-THC exposure on adult behavior

may rely on its ability to trigger different region-dependent changes in glutamate synapse and glial cells.

Cannabis use confers a twofold increase in the risk for psychosis, with adolescent use conferring an even greater risk. A high-low activity catechol-O-methyltransferase (COMT) polymorphism may modulate the effects of adolescent delta-9-tetrahydrocannabinol exposure on the risk for adult psychosis. Mice with the knockout of the COMT gene were treated chronically with delta-9-THC (4.0 and 8.0 mg/kg over 20 days) during either adolescence (postnatal days (PDs) 32–52) or adulthood (PDs 70–90). The effects of delta-9-THC exposure were assessed in adulthood across behavioral phenotypes relevant to psychosis: exploratory activity, spatial working memory (spontaneous and delayed alternation), object recognition memory, social interaction (sociability and social novelty preference), and anxiety (elevated plus maze) [114].

Adolescent delta-9-THC administration induced a larger increase in exploratory activity, greater impairment in spatial working memory, and a stronger antianxiety effect in COMT knockouts than in wild types, primarily among males. No such effects of selective adolescent delta-9-THC administration were evident for other behaviors. Both object recognition memory and social novelty preference were disrupted by either adolescent or adult delta-9-THC administration, independent of genotype. The COMT genotype modifies responsivity to chronic delta-9-THC administration during adolescence in exploratory activity, spatial working memory, and anxiety. The findings illuminate the interaction between genes and adverse environmental exposures over a particular stage of development in the expression of the psychosis phenotype.

Although several findings indicate an association with adolescent *Cannabis* abuse and the risk of developing schizophrenia later in life, the evidence for a causal relationship is still inconclusive. The emergence of psychotic-like behavior in adult female rats chronically exposed to delta-9-tetrahydrocannabinol during adolescence was investigated. Female Sprague-Dawley rats were treated with delta-9-THC during adolescence (PND 35–45) and adulthood (PND 75). A series of behavioral tests and biochemical assays were performed to investigate the long-term effects of adolescent delta-9-THC exposure. Adolescent delta-9-THC pretreatment leads to long-term behavioral alterations characterized by recognition memory deficits, social withdrawal, altered emotional reactivity, and sensitization to the locomotor activating effects of acute PCP.

Moreover, since cortical disinhibition seems to be a key feature of many different animal models of schizophrenia, GABAergic hypofunction in the prefrontal cortex (PFC) has been observed in the postmortem brains of schizophrenic patients. The long-lasting consequences of adolescent delta-9-THC exposure on GABAergic transmission in the adult rat PFC were investigated.

Biochemical analyses revealed that adolescent delta-9-THC exposure reduces GAD67 and basal GABA levels within the adult PFC. GAD67 expression was reduced in parvalbumin (PV)- and cholecystokinin (CCK)-containing interneurons; the alteration may be related to the altered emotional reactivity triggered by adolescent delta-9-THC, as silencing PFC GAD67 expression through a siRNA-mediated

approach is sufficient to impact rats' behavior in the forced swim test [118]. Finally, the cellular underpinnings of the observed sensitized response to acute PCP in adult delta-9-THC-treated rats could be ascribed to the increased cFos immunoreactivity and glutamate levels in the PFC and dorsal striatum.

To determine whether the use of *Cannabis* in adolescence increases the risk for psychotic outcomes by affecting the incidence and persistence of subclinical expression of psychosis in the general population, an analysis from a prospective population-based cohort study was conducted. The Population study includes 1923 individuals from the general population, aged 14–24 years at baseline. *Cannabis* use and psychotic symptoms were assessed at three time points (baseline, T2 (3.5 years), T3 (8.4 years)) over a 10-year follow-up period with the Munich version of the composite international diagnostic interview (M-CIDI). Incidence and persistence of subthreshold psychotic symptoms after the use of *Cannabis* in adolescence were examined.

Cannabis use is a risk factor for the development of incident psychotic symptoms [22]. In the individuals who had no reported lifetime psychotic symptoms and no reported lifetime *Cannabis* use at baseline, the incident of *Cannabis* use over the period from baseline to T2 increased the risk of later incidents of psychotic symptoms over the period from T2 to T3 (adjusted odds ratio 1.9, 95% confidence interval 1.1–3.1; $p = 0.021$). Continued use of *Cannabis* increased the risk of persistent psychotic symptoms over the period from T2 to T3 (2.2, 1.2–4.2; $p = 0.016$). The incidence rate of psychotic symptoms over the period from baseline to T2 was 31% (152) in exposed individuals versus 20% (284) in nonexposed individuals; over the period from T2 to T3, the rates were 14% (108), and 8% (49), respectively. Continued *Cannabis* use might increase the risk for psychotic disorder by impacting the persistence of symptoms.

Posttraumatic Stress Disorder

Posttraumatic stress disorder may stem from the formation of aberrant and enduring aversive memories. Some PTSD patients have recreationally used *Cannabis*, probably aiming at relieving their symptomatology. It is unknown whether and how *Cannabis* or its psychotomimetic compound, delta-9-tetrahydrocannabinol, attenuates the aversive/traumatic memory outcomes. A review examined the effects of delta-9-THC on aversive memory extinction and anxiety in healthy humans and PTSD patients. At low doses, delta-9-THC can enhance the extinction rate and reduce anxiety responses [6]. Both effects involve activating cannabinoid type 1 receptors in discrete components of the corticolimbic circuitry, which could counterbalance the low “endocannabinoid tonus” reported in PTSD patients.

The advantage of associating CBD with delta-9-THC to attenuate anxiety while minimizing the potential psychotic or anxiogenic effect of high doses of delta-9-THC has been reported. Evidence from healthy humans and PTSD patients supports the delta-9-THC value to suppress anxiety and aversive memory expression without

producing significant adverse effects if used in low doses or when associated with CBD. However, the effects of delta-9-THC, alone or combined with CBD, on aversive memory reconsolidation are still unknown. Future studies are guaranteed to address open questions related to their dose ratios, administration routes, pharmacokinetic interactions, sex-dependent differences, and prolonged efficacy.

Schizophrenia-Like Psychoses

Cannabis use is associated with an increased risk of psychotic symptoms, and in a small number of cases, it can lead to psychoses [4]. The neurobiological mechanisms that mediate the link between *Cannabis* use and psychosis risk were examined in an established preclinical model of psychosis. The methylazoxymethanol acetate rodent model was used to examine if psychosis risk in some *Cannabis* users is mediated by the effects of *Cannabis* on the hippocampus and the role of the hippocampus in the regulation of mesolimbic dopamine.

The mechanism of how *Cannabis* affects excitatory neurotransmission known to regulate hippocampal neural activity and output was examined. While the evidence that *Cannabis*/cannabinoids can affect hippocampal and medial temporal lobe function and structure is clear, the evidence that *Cannabis*/cannabinoids increase striatal dopamine function is less robust. There is evidence that *Cannabis* use affects cortical and striatal glutamate levels.

Prospective epidemiological studies have demonstrated that *Cannabis* use is associated with an increased subsequent risk of both psychotic symptoms and schizophrenia-like psychoses [8]. Early onset of use, daily use of high-potency *Cannabis*, and synthetic cannabinoids carry the greatest risk. A shared genetic predisposition does not explain the risk-increasing effects between schizophrenia and *Cannabis* use.

Experimental studies in healthy humans indicated that *Cannabis* and delta-9-tetrahydrocannabinol could produce transient, dose-dependent, psychotic symptoms and an array of psychosis-relevant behavioral, cognitive, and psychophysiological effects that could be ameliorated by cannabidiol. Findings from structural imaging studies in *Cannabis* users have been inconsistent. However, functional MRI studies have linked the psychotomimetic and cognitive effects of delta-9-THC to activation in brain regions implicated in psychosis. Human PET studies have shown that acute administration of delta-9-THC releases dopamine in the striatum, but chronic users are characterized by low striatal dopamine.

Acute exposure to *Cannabis* is associated with an array of cognitive alterations, increased risk for neuropsychiatric illness, and other neuropsychiatric sequelae, including the emergence of acute psychotic symptoms [9]. Chronic exposure studies provide an insight into how *Cannabis* use may be related to long-term outcomes, and acute exposure may reveal information regarding the immediate impact of use and abuse on brain circuits. Understanding those alterations could reveal the connection with symptom dimensions in neuropsychiatric disorders and, more

specifically, with psychosis. A review provides the findings of pharmacological neuroimaging studies that examine the effects of administered cannabinoids and the sensitive window for the emergence of psychosis.

Cannabis exposure has varied effects on the brain, with the principal compounds in *Cannabis*, delta-9-tetrahydrocannabinol, and cannabidiol, altering activity across different brain regions. It was discovered that there are critical gaps in the literature, particularly regarding sex-dependent responses and the long-term effects of chronic exposure. Certain networks often characterized as dysregulated in psychosis, like the default mode network and limbic system, were also impacted by delta-9-THC exposure.

***Cannabis* and Psychosocial Stress**

Cannabis, similar to psychosocial stress, is well known to exacerbate psychotic experiences and can precipitate psychotic episodes in vulnerable individuals. Cannabinoid receptors 1 are widely expressed in the brain and are particularly important to mediate the effects of *Cannabis*. Chronic *Cannabis* use in patients and chronic cannabinoid treatment in animals cause reduced prepulse inhibition. Similarly, chronic psychosocial stress in mice impairs prepulse inhibition.

A study investigated the synergistic effects of substances modulating the CB1 receptors and chronic psychosocial stress on prepulse inhibition. Adult C57Bl/6 J mice were exposed to chronic psychosocial stress using the resident-intruder paradigm. The cannabinoid receptor agonist WIN55212.2 served as a surrogate marker for the effects of *Cannabis* in the brain. After exposure to stress, mice were acutely injected with WIN55212.2 (3 mg/kg) with or without pretreatment with rimonabant (3 mg/kg), a specific CB1 receptor antagonist, and subjected to behavioral testing. Stressed mice displayed a higher vulnerability to WIN55212.2 in the prepulse inhibition test than control animals. The effects of WIN55212.2 on PPI were antagonized by rimonabant, indicating involvement of CB1 receptors in sensorimotor gating.

The data imply that CB1 receptors might play a crucial role in the synergistic effects of psychosocial stress and cannabinoids in the brain [104]. WIN55212.2 increased prepulse inhibition in psychosocially stressed mice, although previous studies in rats showed the opposite effects. Thus, opposite effects can be evoked in different experimental animals depending on the cannabinoids/CB1-receptor agonists applied and environmental conditions (psychosocial stress).

Synthetic Cannabinoids

Personal battery-powered vaporizers, or e-cigarettes, were developed to deliver a nicotine vapor so smokers could simulate smoking tobacco without the inherent pathology of inhaled tobacco smoke [98]. The liquid formulations in the devices

comprise active ingredients such as nicotine mixed with vegetable glycerin, propylene glycol, and flavorings. Cannabidiol has been purported to have anticonvulsant, antinociceptive, and antipsychotic properties. The properties have potential medical therapies such as the intervention of addictive behaviors, treatments for epilepsy, management of pain for cancer patients, and treatments for schizophrenia.

However, cannabidiol extracted from *Cannabis* remains a DEA Schedule I drug since the FDA has not approved it for medical purposes. Two commercially available e-cigarette liquid formulations reported containing 3.3 mg/mL of cannabidiol as the active ingredient were evaluated. The products are not regulated by the FDA in manufacturing or labeling and were found to contain 6.5 and 7.6 mg/mL of cannabidiol in vegetable glycerin and propylene glycol with various flavoring agents.

With the increasing number of synthetic cannabinoids on the market, health professionals have seen a corresponding increase in the use of those compounds among patients with psychiatric disorders. Synthetic cannabinoids are functionally similar to but structurally different from delta-9-tetrahydrocannabinol. The psychotropic action of synthetic cannabinoids in patients with schizophrenia is unpredictable, with very diverse clinical presentations. The drugs can be much more potent than delta-9-tetrahydrocannabinol; they are readily available and difficult to detect. The gold standard for identifying synthetic cannabinoids is gas chromatography with mass spectrometry, but even this is difficult because new formulations of designer drugs are constantly emerging.

Four patients with synthetic cannabinoid intoxication were hospitalized in an intensive psychiatric unit. The patient had a history of schizophrenia and had been hospitalized several times previously. While hospitalized, they smoked an unknown substance brought in by a visitor, which was then confirmed using gas chromatography with mass spectrometry to be the synthetic cannabinoid AM-2201. The patients experienced predominantly psychiatric adverse clinical effects. There was the appearance of new psychotic phenomena, without exacerbation of their previously known psychotic symptoms, as well as the occurrence or marked worsening of mood and anxiety symptoms.

Despite several similar reactions, the clinical picture differed markedly between individual patients even though they ingested the same substance. The reasons for this difference could be the actual symptomatology of the presenting disorder, the impact of psychopharmacotherapy, individual patient differences, and probably unknown factors [116]. It was assumed that the acute effects of synthetic cannabinoids in patients with schizophrenia would be different from those in persons without psychotic disorders.

Cannabidiol and Parkinson's Patients

Cannabidiol reverses L-dopa-induced psychotic symptoms and improves motor function in Parkinson's patients. The effect raised the possibility that CBD could benefit motor-related striatal disorders [117]. The possibility of CBD preventing

catalepsy induced by drugs with distinct pharmacological mechanisms was evaluated. The catalepsy test is largely used to investigate motor function impairments caused by interference with striatal function.

Male Swiss mice received acute pretreatment with CBD (5, 15, 30, or 60 mg/kg, ip) 30 min prior to the D2 receptor antagonist haloperidol (0.6 mg/kg), the nonselective nitric oxide synthase (NOS) inhibitor L-nitro-N-arginine (L-NOARG, 80 mg/kg), or the CB1 receptor agonist WIN55212-2 (5 mg/kg). The mice were tested 1, 2, or 4 h after injection of haloperidol, L-NOARG, or WIN55212-2. The drugs significantly increased catalepsy time, and the effect was attenuated dose-dependently by CBD. CBD, by itself, did not induce catalepsy.

The mechanism of CBD effects was investigated in the second set of experiments. Thirty minutes before CBD (30 mg/kg), the animals received the 5-HT1A receptor antagonist WAY100635 (0.1 mg/kg). WAY100635 prevented the anticataleptic effect of CBD. The findings indicate that CBD can attenuate catalepsy caused by different mechanisms (D2 blockade, NOS inhibition, and CB1 agonism) via 5-HT1A receptor activation, suggesting that it could be useful in the treatment of striatal disorders.

Metabolic Dysregulation

Cannabis use has been associated with favorable outcomes on metabolic risk factors and the cause of this relation is still unknown. A study investigates whether the effect is mediated by the AKT1 gene, as activating the related enzyme by *Cannabis* may cause metabolic changes [15]. Six Single Nucleotide Polymorphisms (SNPs) of the AKT1 gene (rs1130214, rs1130233, rs2494732, rs2498784, rs3730358, and rs3803300) of patients with psychotic disorders ($n = 623$) were related to Body Mass Index (BMI), levels of glycosylated hemoglobin (HBA1c), and total metabolic risk. Mediation analysis was performed with BMI as the outcome, *Cannabis* as a predictor, and AKT1 as a mediator.

The results indicated that *Cannabis* use is likely associated with a lower BMI in patients with a psychotic disorder. *Cannabis* use was inversely related to BMI but not to levels of HBA1c and total metabolic risk. Out of 6 AKT1 SNPs, rs2494732 was associated with *Cannabis* use, but AKT1 did not mediate the effect of *Cannabis* on BMI. AKT1 risk alleles may increase the incidence of *Cannabis* use in patients with a psychotic disorder, but AKT1 does not appear to mediate the effect of *Cannabis* on BMI.

Cannabis use is associated with lower rates of metabolic dysregulation. Since *Cannabis* impacts each sex differently, a study examined the metabolic profile of male and female *Cannabis* users separately. Sex differences and the impact of *Cannabis* use on metabolic syndrome in adults with psychotic illness were investigated. Data from 1078 men and 735 women interviewed in the second Australian national survey of psychosis were analyzed using multiple logistic regression to model separately, for each sex, the influence of no, occasional, and frequent

past-year *Cannabis* use on metabolic syndrome, adjusting for potential covariates including antipsychotic medication, smoking, and physical activity.

The proportion of women and men with metabolic syndrome was 58.1% and 57.6%, respectively. Unadjusted analyses showed that frequent *Cannabis* use was associated with significantly lower odds of metabolic syndrome for both sexes [23]. In adjusted analyses, the association between metabolic syndrome and frequent *Cannabis* use remained significant for men (AOR = 0.49, 95% CI = 0.31–0.78) but not for women (AOR = 0.68, 95% CI = 0.37–1.24). Frequent *Cannabis* use was associated with lower odds of abdominal obesity, hypertension, and elevated triglyceride levels in men. The differences suggest that cannabinoid regulation of energy balance may be sex-dependent and highlight the importance of examining *Cannabis* use in men and women separately. At the same time, attention should be maintained on the negative association between *Cannabis* and psychosis onset and relapse.

Colic is a common digestive disorder in horses and one of the most urgent problems in equine medicine. Activating cannabinoid receptors could benefit gastrointestinal inflammation and visceral hypersensitivity. The localization of cannabinoid and cannabinoid-related receptors in the horse's intestine has not been investigated. A study was conducted to immunohistochemically localize the cellular distribution of canonical and putative cannabinoid receptors in the ileum of healthy horses [38]. Distal ileum specimens were collected from six horses at the slaughterhouse. The tissues were fixed and processed to obtain cryosections used to investigate the immunoreactivity of canonical cannabinoid receptors 1 and 2 and three putative cannabinoid-related receptors: nuclear peroxisome proliferator-activated receptor- α (PPAR α), transient receptor potential ankyrin 1, and serotonin 5-HT1a receptor (5-HT1aR).

Cannabinoid and cannabinoid-related receptors showed a wide distribution in the ileum of the horse. The epithelial cells showed immunoreactivity for CB1R, CB2R, and 5-HT1aR. Lamina propria inflammatory cells showed immunoreactivity for CB2R and 5-HT1aR. The enteric neurons showed immunoreactivity for CB1R, transient receptor potential ankyrin 1, and PPAR α . The enteric glial cells showed immunoreactivity for CB1R and PPAR α . The smooth muscle cells of the tunica muscularis and the blood vessels showed immunoreactivity for PPAR α .

The study represents a histological basis that could support additional studies regarding the distribution of cannabinoid receptors during gastrointestinal inflammatory diseases, as well as studies assessing the effects of nonpsychotic *Cannabis*-derived molecules in horses for managing intestinal diseases.

Creatine Kinase Elevation

Cannabinoid use is associated with increased frequency and duration of hospitalizations. Elevated serum levels of creatine kinase enzymes have been found in brain injuries and psychosis. A study was conducted to determine whether creatine kinase

levels differ in psychotic cannabinoid users and the association between creatine kinase levels and clinical measures (duration of hospitalization and need for mechanical restraint) [42]. Computerized medical records of 124 men hospitalized due to acute psychotic episodes were reviewed. Creatine kinase levels and various clinical measures at admission were documented. *Cannabis* users were significantly younger than nonusers. The duration of illness was longer among nonusers.

Log creatine kinase among cannabinoid users ($n = 32$) was numerically higher compared to nonusers ($n = 92$) (5.6 ± 1 vs. 5.2 ± 0.9 , respectively). A significantly higher rate of elevated creatine kinase levels (creatin kinase > 195 U/l) was detected in the cannabinoid users compared to nonusers (59.4% vs. 38%, respectively; $p < 0.04$). A higher rate of elevated creatine kinase levels was observed in hospitalized psychotic cannabinoid users, possibly due to *Cannabis* activity in peripheral or brain tissues. No association was found between creatine kinase levels, mechanical restraints, and hospitalization days.

The Cardiac Electrophysiological Effects of Cannabidiol

Cannabidiol has been shown to exert antinociceptive, antipsychotic, and anticonvulsant effects and influence the cardiovascular system. The effects of cannabidiol on major ion currents were investigated using the patch-clamp technique in rabbit ventricular myocytes. Cannabidiol inhibited voltage-gated Na^+ and Ca^{2+} channels with IC_{50} values of 5.4 and 4.8 μM , respectively [99]. In addition, cannabidiol, at lower concentrations, suppressed ion currents mediated by rapidly and slowly activated delayed rectifier K^+ channels with IC_{50} of 2.4 and 2.1 μM , respectively. Cannabidiol, up to 10 μM , did not significantly affect inward rectifier *I*_{K1} and transient outward *I*_{to} currents.

The effects of cannabidiol on the currents developed gradually, reaching steady-state levels within 5–8 min, and recoveries were usually slow and partial. Hill coefficients higher than unity in concentration-inhibition curves suggested multiple cannabidiol binding sites on the channels. The findings indicate that cannabidiol affects cardiac electrophysiology by acting on a diverse range of ion channels and suggest that caution should be exercised when cannabidiol is administered to carriers of cardiac channelopathies or individuals using drugs known to affect the rhythm or the contractility of the heart.

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Chapter 7

The Analgesic Effects of *Cannabis*



Drugs derived from *Cannabis* were used until the 1940s for their stimulant and depressant effects in treating somatic and psychiatric illnesses. Renewed interest in *Cannabis* research began in the 1970s and again pointed to the therapeutic potential of cannabinoids which are similar to morphine, lysergic acid diethylamide, and cocaine and are structurally related as analgesics, oxytocics, and local anesthetics and may be safer and provide more useful therapeutic agents. It has been shown that the C-ring in cannabinoids can be substituted with a variety of nitrogen- and sulfur-containing rings without loss of central nervous system activity. Cannabinoids have been shown to inhibit prostaglandin synthesis, intensify the pressor effects of endogenous amines like norepinephrine, and enhance the stimulant effects of amphetamine [64]. Cannabinoids' therapeutic potential is indicated in analgesics and anticonvulsants and for use as a sedative-hypnotic, antiglaucoma agent, anti-asthmatic agent, antidiarrheal agent, and possibly as an anticancer, and immunosuppressant agent.

Cannabinoids produce some central nervous system effects via the CB2 receptor, including analgesia, antianxiety, antireward, hypoactivity, and attenuation of opioid-induced respiratory depression. However, the cellular distributions of the CB2Rs in the brain remain unclear. CB2Rs are expressed in midbrain dopamine (DA) neurons and functionally regulate DA-mediated behavior(s). High densities of CB2-like signaling were also found in a neighboring motor structure – the midbrain's red nucleus (RN). Immunohistochemistry and in situ hybridization assays indicated high densities of CB2R-immunostaining and mRNA signal in RN magnocellular glutamate neurons in wild-type and CB1-knockout, but not CB2-knockout mice [65].

Ex vivo electrophysiological recordings in midbrain slices demonstrated that CB2R activation by JWH-133 dose-dependently inhibited firing rates of RN magnocellular neurons in wild-type, but not CB2-knockout mice while having no effect on RN GABA neurons in transgenic GAD67-GFP reporter mice, suggesting CB2-mediated effects on glutamatergic neurons. In addition, microinjection of JWH-133 into the RN produced robust ipsilateral rotations in wild-type but not CB2-knockout

mice, which was blocked by pretreatment with either a CB2 or DA D1 or D2 receptor antagonist, suggesting a DA-dependent effect. Finally, fluorescent tract tracing revealed glutamatergic projections from the RN to multiple brain areas, including the ventral tegmental area, thalamus, and cerebellum. The findings suggest that CB2Rs in RN glutamate neurons functionally modulate motor activity and constitute a new target in *Cannabis*-based medication development for motor disorders.

Endocannabinoids have been found to have many physiological and pathophysiological functions, including mood alteration and analgesia, control of energy balance, gut motility, motor and coordination activities, and alleviation of neurological, psychiatric, and eating disorders. Plants offer a wide range of chemical diversity and have been a growing domain in the search for effective cannabinoid ligands. Delta-9-tetrahydrocannabinol and *Echinacea* species with the cannabinoid receptor-binding lipophilic alkamides are the best-known herbal cannabimimetic.

The isolation of the endogenous CB1 receptor ligand, arachidonylethanolamide (anandamide), from the porcine brain revealed that it is metabolized by the enzyme arachidonylethanolamide amidohydrolase or fatty acid amide hydrolase [36]. It has been characterized as a reuptake system for transporting anandamide across the cell membrane. It has shown that selective inhibition of the transporter is associated with analgesia and peripheral vasodilation. The four cannabinoid system proteins, including the CB1 and CB2 receptors, fatty acid amide hydrolase, and the anandamide transporter, are excellent targets for developing novel medications for various conditions, including pain, immunosuppression, peripheral vascular disease, appetite enhancement or suppression, and motor disorders.

The compounds that modulate the endocannabinoid system functions can be collectively described under cannabinergics, regardless of chemical classification or type of resultant pharmacological action. Numerous selective ligands of the proteins were designed and synthesized. Many of the agents serve as important molecular probes, providing structural information about their binding sites and as pharmacological tools imparting information about the roles of their targets in physiological and disease states.

Chronic pain is the most commonly cited indication for the use of *Cannabis*. Cannabinoids act via cannabinoid receptors, but they also affect the activities of many other receptors, ion channels, and enzymes. Preclinical studies in animals using pharmacological and genetic approaches have improved our understanding of the mechanisms of cannabinoid-induced analgesia, and they provided therapeutic strategies for treating pain in humans. The mechanisms of the analgesic effect of cannabinoids include inhibition of the release of neurotransmitters and neuropeptides from presynaptic nerve endings, modulation of postsynaptic neuron excitability, activation of descending inhibitory pain pathways, and reduction of neural inflammation.

The synergistic contributions of cannabidiol to *Cannabis* pharmacology and analgesia have been scientifically demonstrated. The phytocannabinoids, including tetrahydrocannabivarin, cannabigerol, and cannabichromene also exert additional effects of therapeutic interest. Innovative, conventional plant breeding has produced *Cannabis* chemotypes with high titers of the components [1]. *Cannabis* terpenoids:

limonene, myrcene, α -pinene, linalool, β -caryophyllene, caryophyllene oxide, nerolidol, and phytol have also been examined.

Terpenoids share a precursor with phytocannabinoids as flavor and fragrance components common in human diets that have been designated Generally Recognized as Safe by the US Food and Drug Administration. Terpenoids are potent and affect animal and human behavior when inhaled from ambient air at serum levels in the single digits ng/mL. They display therapeutic effects that may contribute to the entourage effects of *Cannabis*-based medicinal extracts. The phytocannabinoid–terpenoid interactions produced synergy in treating pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, and fungal and bacterial infections, including methicillin-resistant *Staphylococcus aureus*.

Since the legalization of *Cannabis* products by several countries, the use of cannabinoids for medical purposes is constantly growing. Clinical reports have indicated enhanced requirements for propofol and analgesics used for general anesthesia and perioperative pain management in *Cannabis* users. There is also an increased requirement for propofol and fentanyl during anesthesia and postoperative analgesia, in which the risk for tachycardia and pulmonary and cardiovascular complications was observed. Concerning the data, it is recommended that patients should be asked preoperatively about recreational or medical *Cannabis* use or cannabinoid-based medication.

Delta-9-tetrahydrocannabinol produces the pharmacological effects caused by the consumption of *Cannabis*, both the *Cannabis*-like action and the medicinal benefits of the plant. Its acidic metabolite delta-9-THC-COOH, the nonpsychotropic cannabidiol, several cannabinoid analogs, and other modulators of the endogenous cannabinoid system are also promising for clinical and therapeutic uses. Cannabinoids exert many effects by activating G-protein-coupled cannabinoid receptors in the brain and peripheral tissues [2].

Additionally, there is evidence of nonreceptor-dependent mechanisms. Natural *Cannabis* products and individual cannabinoids are usually inhaled or taken orally. The rectal route, sublingual administration, transdermal delivery, eye drops, and aerosols have also been used and are less relevant in practice today.

Routes of Administration

The pharmacokinetics of delta-9-THC varies depending on its route of administration. Pulmonary assimilation of inhaled delta-9-THC causes a maximum plasma concentration within minutes. The psychotropic effects started within seconds to a few minutes and reached a maximum after 15–30 min, then tapered off within 2–3 h. Following oral ingestion, psychotropic effects set in with a delay of 30–90 min, reach a maximum after two to 3 h, and last for about four to 12 h, depending on the dose and specific effect.

At doses exceeding the psychotropic threshold, ingestion of *Cannabis* usually causes enhanced well-being and relaxation with an intensification of ordinary sensory experiences. The most acute adverse effects caused by overdosing are anxiety and panic attacks with increased heart rate and changes in blood pressure. Regular use of *Cannabis* may lead to dependency and mild withdrawal syndrome. The existence and the intensity of possible long-term adverse effects on the psyche and cognition, immune system, fertility, and pregnancy remain controversial. They are reported to be low in humans and do not preclude the legitimate therapeutic use of *Cannabis*-based drugs.

Precise *Cannabis* dosage is a major challenge for physicians to prescribe *Cannabis*. A investigated the pharmacokinetics, analgesic effect, cognitive performance, and safety effects of an innovative medical device that delivers inhaled therapeutic doses of delta-9-tetrahydrocannabinol in patients with chronic pain randomized, three-arms, double-blinded, placebo-controlled, crossover trial. Twenty-seven patients received a single inhalation of delta-9-THC: 0.5 mg, 1 mg, or a placebo. Delta-9-THC plasma levels were measured at baseline and up to 150 min postinhalation. Pain intensity and safety parameters were recorded on a 10-cm visual analog scale (VAS) at predefined time points.

Their limited bioavailability and undesirable psychoactivity often hinder the therapeutic use of cannabinoids. Observational studies investigated the effectiveness of Trokie lozenges, a standardized formulation containing *Cannabis* extracts, in delivering cannabinoids via buccal absorption and its long-term safety [28]. The participants were Palliative Care Corporation health clinic members and registered California *Cannabis* patients diagnosed with chronic noncancer pain. For the effectiveness study, 49 participants were required to self-report pain perception before and after one to 12 weeks of taking Trokie lozenges, using an 11-point pain intensity numeric rating scale (PI-NRS).

A mean reduction in PI-NRS score of 4.9 ± 2.0 points was observed. The onset of analgesia typically varied between 5 and 40 min, which was consistent with partial buccal absorption. In the safety study, 35 participants were asked to complete a questionnaire about adverse events (AEs) associated with Trokie lozenges. AEs were reported by 16 subjects (46%), the most common being dizziness/unsteadiness ($n = 7$), bad taste ($n = 5$), and throat irritation/dry mouth ($n = 4$). None of the self-reported AEs resulted in any serious medical situation, and most had a limited impact on daily functions. Despite the AEs, 90% of participants reported being satisfied or very satisfied with the product. The observations suggest that buccal administration of standardized extracts via Trokie lozenges may represent an efficacious and safe approach to *Cannabis* administration [29].

Smoked *Cannabis* is generally well tolerated and effective when added to concomitant analgesic therapy in patients with medically refractory pain due to HIV DSPN. Among the completers, pain relief was greater with *Cannabis* than with placebo (median difference in DDS pain intensity change, 3.3 points, effect size = 0.60; $p = 0.016$). The proportions of subjects achieving at least 30% pain relief with *Cannabis* versus placebo were 0.46 (95% CI: 0.28, 0.65) and 0.18 (0.03, 0.32). Mood and daily functioning improved to a similar extent during both

treatment periods. Although most side effects were mild and self-limited, two subjects experienced treatment-limiting toxicities.

Smoking is the most common route of administration for *Cannabis*; however, vaping *Cannabis* extracts and synthetic cannabinoids (“fake *Cannabis*”) in electronic cigarette devices have become increasingly popular. Most animal models used to investigate biological mechanisms underlying *Cannabis* use employ injection as the route of administration. This study evaluated a novel e-cigarette device that delivers aerosolized cannabinoids to mice. The effects of aerosolized and injected synthetic cannabinoids (CP 55940, AB-CHMINACA, XLR-11, and JWH-018) in mice were compared in a battery of bioassays in which psychoactive cannabinoids produce characteristic effects. The most potent cannabinoids (CP 55940 and AB-CHMINACA) produced the full cannabinoid profile (i.e., hypothermia, hypolocomotion, and analgesia), regardless of the route of administration.

The ability to administer cannabinoids to rodents using the most common route of administration among humans provides a method for collecting preclinical data with enhanced translational relevance [53]. Results of time course analysis for hypothermia showed that aerosol exposure to CP 55940 and AB-CHMINACA produced faster onset of effects and shorter duration of action than injection. In contrast, aerosolized JWH-018 and XLR-11 did not produce the full profile of cannabimimetic effects.

A study was conducted to determine the tolerance of male mice to delta-9-tetrahydrocannabinol and cannabinolic acid-induced antinociception with long-term treatment [59]. Using behavioral tests of mechanical and thermal nociception; it was revealed that systemic (intraperitoneal) administration of delta-9-THC and CBNA resulted in strong antinociception on day one of the experiment. Over the next 4 days, the behavior indices of antinociception to mechanical and thermal stimuli gradually decreased, indicating the development of tolerance following the systemic administration of the treatment. Thus, the two main components of *Cannabis*, delta-9-THC and CBNA, are characterized by the development of tolerance in mice due to their repeated i.p. administration.

The systemic administration of cannabinoids produces antinociception and their chronic use leads to analgesic tolerance and cross-tolerance to morphine. The effects are mediated by cannabinoids binding to peripheral, spinal, and supraspinal CB1 and CB2 receptors, making it difficult to determine the relevance of each receptor type to the phenomena. In the brain, the CB1 receptors (CB1Rs) are expressed at high levels in neurons, whereas the expression of CB2Rs is marginal. CB1Rs mediate the effects of smoked *Cannabis* and are also implicated in emotional behaviors. The production of supraspinal analgesia and the development of tolerance at CB1Rs by directly injecting a series of cannabinoids into the brain were analyzed.

The influence of the activation of CB1Rs on supraspinal analgesia evoked by morphine was also evaluated. Intracerebroventricular (icv) administration of cannabinoid receptor agonists, WIN55212-2, ACEA, or methanandamide generated dose-dependent analgesia [70]. A single administration of the compounds brought about profound analgesic tolerance lasting more than 14 days. This decrease in the effect of

cannabinoid receptor agonists was not mediated by the depletion of CB1Rs or the loss of regulated G proteins; it was accompanied by reduced morphine analgesia.

Acute morphine administration produced tolerance that lasted only 3 days and did not affect the CB1R. It was revealed that both neural mu-opioid receptors (MORs) and CB1Rs interact with the HINT1-RGSZ module, thereby regulating pertussis toxin-insensitive Gz proteins. In mice with reduced levels of the Gz proteins, the CB1R agonists produced no such desensitization or morphine cross-tolerance.

Experimental enhancement of Gz signaling enabled an acute icv administration of morphine to produce a long-lasting tolerance at MORs that persisted for more than 2 weeks. It also impaired the analgesic effects of cannabinoids. In the brain, cannabinoids can produce analgesic tolerance that is not associated with the loss of surface CB1Rs or uncoupling from regulated transduction. Neural-specific Gz proteins are essential mediators of the analgesic effects of supraspinal CB1R agonists and morphine. The Gz proteins are also responsible for the long-term analgesic tolerance produced by single doses of *the* agonists and the cross-tolerance between CB1Rs and MORs.

The cannabinoid activity of *Cannabis* smoke-exposed mice was tested to confirm the hypothesis that delta-9-THC mediates its effects through a CB1 receptor mechanism of action. To assess doses, delta-9-THC levels in the blood and brains of drug-exposed mice were determined following both intravenous and inhalation routes. The effects of delta-9-THC on analgesia, hypothermia, and catalepsy were compared with those of a *Cannabis* extract with equated delta-9-THC content after either intravenous administration or inhalation exposure.

Mice exposed to the smoke of an ethanol-extracted placebo plant material or low-grade *Cannabis* with minimal delta-9-THC but similar levels of other cannabinoids were impregnated with varying quantities of delta-9-THC. Both *Cannabis* and delta-9-THC produced comparable levels of antinociception, hypothermia, and catalepsy regardless of the route of administration, and the effects were blocked by pretreatment with the CB1 antagonist SR141716 [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl].

The blood and brain levels of delta-9-THC were similar in mice exhibiting similar pharmacological effects, regardless of the presence of non-delta-9-THC *Cannabis* constituents. The experiments prove that the acute cannabinoid effects of *Cannabis* smoke exposure on analgesia, hypothermia, and catalepsy in mice result from delta-9-THC content acting at CB1 receptors and the non-delta-9-THC constituents of *Cannabis* (at concentrations relevant to those typically consumed) influence the effects minimally [74].

Delta-9-tetrahydrocannabinol pain relief is believed to be mediated through central and peripheral CB1 and peripheral CB2 receptors. The possible antinociceptive effect of a CB2 receptor agonist, JWH-133, using the formalin test in mice was explored. The intracerebroventricular and intraperitoneal routes were used to administer the drug. Although no antinociceptive effect was observed after the intracerebroventricular administration of JWH-133, the intraperitoneal route produced an analgesic effect when the drug was administered [80]. The influence of nicotinic

cholinergic receptor modulators, nicotine, and mecamylamine, on the antinociceptive effect of JWH-133 was also studied. Nicotine increased, and mecamylamine decreased the antinociceptive effect of JWH-133.

The cognitive performance was evaluated using the selective subtests of the Cambridge Neuropsychological Test Automated Battery (CANTAB) [3]. Following inhalation of 0.5 mg or 1 mg, delta-9-THC plasma $C_{max} \pm SD$ was 14.3 ± 7.7 and 33.8 ± 25.7 ng/mL. $T_{max} \pm SD$ was 3.7 ± 1.4 and 4.4 ± 2.1 min, and $AUC_0 \rightarrow \infty \pm SD$ were 300 ± 144 and 769 ± 331 ng min/mL, respectively. The treatment groups, but not the placebo, demonstrated a significant reduction in pain intensity compared with baseline and remained stable for 150 min. The 1 mg dose significantly decreased pain compared to the placebo.

Adverse events were mild and resolved spontaneously. There was no evidence of consistent impairments in cognitive performance. The delivery method demonstrated that a metered-dose *Cannabis* inhaler delivered precise and low delta-9-THC doses and produced a dose-dependent and safe analgesic effect in patients with neuropathic pain/complex-regional pain syndrome; it enables individualization of medical *Cannabis* regimens that can be evaluated pharmacokinetically and pharmacodynamically by accepted pharmaceutical models.

Modulation of Chronic Neuropathic Pain

To characterize the functional brain changes involved in delta-9-tetrahydrocannabinol modulation of chronic neuropathic pain, 15 patients with chronic radicular neuropathic pain participated in a randomized, double-blind, placebo-controlled study employing a counterbalanced, within-subjects design. Pain assessments and functional resting-state brain scans were performed at baseline and after sublingual delta-9-THC administration. The functional connectivity of the anterior cingulate cortex and pain-related network dynamics using graph theory measures were evaluated. The delta-9-THC treatment significantly reduced the patients' pain compared to the placebo.

Delta-9-THC-induced analgesia was correlated with a reduction in functional connectivity between the anterior cingulate cortex and the sensorimotor cortex. The degree of reduction was predictive of the response to delta-9-THC. Graph theory analyses of local measures demonstrated a reduction in network connectivity in areas involved in pain processing, specifically in the dorsolateral prefrontal cortex, correlated with individual pain reduction [4].

The results indicated that the anterior cingulate cortex and dorsolateral prefrontal cortex, two major cognitive-emotional modulation areas, and their connections to somatosensory areas are functionally involved in the analgesic effect of delta-9-THC in chronic pain. The induction of functional disconnection between regulatory high-order affective regions and the sensorimotor cortex may mediate the effect. Baseline functional connectivity between those brain areas may predict the extent of pain relief induced by delta-9-THC.

Cannabinoids mimic the effects of endogenous cannabinoids (endocannabinoids), activating specific cannabinoid receptors, particularly CB1, found predominantly in the central nervous system, and CB2, found predominantly in cells involved with immune function. Cannabinoids have been recognized as beneficial in treating cancer-related pain, possibly synergistic with opioid analgesics [5]. Delta-9-tetrahydrocannabinol has been available as a prescription medication for the treatment of cancer chemotherapy-induced nausea, vomiting, and anorexia associated with AIDS wasting syndrome. Cannabinoids are beneficial in treating HIV-related peripheral neuropathy. They have a favorable drug safety profile, but their psychoactive effects and limited bioavailability predominantly limit their medical use.

A double-blind, placebo-controlled, crossover study evaluated the analgesic efficacy of vaporized *Cannabis* in 39 patients with central and peripheral neuropathic pain who underwent a standardized procedure for inhaling medium-dose (3.53%), low-dose (1.29%), or placebo *Cannabis*. The primary outcome was a visual analog scale of pain intensity. Psychoactive side effects and neuropsychological performance were also evaluated. Mixed-effects regression models demonstrated an analgesic response to vaporized *Cannabis*. There was no significant difference between the two dose groups' results. The number needed to treat to achieve 30% pain reduction was 3.2 for placebo versus low-dose, 2.9 for placebo versus medium-dose, and 25 for medium- versus low-dose.

As the numbers needed to treat were comparable to those of traditional neuropathic pain medications, *Cannabis* has analgesic efficacy, with the low dose being as effective a pain reliever as the medium dose. Psychoactive effects were minimal and well tolerated, and neuropsychological effects were of limited duration and readily reversible within 1–2 h. Vaporized *Cannabis*, at low doses, presents an effective option for patients with treatment-resistant neuropathic pain [8]. The analgesia obtained from a low dose of delta-9-tetrahydrocannabinol (1.29%) in patients experiencing neuropathic pain despite conventional treatments is a clinically significant outcome.

Preclinical and clinical studies have indicated that *Cannabis* extracts and synthetic cannabinoids may be useful for treating diverse diseases related to acute or chronic pain. The discovery of cannabinoid receptors, their endogenous ligands, and the machinery for the synthesis, transport, and degradation of retrograde messengers, has provided the neurochemical tools for drug design. Agonist-activated cannabinoid receptors modulate nociceptive thresholds, inhibit the release of proinflammatory molecules, and display synergistic effects with other systems that influence analgesia, especially the endogenous opioid system. Cannabinoid receptor agonists have shown therapeutic value against inflammatory and neuropathic pain, often refractory to therapy [81].

Cannabis extracts containing delta-9-tetrahydrocannabinol and cannabidiol have been approved in Canada to relieve neuropathic pain in Multiple sclerosis. Although the psychoactive effects of the extracts have limited clinical progress in studying cannabinoid actions in pain mechanisms, preclinical research is progressing rapidly. For example, CB1-mediated suppression of mast cell activation responses,

CB2-mediated indirect stimulation of opioid receptors located in primary afferent pathways, and the discovery of inhibitors for either the transporters or the enzymes degrading endocannabinoids are recent findings that suggest new therapeutic approaches to avoid central nervous system side effects.

A randomized, placebo-controlled crossover trial utilizing vaporized *Cannabis* containing placebo and 6.7% and 2.9% delta-9-tetrahydrocannabinol was performed on 42 subjects with central neuropathic pain related to spinal cord injury and disease [86]. The subjects received two administrations of the study medication at a 4-h interval. Blood samples for pharmacokinetic evaluation were collected, and pain assessment tests were performed immediately after the second administration and 3 h later.

Using mixed model regression, an evaluation of the relationship between plasma concentrations of selected cannabinoids and percent change in items from the Neuropathic Pain Scale was conducted. Pharmacokinetic data, although limited, were consistent with literature reports, namely dose-dependent increase in systemic exposure followed by the rapid disappearance of delta-9-THC. Dose-dependent improvement in pain score was evident across all pain scale elements. Changes in the concentration of delta-9-THC and its nonpsychotropic metabolite, 11-nor-9-carboxy-delta-9-THC, were related to percent change from baseline of several descriptors (e.g., itching, burning, and deep pain).

Given a large number of multiple comparisons, false-discovery-rate-adjusted *p*-values were not significant. Future work is required to explore the relationship of plasma concentrations with the analgesic response to different cannabinoids. Such an appraisal of descriptors might contribute to identifying distinct pathophysiologic mechanisms and, ultimately, the development of mechanism-based treatment approaches for neuropathic pain. This condition remains difficult to treat.

Peripheral nerve stimulation (PNS) is a form of neuromodulation used to treat chronic and refractory neuropathic pain. Peripheral nerve stimulation was first described in the early 1960s when Shelden implanted a PNS device for trigeminal neuralgia. Despite PNS being known since the 1960s, a technology designed specifically for PNS was lacking. Design-specific PNS devices, with favorable efficacy and safety profiles, have become widely available within the past few years. A case of design-specific PNS that provided 2 years of pain relief in a patient with lower extremity neuropathic pain was examined [87].

Chronic neuropathic pain is often refractory to standard pharmacological treatments. Although growing evidence supports the use of inhaled *Cannabis* for neuropathic pain, the lack of standard inhaled dosing plays a major obstacle to *Cannabis* becoming a pharmacological treatment for neuropathic pain. A study explored the pharmacokinetics, safety, tolerability, efficacy, and ease of use of a novel portable thermal-metered-dose inhaler (tMDI) for *Cannabis* in a cohort of eight patients suffering from chronic neuropathic pain and on a stable analgesic regimen including medicinal *Cannabis* [47].

In a single-dose, open-label study, the patients inhaled a single 15.1 ± 0.1 mg dose of *Cannabis* using the Syqe Inhaler device. Blood samples for delta-9-tetrahydrocannabinol and 11-hydroxy-delta-9-THC were taken at baseline and up to

2 h. Pain intensity (0–10 VAS), adverse events, and satisfaction scores were monitored following the inhalation. A uniform pharmacokinetic profile was exhibited across all participants (delta-9-THC plasma $C_{max} \pm SD$ was 38 ± 10 ng/mL, $T_{max} \pm SD$ was 3 ± 1 min, $AUC_0 \rightarrow \infty \pm SD$ was 607 ± 200 ng/min/mL). Higher plasma C_{max} increases per mg delta-9-THC administered (12.3 ng/mL/mg delta-9-THC) and lower interindividual variability of C_{max} (25.3%), compared with reported alternative modes of delta-9-THC delivery, were measured.

A significant 45% reduction in pain intensity was noted 20 min post inhalation ($p = 0.001$), returning to baseline within 90 min. Tolerable lightheadedness, lasting 15–30 min and requiring no intervention, was the only reported adverse event. The trial suggests the potential use of the Syqe Inhaler device as a smokeless delivery system of medicinal *Cannabis*, producing a delta-9-THC pharmacokinetic profile with a low interindividual variation of C_{max} , achieving pharmaceutical standards for inhaled drugs.

Despite management with opioids and other pain-modifying therapies, neuropathic pain continues to reduce the quality of life and daily functioning in HIV-infected individuals. Cannabinoid receptors in the central and peripheral nervous systems have modulated pain perception. A clinical trial was conducted to assess the impact of smoked *Cannabis* on neuropathic pain in HIV [50]. In phase II, a double-blind, placebo-controlled, crossover trial of analgesia with smoked *Cannabis* in HIV-associated distal sensory predominant polyneuropathy (DSPN), eligible subjects had neuropathic pain refractory to at least two previous analgesic classes; they continued on their prestudy analgesic regimens throughout the trial. Regulatory considerations dictated that the subjects smoke under direct observation in a hospital setting.

Of 127 volunteers screened, 34 eligible subjects enrolled, and 28 completed both *Cannabis* and placebo treatments. Treatments were placebo and active *Cannabis* ranging in potency between 1% and 8% Delta-9-tetrahydrocannabinol, four times daily for five consecutive days during each of the two treatment weeks, separated by a 2-week washout. The primary outcome was a change in pain intensity measured by the Descriptor Differential Scale (DDS) from a pretreatment baseline to the end of each treatment week. Secondary measures included assessments of mood and daily functioning.

Pain is one of the most common reasons to seek medical attention, and chronic pain is a worldwide epidemic. Anecdotal reports suggest *Cannabis* may be an effective analgesic. In a mouse model for neuropathic pain, the terpenes α -terpineol, β -caryophyllene, and γ -terpinene were evaluated for their analgesic effects [58]. The chronic constriction injury of the sciatic nerve mouse model, which produces mechanical allodynia, was accessed via the von Frey assay, and thermal hyperalgesia was assessed via the hotplate assay.

Compounds were further assessed in locomotor activity tests, hypothermia, and acute antinociception. Each terpene produced a dose-related reversal of mechanical allodynia and thermal hyperalgesia. Thermal hyperalgesia displayed higher sensitivity to the effects of each terpene than mechanical allodynia, and the rank order potency of the terpenes was α -terpineol > β -caryophyllene > γ -terpinene. To

examine the involvement of cannabinoid receptors, tests were conducted in mice lacking either functional cannabinoid type 1 receptors (CB1R $(-/-)$) or cannabinoid type 2 receptors (CB2R $(-/-)$). Compared to wild-type mice, CB1R $(-/-)$ mice treated with α -terpineol displayed a 2.91-fold decrease in potency to reverse mechanical allodynia; in CB2R $(-/-)$ mice, the potency of α -terpineol was decreased by 11.73-fold. The potency of β -caryophyllene to reverse mechanical allodynia decreased 1.80-fold in CB2R $(-/-)$ mice. Each terpene produced a subset of locomotor activity tests, hypothermia, and acute antinociception effects. The findings suggest α -terpineol, β -caryophyllene, and γ -terpinene may have differential cannabinoid receptor activity and a pharmacological profile that may yield new efficacious analgesics.

Cannabidiol exhibits therapeutic potential for various human diseases, including chronic neurodegenerative diseases, such as Alzheimer's and Parkinson's, ischemic stroke, epilepsy, and other convulsive syndromes, neuropsychiatric disorders, neuropathic allodynia, and certain types of cancer [62]. Cannabidiol does not bind directly to endocannabinoid receptors 1 and 2, and despite research efforts, its specific targets remain to be fully identified. Notably, sigma 1 receptor (σ 1R) antagonists inhibit glutamate N-methyl-D-aspartate acid receptor (NMDAR) activity and positively affect most diseases discussed.

The effects of CBD on three animal models in which NMDAR overactivity plays a critical role: opioid analgesia attenuation, NMDA-induced convulsive syndrome, and ischemic stroke, were investigated. In an *in vitro* assay, CBD disrupted the regulatory association of σ 1R with the NR1 subunit of NMDAR, an effect shared by σ 1R antagonists, such as BD1063 and progesterone prevented by σ 1R agonists, such as 4-IBP, PPCC, and PRE084. The *in vivo* administration of CBD or BD1063 enhanced morphine-evoked supraspinal antinociception alleviated NMDA-induced convulsive syndrome and reduced the infarct size caused by permanent unilateral middle cerebral artery occlusion. The positive effects of CBD were reduced by the σ 1R agonists PRE084 and PPCC and were absent in σ 1R $-/-$ mice. Thus, CBD displays antagonist-like activity toward σ 1R to reduce the negative effects of NMDAR overactivity in the abovementioned experimental situations.

Given the anecdotal observations that several patients have reported using CBD for spine-related pain, a study was designed to characterize CBD consumption patterns and perceived effects in patients with spine-related complaints [63]. The study design was a cross-sectional survey. Over 4 weeks, one of nine spine surgeons at a single institution administered an anonymous paper survey to all patients presenting for evaluation. Upon registration for the office visit, surveys were given and collected by the office manager or nurse before evaluation by the surgeon. Patients were included regardless of surgical status (i.e., preoperative, postoperative, or non-operative) or region of pathology (lumbar, thoracic, or cervical).

The survey consisted of multiple-choice questions on patient patterns of CBD use. Out of 300 surveys, 214 (71%) were completed, and 54 (25.2%) patients reported CBD use for spine-related pain. CBD was initially used for the potential relief of back pain (66.7%), neck pain (37.0%), leg pain (35.2%), and arm pain (9.3%). Users also sought improvements in insomnia (25.9%) and mood (18.5%).

Oil was the most popular formulation (64.8%). Cannabidiol was consumed 2–5 times (40.7%) or 6–10 times (31.5%) per week. The most common source of initial recommendation for CBD was friends or family (75.9%). Reported benefits were pain relief (46.3%), improved sleep (33.3%), and reduced anxiety (20.4%); however, 24.1% of patients reported no benefit from CBD use. The most reported side effect was fatigue (7.4%). Most users (63.0%) would recommend CBD to a friend for pain relief.

Preclinical studies demonstrate that cannabidiol elicits an antinociceptive response in animal models of neuropathic pain. A double-blind, placebo-controlled, within-subject outpatient clinical laboratory study sought to determine the analgesic effects, abuse liability, safety, and tolerability of acute CBD (0, 200, 400, and 800 mg orally) in healthy non-*Cannabis*-using volunteers ($n = 17$; eight men, nine women) [67].

The outcomes included experimental pain threshold and pain tolerance using the cold pressor test (CPT), subjective ratings of CPT painfulness and bothersomeness, subjective ratings of abuse liability and mood, and cardiovascular measures, which were assessed at baseline and several time points after drug administration. Data analyses included repeated measures analysis of variance (ANOVA) with planned comparisons. CBD failed to consistently affect pain threshold and tolerance in the CPT relative to placebo. All doses of CBD increased ratings of painfulness compared to placebo ($p < 0.01$). CBD had dose-dependent, modest effects on mood and subjective drug effects associated with abuse liability. Oral CBD was safe and well tolerated, producing small decreases in blood pressure ($p < 0.01$). CBD did not elicit consistent dose-dependent analgesia but increased pain on some measures.

The G protein-coupled receptors CB1 and CB2 are targets for the psychoactive constituents of *Cannabis*, chief among them delta-9-THC. They are also key components of the multifunctional endogenous cannabinoid signaling system. CB1 and CB2 receptors modulate various physiological systems, including analgesia, memory, mood, reward, appetite, and immunity. Identifying and characterizing selective CB1 and CB2 receptor agonists and antagonists will facilitate understanding the precise physiological and pathophysiological roles of cannabinoid receptors in the systems. This is particularly necessary in the case of CB2 because the receptors are sparsely expressed and problematic to detect using traditional immunocytochemical approaches. 1-Propyl-2-methyl-3-(1-naphthoyl)indole (JWH015) is an aminoalkyl-indole employed as a CB2-selective agonist. However, it was revealed that JWH015 potently and efficaciously activates CB1 receptors in neurons.

Using murine autaptic hippocampal neurons, which express CB1 but not CB2 receptors, it was found that JWH015 inhibits excitatory postsynaptic currents with an EC_{50} of 216 nM. JWH015 inhibition was absent in neurons from CB1(–/–) cultures and was reversed by the CB1 antagonist, SR141716 [200 nM]. JWH015 partially occludes CB1-mediated DSE (~35% remaining), an action reversed by the CB2 antagonist, AM630 [1 and 3 μ M], suggesting that high concentrations of AM630 also antagonize CB1 receptors [78]. It was concluded that JWH015 is a CB2-preferring agonist; it activates CB1 receptors at experimentally encountered

concentrations. Thus, the CB1 agonism of JWH015 needs to be considered in designing and interpreting experiments that use JWH015 to probe CB2-signaling.

Pathological or Injury-Related Persistent Pain

A meta-analysis of studies assessed the antinociceptive efficacy of cannabinoids, *Cannabis*-based medicines, and endocannabinoid system modulators on pain-associated behavioral outcomes in animal models of pathological or injury-related persistent pain support the evidence of cannabinoid-induced analgesia [6]. The meta-analyzed 374 studies in which 171 interventions were assessed for antinociceptive efficacy in rodent models of pathological or injury-related pain; antinociceptive efficacy was most frequently measured by attenuation of hypersensitivity to evoked limb withdrawal. Particular cannabinoid type 1, cannabinoid type 2, nonselective cannabinoid receptor agonists, including delta-9-tetrahydrocannabinol and peroxisome proliferator-activated receptor- α agonists, predominantly palmitoylethanolamide, significantly attenuated pain-associated behaviors in a broad range of inflammatory and neuropathic pain models. Fatty acid amide hydrolase inhibitors, monoacylglycerol lipase inhibitors, and cannabidiol significantly attenuated pain-associated behaviors in neuropathic pain models but yielded mixed results in inflammatory pain models.

The Antinociceptive Effects of Delta-9-THC

Activation of cannabinoid CB1 receptors by delta-9-tetrahydrocannabinol produces a variety of negative effects with major consequences in *Cannabis* users that constitute important drawbacks for using cannabinoids as therapeutic agents. Behavioral studies carried out in mice lacking 5-HT_{2A} receptors (5-HT_{2AR}) revealed a remarkable 5-HT_{2AR}-dependent dissociation in the beneficial antinociceptive effects of delta-9-THC and its detrimental amnesic properties [84].

It was revealed that specific effects of delta-9-THC, such as memory deficits, anxiolytic-like effects, and social interaction, are under the control of 5-HT_{2AR}. However, the acute hypolocomotor, hypothermic, anxiogenic, and antinociceptive effects are not. In biochemical studies, it was shown that CB1R and 5-HT_{2AR} form heteromers that are expressed and functionally active in specific brain regions involved in memory impairment.

The data indicated that costimulation of both receptors by agonists reduces cell signaling, antagonist binding to one receptor, blocks signaling of the interacting receptor, and heteromer formation that leads to a switch in G-protein coupling for 5-HT_{2AR} from G_q to G_i proteins. Synthetic peptides with the sequence of transmembrane helices 5 and 6 of CB1R, fused to a cell-penetrating peptide, were able to disrupt receptor heteromerization in vivo, leading to a selective abrogation of

memory impairments caused by exposure to delta-9-THC. The data reveal a novel molecular mechanism for the functional interaction between CB1R and 5-HT2AR mediating cognitive impairment. CB1R-5-HT2AR heteromers are thus good targets to dissociate the cognitive deficits induced by delta-9-THC from its beneficial antinociceptive properties.

The effects of hashish extract on the adaptive behavior of male mice were studied in a paradigm that allows the investigation of learning mechanisms in a social context [94]. Mice of the C3H strain, which were not submissive in a confrontation with a nonaggressive DBA mouse on day one, were defeated on day 2 over 3 min by aggressive, isolated DBA mice and demonstrated conditioned submissive behavior upon mere contact with a nonaggressive DBA mouse on day three.

A hashish extract containing 38.6–39.4% delta-9-THC, 11.6–12.0% cannabinalol, and 47.7–48.5% cannabidiol was administered orally in all experiments. Hashish extract was given 90 min before defeat on day two, in dosages corresponding to 1, 5, and 10 mg delta-9-THC/kg, with impaired retention of defensive upright, defensive sideways, and immobility on day three (experiment 1). Experiment 2 indicated that the drug (5 and 10 mg delta-9-THC/kg) had no antinociceptive potency in mice and did not modify defeat-induced analgesia. Experiment 3, with drug (5 mg delta-9-THC/kg) or solvent administration on day two and day three, indicated that the retention deficit was neither due to state-dependent learning nor impaired retrieval. Hashish extract administered before learning might interfere with memory processing.

The interaction between delta-9-tetrahydrocannabinol and PGE1 was studied using two pharmacological parameters – the rate of passage of a charcoal meal through a mouse's small intestine and the abdominal constriction response in the mouse [95]. PGE1 administered intraperitoneally produced a dose-dependent decrease in intestinal motility, and the effect was antagonized by low (0.25 mg/kg) doses of delta-9-THC and potentiated by higher doses of delta-9-THC (1 mg/kg).

In the two pharmacological parameters studied, delta-9-THC appears to interact with PGE1 at the same receptor site. Kinetic analysis indicated that the interaction was mixed but predominantly competitive. PGF2alpha produced an increase in intestinal motility that was not dose-dependent. Delta-9-THC antagonized the effect of PGF2alpha dose-dependent, indicative of a physiological antagonism. Delta-9-THC (0.25–2 mg/kg) antagonized the dose-dependent PGE1 abdominal constriction response, indicating a mixed, though mainly competitive antagonism. Although the doses of delta-9-THC used are within the range of those used in man, it is not implied that the results are necessarily implicated in the psychoactivity of the drug.

Terpenes in *Cannabis*-Mediated Analgesia

The contributions of terpenes and cannabinoids in *Cannabis*-mediated analgesia in rats were investigated. Animals received intraperitoneal administration of control, 10.0 or 18.0 mg/kg morphine, or various doses of the extract without terpenes,

isolated terpenes, delta-9-tetrahydrocannabinol, or the full extract [7]. Thirty minutes later, the animals were tested on a hotplate and tail-flick tests of thermal nociception. One week later, the rats received a second administration of the test articles and were tested 30 min later in the abdominal writhing test of inflammatory nociception. In the thermal assays, hotplate and tail-flick latencies for morphine-treated rats were dose-dependent and significantly higher than vehicle-treated animals.

The cannabinoid compounds, except for the isolated terpenes, produced dose-dependent increases in hotplate and tail-flick latencies. In the inflammatory nociceptive assay, animals dosed with the vehicle and isolated terpenes demonstrated increased abdominal writhing. The animals dosed with the cannabinoid compounds significantly decreased abdominal writhing responses. Delta-9-tetrahydrocannabinol alone produced robust analgesia equivalent to the full *Cannabis* extract. Terpenes alone did not produce analgesia. The data suggest that the analgesic activity of *Cannabis* is largely mediated by delta-9-THC, whereas terpenes alone do not cause alterations in *Cannabis*-mediated analgesia.

Coadministration of *Cannabis* and Opioid

Cannabinoids combined with opioids produce synergistic antinociceptive effects, decreasing the lowest effective antinociceptive opioid dose in laboratory animals. Although patients treated for pain reported greater analgesia when *Cannabis* with opioids, no placebo-controlled studies have assessed the direct effects of opioids combined with *Cannabis* in humans and the impact of the combination on abuse liability. A double-blind, placebo-controlled, within-subject study determined if *Cannabis* enhances the analgesic effects of low-dose oxycodone using a validated experimental model of pain and its effects on abuse liability [9].

Healthy *Cannabis* smokers ($n = 18$) were administered oxycodone (0, 2.5, and 5.0 mg, P.O.) with smoked *Cannabis* (0.0, 5.6% delta-9-THC and analgesia was assessed using the Cold-Pressor Test. The participants immersed their hands in cold water (4 °C). Times were recorded to report pain (pain threshold) and withdraw the hand from the water (pain tolerance). Abuse-related effects were measured, and the effects of oxycodone on *Cannabis* self-administration were determined. Alone, 5.0 mg of oxycodone increased pain threshold and tolerance.

Although *Cannabis* and 2.5 mg oxycodone, separately, failed to elicit analgesia, when combined, they increased pain threshold and tolerance. Oxycodone did not increase subjective ratings associated with *Cannabis* abuse, nor did it increase *Cannabis* self-administration. However, the 2.5 mg oxycodone and active *Cannabis* produced small yet significant increases in oxycodone abuse liability. *Cannabis* enhances the analgesic effects of subthreshold oxycodone, suggesting synergy without increases in *Cannabis* abuse liability. The evidence supports future research into the therapeutic use of opioid-cannabinoid combinations for pain.

A phase I open-label study examined the pharmacokinetics, safety, and tolerability of escalating doses of a combination of cannabinoid medications (1:1

delta-9-THC to cannabidiol) in patients with chronic noncancer pain who are on high-dose opioid analgesia [10]. Nine people with noncancer pain and oral morphine equivalent daily at a dose of 60 mg or higher were recruited. Blood concentrations of delta-9-THC, 11-hydroxytetrahydrocannabinol (OH-delta-9-THC), 11-nor-9-carboxy-tetrahydrocannabinol (COOH-delta-9-THC), and CBD were assayed weekly. The concentrations were measured after a single dose of 2.5 mg delta-9-THC/2.5 mg CBD on day one and escalating daily doses up to a single dose of 12.5 mg delta-9-THC/12.5 mg CBD on day 29. Follow-up was on day 36 after a seven-day washout. Secondary outcome data encompassed pain, mood, and sleep parameters.

The parent compounds delta-9-THC and CBD and metabolites OH-delta-9-THC and COOH-delta-9-THC were detected most times. The concentration of all of the analytes increased until 2 h postadministration, decreasing to approximately predose concentrations by 8 h. There was considerable inter- and intraindividual variability. The studied medications were well tolerated. Eight participants reported at least one adverse event with a total of 62 adverse events, the most common were euphoric mood, headache, and agitation, and no one was classified as severe. There was no significant change in pain severity self-ratings or use of pain medications. Improvements in pain interference scores, mood, and some sleep parameters were observed. The delta-9-THC/CBD formulation was tolerated well in a group of patients with chronic non-cancer pain (CNCP). Between-participant variability supports personalized dosing and “start low-go slow” titration.

Cannabinoids and their potential for enhancing opioid analgesia could be used to reduce the reliance on opioids for analgesia. A study was conducted to evaluate whether the concurrent administration of *Cannabis* influences opioid consumption using a behavioral economic demand framework [18]. An online survey to assess *Cannabis* and opioid use frequency and dependence measured, pain severity, and demand for both *Cannabis* and opioids alone, and when concurrently available using hypothetical purchase tasks. Adults using opioids for pain management completed two hypothetical purchase tasks in which only grams of *Cannabis* or units of participants’ index opioids were available for purchase and two hypothetical tasks in which both were concurrently available. The price of one drug increased, whereas the other was kept constant.

Paired-sample *t*-tests compared the demand for each drug alone when it was available concurrently with an alternative. The demand intensity was significantly reduced, and demand elasticity was significantly increased for both *Cannabis* and opioids when the alternate commodity was available. The reductions in *Cannabis* consumption were more pronounced than they were for opioid consumption in the presence of the alternate commodity. The data provide behavioral economic evidence that *Cannabis* access may modestly reduce demand for opioids in persons who have pain.

Studies indicated that cannabinoids provide adjunctive analgesia in advanced cancer patients with uncontrolled pain. A study assessed adjunctive nabiximols (Sativex), an extract of *Cannabis sativa* containing two potentially therapeutic cannabinoids (delta-9-tetrahydrocannabinol [27 mg/mL] and cannabidiol [25 mg/mL]),

on advanced cancer patients with chronic pain unalleviated by optimized opioid therapy [19]. The trial was a double-blind, randomized, placebo-controlled in patients with advanced cancer and moderate pain with Numerical Rating Scale scores ≥ 4 and ≤ 8 , despite optimized opioid therapy. The patients were randomized to nabiximols ($n = 199$) or placebo ($n = 198$) self-titrated study medications over 2 weeks, followed by a 3-week treatment period at the titrated dose. The median percent improvements in average pain Numerical Rating Scale score from baseline to end of treatment in the nabiximols and placebo groups were 10.7% vs. 4.5% ($p = 0.0854$) in the intention-to-treat population (primary variable), and 15.5% vs. 6.3% ($p = 0.0378$) in the per-protocol population.

Nabiximols were statistically superior to placebo on two of three quality-of-life instruments in Week 3 and on all three in Week five. In exploratory post hoc analyses, US patients, but not patients from the rest of the world, experienced significant benefits from nabiximols on multiple secondary endpoints. The contributing factors to differences in nabiximols efficacy are that the US participants received lower doses of opioids at baseline than the rest of the world, and the subgroups had a different distribution of cancer pain types, which may have been related to differences in the pathophysiology of the pain. The safety profile of nabiximols was consistent with other studies. Although not superior to placebo on the primary efficacy endpoint, nabiximols had benefits on multiple secondary endpoints, particularly in the United States.

A study was conducted to determine whether cannabidiol or a cannabidiol derivative, cannabidiol monovalinate monohemisuccinate (CBD-val-HS), could attenuate the development of oxycodone reward while retaining its analgesic effects [28]. To determine the effect on oxycodone reward, animals were used in the conditioned place preference paradigm. They received either saline or oxycodone (3.0 mg/kg) in combination with either CBD or CBD-val-HS utilizing three sets of drug–no drug-conditioning trials. To determine if the doses of CBD or CBD-val-HS that blocked opioid reward would affect nociceptive processes, animals were studied in the hot plate and abdominal writhing assays when administered alone or in combination with a subanalgesic (1.0 mg/kg) or analgesic (3.0 mg/kg) doses of oxycodone.

The results demonstrated that CBD could not attenuate oxycodone place preference while CBD-val-HS attenuated the rewarding effects at 8.0 mg/kg and was void of rewarding or aversive properties. In contrast to CBD, CBD-val-HS alone produced analgesic effects in both nociceptive assays and was most effective compared with oxycodone against thermal nociception. There was a differential interaction of CBD and CBD-val-HS oxycodone across the two nociceptive assays producing subadditive responses on the hot plate assay. In contrast, additive responses were observed in the abdominal writhing assay. The findings indicated that CBD-val-HS alone, a nonrewarding analgesic compound, could be useful in pain management and addiction treatment settings.

The use of *Cannabis* for the treatment of pain is confirmed, but the potential for psychotropic effects limits its use. The approaches to circumvent this problem emphasized the CB2 receptor agonists. Selective CB2 agonists and peripherally restricted CB1 or CB1/CB2 dual agonists have been explored to treat inflammatory

and neuropathic pain, demonstrating efficacy in various pain models. CB2 receptors were originally described as being restricted to cells of immune origin. However, their expression in human primary sensory neurons and increased CB2 receptors in human peripheral nerves have been seen after injury, particularly in painful neuromas [33].

CB2 receptor agonists produce antinociceptive effects in inflammatory and nociceptive pain models, and in some cases, the effects involve activating the opioid system. CB receptor agonists also enhance the effect of mu-opioid receptor agonists in various models of analgesia, and combinations of cannabinoids and opioids may produce synergistic effects. Antinociceptive effects of compounds blocking the metabolism of anandamide have been reported in inflammatory pain models. There is also evidence that such compounds increase the analgesic effect of nonsteroidal anti-inflammatory drugs (NSAIDs), raising the possibility that a combination of suitable agents also could do the same by reducing the NSAID dose needed and providing an efficacious treatment strategy while minimizing the potential for NSAID-induced gastrointestinal and cardiovascular disturbances.

There has been increasing awareness of the impact of excessive opioid prescribing on long-term narcotic use and the development of adverse narcotic-related events. Studies have identified significant knowledge gaps in acute and chronic pain management among graduating urology residents as of 2018. However, it is unclear whether the experience of graduating urology residents has changed. To evaluate the attitudes and experience of graduating urology residents in prescribing opioid/nonopioid analgesia for acute (AP), chronic noncancer (CnC), and chronic cancer (CC) pain, a survey was conducted in a review course [48]. The survey consisted of open-ended and close-ended five-point Likert scale questions, and descriptive statistics, Mann-Whitney U-test, and Student's t-test were used to analyze the data.

Thirty-two postgraduate year-five (PGY5) urology residents completed the survey (92% response rate); most agreed that formal training in managing AP/CnC/CC is valuable (91/78/81%); most find their CnC/CC management training inadequate and are unaware of opioid prescribing guidelines; 66% never counsel patients on how to dispose of excess opioids. In general, 88% are comfortable prescribing opioids, whereas most are uncomfortable prescribing *Cannabis* or antidepressants (100% and 78%, respectively). Residents reported the acute pain service as the highest-rated resource for information and dedicated textbooks as the least. The survey demonstrated that experience in pain management remains variable among urology residents. Knowledge gaps remain, particularly in the management of CC/CnC pain.

The number of fatalities resulting from opioid overdoses is typically attributed to respiratory depression due to decreased reflexive inspiration within the pre-Bötzinger complex in the brainstem. Cannabinoid receptor agonism is reported to enhance opioid analgesia, but cannabinoid enhancement or inhibition of opioid-induced respiratory depression has not been determined. The roles of the cannabinoid-1 and cannabinoid-2 receptors on respiratory depression were evaluated using selective agonists alone and in combination with morphine in male mice.

Using whole-body plethysmography, the nonselective CB1R and CB2R agonist (delta-9-tetrahydrocannabinol) and the CB1R synthetic cannabinoid, AM356,

induced respiratory depression, whereas the well-published selective CB2 agonist JWH-133, and the CB2 agonist (AM2301) did not [52]. A selective CB2R agonist (AM2301) significantly attenuated morphine sulfate-induced respiratory depression. The findings suggest that attenuation of opioid-induced respiratory depression relies on CB2R activation, supporting selective CB2R agonism as an opioid adjunct therapy.

In an 8-h laboratory study, the analgesic efficacy of vaporized *Cannabis* in patients with neuropathic pain related to injury or disease of the spinal cord, most of whom were experiencing pain despite traditional treatment, was evaluated [56]. After obtaining baseline data, 42 participants underwent a standardized procedure for inhaling four puffs of vaporized *Cannabis* containing either placebo, 2.9%, or 6.7% delta-9-THC on three separate occasions. Double dosing occurred 3 h later; participants chose to inhale four to eight puffs. The flexible dosing was used in an attempt to reduce the placebo effect. Using an 11-point numerical pain intensity rating scale as the primary outcome, a mixed effects linear regression model indicated a significant analgesic response for vaporized *Cannabis*. When subjective and psychoactive side effects (e.g., drug effect, feeling high) were added as covariates to the model, the reduction in pain intensity remained significant above and beyond any effect of the measures ($p < 0.0004$).

Measurement of neuropsychological performance proved challenging because of the disabilities of the studied population. Psychoactive and subjective effects were dose-dependent. The two active doses did not significantly differ in terms of analgesic potency; the lower dose appears to offer the best risk-benefit ratio in patients with neuropathic pain associated with injury or disease of the spinal cord. The study supports the consideration of future research that would include longer studies over weeks to months to evaluate the efficacy of medicinal *Cannabis* in patients with central neuropathic pain.

The systemic administration of cannabinoids produces antinociception and their chronic use leads to analgesic tolerance and cross-tolerance to morphine. The effects are mediated by cannabinoids binding to peripheral, spinal, and supraspinal CB1 and CB2 receptors, making it difficult to determine the relevance of each receptor type to the phenomena. In the brain, the CB1 receptors (CB1Rs) are expressed at high levels in neurons, whereas the expression of CB2Rs is marginal. CB1Rs mediate the effects of smoked *Cannabis* and are also implicated in emotional behaviors. The production of supraspinal analgesia and the development of tolerance at CB1Rs by directly injecting a series of cannabinoids into the brain were analyzed.

The influence of the activation of CB1Rs on supraspinal analgesia evoked by morphine was also evaluated. Intracerebroventricular (icv) administration of cannabinoid receptor agonists, WIN55212-2, ACEA, or methanandamide generated dose-dependent analgesia [70]. A single administration of the compounds brought about profound analgesic tolerance lasting more than 14 days. This decrease in the effect of cannabinoid receptor agonists was not mediated by the depletion of CB1Rs or the loss of regulated G proteins; it was accompanied by reduced morphine analgesia.

Acute morphine administration produced tolerance that lasted only 3 days and did not affect the CB1R. It was revealed that both neural mu-opioid receptors (MORs) and CB1Rs interact with the HINT1-RGSZ module, thereby regulating pertussis toxin-insensitive Gz proteins. In mice with reduced levels of *the* Gz proteins, the CB1R agonists produced no such desensitization or morphine cross-tolerance. Experimental enhancement of Gz signaling enabled an acute icv administration of morphine to produce a long-lasting tolerance at MORs that persisted for more than 2 weeks. It also impaired the analgesic effects of cannabinoids. In the brain, cannabinoids can produce analgesic tolerance that is not associated with the loss of surface CB1Rs or uncoupling from regulated transduction. Neural-specific Gz proteins are essential mediators of the analgesic effects of supraspinal CB1R agonists and morphine. The Gz proteins are also responsible for the long-term analgesic tolerance produced by single doses of *the* agonists and the cross-tolerance between CB1Rs and MORs.

After oral administration to mice, pethidine, delta-8-tetrahydrocannabinol (delta-8-THC), delta-9-THC, and cannabidiol had a dose-dependent antinociceptive effect when measured by the hot-plate method. Cannabidiol was inactive at 30 mg/kg. Delta-8-THC, delta-9-THC, and pethidine did not differ significantly in potency, but delta-9-THC was 6.5 times more active than cannabidiol [73].

After oral administration, three different *Cannabis* extracts, delta-8-THC, delta-9-THC, and morphine, produced dose-dependent depressions of the passage of a charcoal meal in mice. The delta-9-THC content could account for the effect of the three *Cannabis* extracts on intestinal motility. Delta-8-THC and delta-9-THC were equipotent and were about five times less potent than morphine. Cannabidiol was inactive up to 30 mg/kg.

The antinociceptive effect of pethidine and the effect of morphine on intestinal motility were antagonized by nalorphine, while the effects of the *Cannabis* extracts and the pure cannabinoids were not. The findings conclude that although *Cannabis* and narcotics share several common pharmacological properties, the mode of action is pharmacologically distinct.

A case report described a patient with preoperative complex pain syndrome who underwent liver transplantation and could reduce his opioid consumption significantly following treatment with medical *Cannabis* [90]. A 57-year-old male with a history of hepatitis C cirrhosis underwent liver transplantation. Preoperatively, the patient took 2–8 mg/day of hydromorphone for chronic abdominal pain. He was given intravenous patient-controlled analgesia postoperatively, receiving hydromorphone 30 mg/day. The multidisciplinary Transitional Pain Service managed his moderate to severe acute postsurgical pain in the hospital and continued weaning him from opioid medications after discharge.

It was difficult to wean the patient from opioids, and he was subsequently given medical *Cannabis* at 6 weeks postoperatively with remarkable effect. By the fifth postoperative month, his use of opioids had tapered to 6 mg/day of hydromorphone, and his functional status was excellent on the regimen. Reductions in opioid consumption were achieved with the administration of medical *Cannabis* in a patient with acute postoperative pain superimposed on a chronic pain syndrome and

receiving high doses of opioids. Concurrent benefits of initiating medical *Cannabis* may include improvements in pain profile and functional status with reductions in opioid-related side effects. The case highlights the potential for medical *Cannabis* as an adjunct medication for weaning patients from opioid use.

A study was conducted to determine whether cannabidiol-opioid pharmacotherapy could attenuate cisplatin-induced tactile allodynia [91]. Mice (C57BL/6) were given six doses of 2.3 mg/kg cisplatin intraperitoneally on alternating days to induce tactile allodynia as quantified using an electric von Frey (eVF). The test groups in experiment 1 received either vehicle, 0.1 or 2.5 mg/kg morphine, 1.0 or 2.0 CBD, or the two drugs in combination. Test groups in experiment two received vehicle 0.1 or 2.5 mg/kg morphine; 1.0, 2.0, 3.0, or 4.0 mg/kg NB2111 (a long-acting CBD analog), or the two drugs combined. The treatments were administered intraperitoneally 45 min before the eVF assessment.

Cisplatin produced tactile allodynia that was attenuated by 2.5 mg/kg morphine. Both CBD and NB2111 produced dose-dependent attenuation of tactile allodynia. Cannabidiol and NB2111 given in combination with subanalgesic doses of morphine produced attenuation of tactile allodynia, equivalent to 2.5 mg/kg morphine. While both CBD and NB2111, either alone or in combination with subanalgesic doses of opioids, exhibited analgesic effects, NB2111 could be capable of superior analgesia over time by enhanced pharmacokinetics.

In animal studies, it has been shown that delta-9-tetrahydrocannabinol has antinociceptive effects or potentiates the antinociceptive effect of morphine. A study measured the analgesic effect of delta-9-THC, morphine, and a delta-9-THC/morphine combination (delta-9-THC/morphine) in humans using experimental pain models [92]. Delta-9-THC (20 mg), morphine (30 mg), delta-9-THC/morphine (20 mg delta-9-THC/30 mg morphine), or placebo were given orally and in single doses. Twelve healthy volunteers were included in the randomized, placebo-controlled, double-blinded, crossover study. The experimental pain tests (order randomized) were heat, cold, pressure, and single and repeated transcutaneous electrical stimulation.

Additionally, reaction time, side effects (visual analog scales), and vital functions were monitored. For the pharmacokinetic profiling, blood samples were collected. Delta-9-THC did not significantly reduce pain. The cold and heat tests produced hyperalgesia which was completely neutralized by delta-9-THC/morphine. A slight additive analgesic effect could be observed for THC-morphine in the electrical stimulation test. No analgesic effect resulted in the pressure and heat test, neither with delta-9-THC nor delta-9-THC-morphine. Psychotropic and somatic side effects (sleepiness, euphoria, anxiety, confusion, nausea, dizziness) were common but usually mild.

Chlornaltrexamine (beta-CNA), a selective, long-acting irreversible opiate antagonist, inhibited the analgesia, hypothermia, tolerance, and physical dependence produced by delta-9-tetrahydrocannabinol in rats. The results suggested some common features between *Cannabis* and opiates, and some actions of delta-9-THC may be mediated by opioid-related mechanisms in the central nervous system [93].

Cannabidiol and the Serotonin (5-HT)_{1A} Receptor

Clinical studies indicated that cannabidiol interacts with the serotonin (5-HT)_{1A} receptor and possesses analgesic and anxiolytic effects. The effects on 5-HT neuronal activity and impact on neuropathic pain models are unclear. In vivo single-unit, extracellular recordings in rats demonstrated that acute intravenously administered doses of CBD (0.1–1.0 mg/kg) decreased the firing rate of 5-HT neurons in the dorsal raphe nucleus [14]. The effect was inhibited by the intravenous administration of the 5-HT_{1A} antagonist, WAY 100635, at a dose of 0.3 mg/kg and the TRPV1 antagonist, capsaizpine, at 1 mg/kg, but not by the CB1 receptor antagonist AM 251 at a dose of 1 mg/kg intravenously.

Repeated treatments with CBD at 5 mg/kg/day, subcutaneously administered for 7 days, increase 5-HT firing through desensitization of 5-HT_{1A} receptors. Seven days of treatment with CBD reduced mechanical allodynia, decreased anxiety-like behavior, and normalized 5-HT activity. Rats subjected to the spared nerve injury model for 24 days demonstrated decreased 5-HT firing activity and mechanical allodynia, increasing anxiety-like behavior in the elevated plus maze, open-field, and novelty-suppressed feeding tests.

Antiallodynic effects of CBD were fully suppressed by capsaizpine at a dose of 10 mg/kg/day, subcutaneously for 7 days, and partially prevented by WAY 100635 at 2 mg/kg/day administered subcutaneously for 7 days. The anxiolytic effect was blocked only by WAY. Repeated treatment with low-dose CBD induces analgesia predominantly through TRPV1 activation, reduces anxiety through 5-HT_{1A} receptor activation, and rescues impaired 5-HT neurotransmission under neuropathic pain conditions.

TRPV1 as a Target of the Therapeutic Potential of Myrcene

Nociceptive Transient Receptor Potential channels such as TRPV1 are targets for treating pain. Both antagonists and agonists of TRP channels can promote analgesia through inactivation and chronic desensitization. Since plant-derived mixtures of cannabinoids and the *Cannabis* component myrcene have been suggested as pain therapeutics, terpenes in *Cannabis* were examined for activity at TRPV1, an inducible expression of TRPV1 to examine TRPV1-dependency of terpene-induced calcium flux responses.

Terpenes contribute differentially to calcium fluxes via TRPV1 induced by *Cannabis*-mimetic cannabinoid/terpenoid mixtures. Myrcene dominates the TRPV1-mediated calcium responses seen with terpenoid mixtures. The TRPV1 inhibitor capsaizpine inhibited myrcene-induced calcium influx, and myrcene elicits TRPV1 currents in the whole-cell patch-clamp configuration. TRPV1 currents were highly sensitive to internal calcium.

When myrcene currents were evoked, they were distinct from capsaicin responses based on I_{max} and their lack of shift to a pore-dilated state. Myrcene preapplication and residency at TRPV1 appear to negatively impact subsequent responses to TRPV1 ligands such as cannabidiol, indicating allosteric modulation and possible competition by myrcene. Molecular docking studies suggested a noncovalent interaction site for myrcene in TRPV1 and identified key residues that form partially overlapping myrcene and cannabidiol binding sites. Several non-*Cannabis* plant-derived sources of myrcene and other compounds targeting nociceptive TRPs were identified [26]. Using a data mining approach focused on analgesics suggested by non-Western Traditional Medical Systems, the data established TRPV1 as a target of myrcene and indicated the therapeutic potential of analgesic formulations containing myrcene.

Cannabinoids and the Glycine Receptors

Another potential for endocannabinoid modulatory agents includes alpha-(2)-adrenoceptor modulators, peroxisome proliferator-activated receptor alpha agonists, and TRPV1 antagonists. An extension of the polypharmacological approach is combining the treatment's desired pharmacological properties within a single molecule. The approaches have the potential of yielding novel analgesics that do not produce the psychotropic effects that limit the medicinal use of *Cannabis*.

Cannabinoids enhance the function of glycine receptors (GlyRs), but little is known about the mechanisms and behavioral implications of cannabinoid–GlyR interaction. Using mutagenesis and NMR analysis, a serine at 296 in the GlyR protein critical for the potentiation of I(Gly) by delta-9-tetrahydrocannabinol, a major psychoactive component of *Cannabis*, had been identified [34]. The polarity of the amino acid residue at 296 and the hydroxyl groups of delta-9-THC are critical for delta-9-THC potentiation.

Removal of the hydroxyl groups of delta-9-THC results in a compound that does not affect I(Gly) when applied alone but selectively antagonizes cannabinoid-induced potentiating effect on I(Gly) and analgesic effect in a tail-flick test in mice. The cannabinoid-induced analgesia is absent in mice lacking $\alpha 3$ GlyRs, but not in those lacking CB1 and CB2 receptors. The findings reveal a new mechanism underlying the cannabinoid potentiation of GlyRs, which could contribute to some of the *Cannabis*-induced analgesic and therapeutic effects.

Gender Differences in Cannabinoid-Mediated Antinociceptive Effects

Preclinical studies demonstrated that cannabinoid-mediated antinociceptive effects vary according to gender, but it is unknown whether the findings extend to humans. A retrospective analysis compared the analgesic, subjective and physiological effects of active *Cannabis* (3.56–5.60% delta-9-THC) and inactive *Cannabis* (0.00% delta-9-THC) in male ($n = 21$) and female ($n = 21$) *Cannabis* smokers under double-blind, placebo-controlled conditions. Pain response was measured using the Cold-Pressor Test (CPT). The participants immersed their hands in cold water (4 °C); times to report pain (pain sensitivity) and withdraw the hand (pain tolerance) were recorded. Subjective drug ratings were also measured.

Among men, active *Cannabis* significantly decreased pain sensitivity relative to inactive *Cannabis* ($p < 0.01$). In women, active *Cannabis* failed to decrease pain sensitivity relative to the inactive [15]. Active *Cannabis* increased pain tolerance in both men and women immediately after smoking ($p < 0.001$); a trend was observed for differences between men and women ($p < 0.10$). Active *Cannabis* also increased the subjective ratings of *Cannabis* associated with abuse liability (“Take again,” “Liking,” “Good drug effect”), drug strength, and “High” relative to inactive in both men and women ($p < 0.01$).

The results indicate that in *Cannabis* smokers, men exhibit greater *Cannabis*-induced analgesia than women. The gender-dependent differences are independent of *Cannabis*-elicited subjective effects associated with abuse-liability, which were consistent between men and women. As such, sex-dependent differences in *Cannabis*'s analgesic effects are an important consideration that warrants further investigation when considering the potential therapeutic effects of cannabinoids for pain relief.

Preclinical and human research consistently produced antinociceptive effects in preclinical models. They heterogeneously influenced the perception of experimentally induced pain in humans and have not provided robust clinical analgesia [16]. The cannabinoid CB1 receptors are widely expressed throughout the nociceptive system. The activation by endogenous or exogenous cannabinoids modulates the release of neurotransmitters reflected in the antinociceptive effects of cannabinoids in preclinical models of inflammatory, cancer, neuropathic pain, and nociceptive hypersensitivity of cannabinoid receptor-deficient mice.

Cannabis-based medications available for humans mainly comprise delta-9-tetrahydrocannabinol, cannabidiol, and nabilone. Controlled studies assessing the analgesic effects of cannabinoid-based drugs in human experimental settings have been reported. An effect on nociceptive processing could be translated to the human setting in functional magnetic resonance imaging studies that indicated reduced connectivity within the brain's pain matrix. Cannabinoid-based drugs heterogeneously influenced the perception of experimentally induced pain, including a reduction in only the affective but not the sensory perception of pain, moderate analgesic effects, or occasional hyperalgesic effects. Controlled studies indicated a

lack of robust analgesic effects. *Cannabis* has always been associated with analgesia in open-label or retrospective reports, possibly indicating an effect on well-being or mood rather than sensory pain.

Cannabis use has been increasing among women, and one of the most common uses of *Cannabis* for medical purposes is pain relief. Pain conditions and response to analgesics have been demonstrated to be influenced by gender, and evidence is emerging that it is also true with cannabinoid-mediated analgesia. The preclinical evidence supporting gender differences in cannabinoid pharmacology, and emerging evidence from human studies, both clinical and observational, were examined. Numerous animal studies have reported gender differences in the antinociceptive response to natural and synthetic cannabinoids that may correlate to gender differences in the expression, and function, of endocannabinoid system components.

Female rodents have generally been found to be more sensitive to the effects of delta-9-THC. The finding is likely a function of both pharmacokinetic and pharmacodynamics factors, including differences in metabolism, cannabinoid receptor expression, and influence of ovarian hormones, including estradiol and progesterone. Preclinical evidence of direct interactions between sex hormones and the endocannabinoid system translates to gender differences in *Cannabis* and cannabinoid use in men and women [21].

Cannabidiol and Osteoarthritis

A study examines CBD as an add-on analgesic therapy in patients with osteoarthritis in the hands or psoriatic arthritis, who are experiencing moderate pain intensity despite therapy, using a randomized, double-blind, placebo-controlled design [43]. The patients received synthetic CBD 20–30 mg or placebo daily for 12 weeks. The primary outcome was pain intensity during the last 24 h (0–100 mm); safety outcomes were the percentage of patients experiencing adverse events and the characterization of serious adverse events. Explorative outcomes included changes in Pittsburgh Sleep Quality Index (PSQI), Hospital Anxiety and Depression Scale (HADS), Pain Catastrophizing Scale (PCS), and Health Assessment Questionnaire (HAQ-DI).

Thirty-six patients were randomized, and 120 were included in the primary analysis. The between-group difference in pain intensity at 12 weeks was 0.23 mm (95% CI: −9.41–9.90; $p = 0.96$). Twenty-two percent of patients receiving CBD and 21% receiving placebo experienced a reduction in pain intensity of more than 30 mm. Compared to placebo, there was neither a clinically nor statistically significant effect of CBD on pain intensity in patients with hand osteoarthritis and psoriatic arthritis. Additionally, no statistically significant effects were found on sleep quality, depression, anxiety, or pain catastrophizing scores.

Rheumatologic Pain

Despite classic analgesic or effective treatments in rheumatic diseases, such as synthetic DMARDs in RA, patients remain in pain and often turn to nonprescribed pharmacological alternatives, such as *Cannabis*. A review determined the incidence of *Cannabis* consumption calculated by metaproportion demonstrated that approximately 20% of the patients suffering from rheumatologic diseases actively consume *Cannabis*, improving pain [17]. It suggests that *Cannabis* use in the management of patients should be addressed during medical consultation, essentially with *Cannabis*-based standardized pharmaceutical products.

In a sample of 10,873 patients, 2900 reported *Cannabis* consumption [incidence 40.4% (95% confidence interval (CI): 0.28, 0.54)] and 15.3% (95% CI: 0.07, 0.27) specified that they were still taking *Cannabis*. *Cannabis* use was higher in the four fibromyalgia studies [68.2% (95% CI: 0.41, 0.90), $n = 611$] compared with seven articles concerning RA or lupus [26.0% (95% CI: 0.14, 0.41), $n = 8168$]. *Cannabis* consumption was associated with a decrease in pain intensity [VAS pain at baseline 8.2 (2.9) vs. 5.6 (3.5) mm over time; pooled effect size -1.75 (95% CI: -2.75 , -0.76)]. *Cannabis* users were younger [58.4 (11.4) vs. 63.6 (12.1) years; $p < 0.001$], more often smokers [OR 2.91 (95% CI: 1.84, 4.60)] or unemployed [OR 2.40 (95% CI: 1.31, 4.40)], and had higher pain intensity [5.0 (2.4) vs. 4.1(2.6) mm; $p < 0.001$] than nonusers.

Preclinical and clinical studies have demonstrated that *Cannabis*-based drugs have therapeutic potential in inflammatory diseases, including rheumatoid arthritis and Multiple sclerosis. A study was conducted to determine whether the key elements of the endocannabinoid signaling system, which produces immunosuppression and analgesia, are expressed in the synovia of patients with osteoarthritis (OA) or RA. Thirty-two OA and 13 RA patients undergoing total knee arthroplasty were included in this study. Clinical staging was conducted from x-rays scored according to Kellgren–Lawrence and Larsen scales, and synovitis of synovial biopsies was graded. Endocannabinoid levels were quantified in synovial fluid by liquid chromatography-mass spectrometry.

The CB1 and CB2 protein and RNA expression in synovial biopsies were investigated. The functional activity of the receptors was determined with mitogen-activated protein kinase assays [75]. To assess the impact of OA and RA on this receptor system, levels of endocannabinoids in the synovial fluid of patients and noninflamed healthy volunteers were compared. The activity of fatty acid amide hydrolase (FAAH), the predominant catabolic endocannabinoid enzyme, was measured in the synovium. CB1 and CB2 protein and RNA were present in the synovia of OA and RA patients.

Cannabinoid receptor stimulation of fibroblast-like cells from OA and RA patients produced time-dependent phosphorylation of extracellular signal-regulated kinase (ERK)-1 and ERK-2, which was significantly blocked by the CB1 antagonist SR141716A. The endocannabinoids anandamide and 2-arachidonyl glycerol were identified in the synovial fluid of OA and RA patients. However, neither AEA nor

2-AG was detected in synovial fluid from normal volunteers. FAAH was active in the synovia of OA and RA patients and was sensitive to inhibition by URB597 (3'-(aminocarbonyl) [1,1'-biphenyl]-3-yl)-cyclohexylcarbamate). The data predict that the cannabinoid receptor system in the synovium may be an important therapeutic target for treating pain and inflammation associated with OA and RA.

A study reports the controlled use of a standardized pharmaceutical preparation of cannabinoids in capsular form.¹⁰² The therapy was assessed in a patient with familial Mediterranean fever who presented with chronic relapsing pain and inflammation of gastrointestinal origin. After determining a suitable analgesic dosage, a double-blind placebo-controlled crossover trial was conducted using 50 mg delta-9-tetrahydrocannabinol daily in five doses in the active weeks and measuring the effects on the parameters of inflammation and pain. Although no anti-inflammatory effects of delta-9-tetrahydrocannabinol were detected during the trial, a highly significant reduction ($p < 0.001$) in additional analgesic requirements was achieved.

***Cannabis* in Acute Pain and Hyperalgesia**

Cannabinoid-induced analgesia has been indicated in animal studies of acute inflammatory and neuropathic pain. In humans, controlled clinical trials with delta-9-tetrahydrocannabinol and other cannabinoids demonstrated analgesic efficacy in chronic pain syndromes, whereas the data in acute pain were less conclusive. In a study to investigate the effects of oral *Cannabis* extract in two different human models of acute inflammatory pain and hyperalgesia, a double-blind crossover study with 18 healthy female volunteers was conducted [24].

Capsules containing delta-9-tetrahydrocannabinol-standardized *Cannabis* extract or active placebo were orally administered. A circular sunburn spot was induced on one upper leg. Heat and electrical pain thresholds were determined at the erythema, the area of secondary hyperalgesia, and the contralateral leg. Intradermal capsaicin-evoked pain and areas of flare and secondary hyperalgesia were measured. Primary outcome parameters were heat pain thresholds in the sunburn erythema and the capsaicin-evoked area of secondary hyperalgesia. Secondary measures were electrical pain thresholds, sunburn-induced secondary hyperalgesia, and capsaicin-induced pain.

The *Cannabis* extract did not affect heat pain thresholds in the sunburn model. Electrical thresholds (250 Hz) were significantly lower than baseline and placebo. In the capsaicin model, the area of secondary hyperalgesia, flare, and spontaneous pain was not altered. The experiments had no analgesic or antihyperalgesic activity of the *Cannabis* extract. The results point to the development of a hyperalgesic state under cannabinoids. The results suggested that cannabinoids are ineffective analgesics for treating acute nociceptive pain in humans.

The frontal-limbic distribution of cannabinoid receptors in the brain suggests that *Cannabis* may preferentially target the affective qualities of pain. This central mechanism of action may be relevant to cannabinoid analgesia in humans. Functional

magnetic resonance imaging was employed to investigate the effects of delta-9-tetrahydrocannabinol on brain activity related to cutaneous ongoing pain and hyperalgesia that were temporarily induced by capsaicin in healthy volunteers [72].

Delta-9-tetrahydrocannabinol reduced the reported unpleasantness but not the intensity of ongoing pain and hyperalgesia. The analgesic effect on hyperalgesia was substantiated by diminished anterior mid-cingulate cortex activity. In individuals, the drug-induced reduction in the unpleasantness of hyperalgesia was positively correlated with right amygdala activity. Delta-9-tetrahydrocannabinol also reduced functional connectivity between the amygdala and primary sensorimotor areas during the ongoing-pain state.

Critically, the reduction in sensory-limbic functional connectivity was positively correlated with the difference in drug effects on the unpleasantness and the intensity of ongoing pain. Peripheral mechanisms alone cannot account for the dissociative effects of delta-9-THC on the pain observed. Instead, the data reveal that amygdala activity contributes to the interindividual response to cannabinoid analgesia and suggests that dissociative effects of delta-9-THC in the brain are relevant to pain relief in humans.

A systematic review critically synthesizes the evidence for cannabinoid analgesic effects on quantitative sensory testing outcomes in both healthy adults and patients with chronic noncancer pain [76]. Consistent evidence of cannabinoid analgesia was observed for inhaled *Cannabis* than synthetic cannabinoids. Analgesic effects were most commonly observed in tests of cold pain sensitivity, and hyperalgesic effects were most commonly observed in tests of electrical stimulation.

Studies with healthy subjects did not differ from those with chronic noncancer pain. However, the observations are qualified by the high degree of inconsistency across studies and methodological heterogeneity. The pharmacological activities of delta-9-THC [(minus)-delta-9-trans-tetrahydrocannabinol], cannabinol, and mixtures of delta-9-THC + Cannabinol were studied in rabbits, rats, and mice. Cannabinol, although in general less active, mimicked the effects of delta-9-THC in several pharmacological tests: Corneal areflexia in rabbits, climbing rope, open-field, irritability, and aggressiveness after REM sleep deprivation in rats and catatonnia, analgesia, and sleeping time in mice.

When the mixture of delta-9-THC + cannabinol was used, a synergistic effect occurred on most of the depressant effects. In the one peripheral test, cannabinol did not alter the delta-9-THC effect [77]. On the other hand, cannabinol did not interfere with or slightly inhibit the excitatory effects of delta-9-THC.

The Endocannabinoid System and Integumentary Wounds

Nonuremic calciphylaxis (NUC) often manifests as intractable and painful integumentary wounds, afflicting patients with a high comorbidity burden. The Endocannabinoid System is a ubiquitous signaling system that is dysregulated within wound beds and associated peri-wound tissues. Preclinical research has

indicated that the dominant chemical classes derived from the *Cannabis* plant, cannabinoids, terpenes, and flavonoids, interact with the integumentary ECS to promote wound closure and analgesia [25].

In an open-label cohort study involving two elderly females with recalcitrant NUC leg ulcers of more than 6 months, Topical *Cannabis*-Based Medicines (TCBM) composed of cannabinoids, terpenes, and flavonoids were applied daily. The wound bed and peri-wound tissues were treated until complete wound closure was achieved. The wounds were photographed regularly, and the digital images were subjected to planimetric analysis to objectively quantify the degree of granulation and epithelialization. Analgesic utilization was also tracked as a surrogate/proxy for pain scores. The cohort had a mean M3 multimorbidity index score of 3.31. Complete wound closure was achieved in a mean of 76.3 days.

Additionally, no analgesics were required after a mean of 63 days. The treatments were well tolerated, with no adverse reactions. The results demonstrated that in very challenging wounds, such as NUC, among highly complex patients, TCBM may have a role in integumentary and wound management.

Pain associated with integumentary wounds is highly prevalent, yet it remains an area of significant unmet healthcare needs. Systemically administered opioids are the mainstay of treatment. Recent publications are casting opioids in a negative light given their high side effect profile, inhibition of wound healing, and association with accidental overdose. The ideal pain relief methods for wound patients are topical modalities, which lack systemic side effects, are noninvasive, self-administered, and display rapid onset of analgesia. Extracts derived from the *Cannabis* plant have been applied to wounds for thousands of years.

The human endocannabinoid system and its dominant presence throughout the integumentary system provide a logical scientific platform to consider topical cannabinoids for wounds. A prospective case series of three patients with pyoderma gangrenosum treated with topical medical *Cannabis* compounded in nongenetically modified organic sunflower oil was reported [83]. Clinically significant analgesia associated with reduced opioid utilization was noted in all three cases. The topical treatment indicated the potential to improve pain management in patients suffering from wounds of all classes.

Epidermolysis bullosa (EB) is a blistering genetic disorder characterized by intense pain related to disease pathology and care-based interventions. Opioid-based therapies underpin pain care in EB; however, they cannot provide adequate analgesia in many patients. Cannabinoid-based medicines (CBMs) have been studied increasingly for pain conditions of various etiologies and pose as a novel dimension for pain care in EB [85]. Three patients with EB were prescribed pharmaceutical-grade sublingually administered CBMs comprising tetrahydrocannabinol and cannabidiol. The patients reported improved pain scores, reduced pruritus, and reduced analgesic drug intake.

The Endocannabinoid System in Female Reproduction

The endocannabinoid system has been discovered in many organs, where it plays multiple physiological roles. In female reproduction, the endocannabinoid system is found in different animals, from sea urchins to humans [27]. Its expression spanning the animal Kingdom indicates that endocannabinoid signaling plays a key role in female reproduction.

In genetically manipulated mouse models, it was revealed that endocannabinoid signaling in both the blastocyst and the uterus is required for normal pregnancy since either silenced or elevated endocannabinoid signaling causes multiple defects during pregnancy. Mouse models with the deletion of various genes within the endocannabinoid system are subfertile, indicating that endocannabinoid signaling functions either as a regulatory rather than a decisive determinant or that deficiency of the signaling are compensated for by other signaling pathways.

Since the genetic backgrounds and diets influence reproductive phenotypes in gene-deleted mice, it would be interesting to understand the genetic background of mice and diet composition impact on fertility phenotypes following mutation of a gene in the endocannabinoid pathway. Pregnancy events are regulated by endocannabinoid signaling, and in most cases, optimal outcomes can only be achieved under normal physiological endocannabinoid levels. Either silenced or enhanced endocannabinoid signaling derails the processes; a trait of endocannabinoid signaling that provides a route for medical manipulation in each pregnancy event.

Advances in understanding the physiology and pharmacology of the endogenous cannabinoid system have potentiated the interest in cannabinoid receptors as potential therapeutic targets. Cannabinoids have been shown to modulate various immune cell functions, have therapeutic implications on central nervous system inflammation and chronic inflammatory conditions such as arthritis, and may be therapeutically useful in treating autoimmune conditions [35]. Many drug effects occur through cannabinoid receptor signaling mechanisms and the modulation of cytokines and other gene products.

Labor Pain

There is increasing use of *Cannabis* during pregnancy, and a survey indicated that women are considering the use of *Cannabis* for labor pain. In a university hospital, 132 women with vaginal deliveries completed a survey exploring attitudes toward labor analgesia and *Cannabis* use [37]. Patients who would and would not consider *Cannabis* for labor pain were compared using the chi-square analysis.

The respondents who reported having epidural or intravenous analgesia were 83% (95% confidence interval [CI]: 76–89) and 30% (95% CI: 23–38), respectively, with 87% (95% CI: 79–92) and 86% (95% CI: 71–94) being satisfied. However, 34% (95% CI: 26–43) of the respondents reported that they would consider the use

of *Cannabis* for labor pain. Of those, 25% (95% CI: 14–41) had previously used *Cannabis* for pain compared with 0% (95% CI: 0–0) who had not, and 72% (95% CI: 56–84) thought it was acceptable to use *Cannabis* nonmedically compared with 35% (95% CI: 26–47) who did not ($P < 0.001$ for both). The greatest worry was the effect of marijuana on the baby, with 26% (95% CI: 19–34) being highly worried and 26% (95% CI: 19–34) being extremely worried. Many women (60%; 95% CI: 51–68) indicated a lack of knowledge of the side effects of *Cannabis* in labor. However, 59% (95% CI: 50–67) of respondents said they would feel comfortable discussing this topic with their obstetrician.

Endometriosis

Endometriosis is associated with a health burden that is difficult to manage. High levels of illicit *Cannabis* use for therapeutic purposes have been reported by endometriosis patients in Australia and New Zealand (N.Z.). Although access to legal medicinal *Cannabis* is available through medical prescription via multiple federal schemes, significant barriers to patient access remain. An anonymous cross-sectional online survey was developed and distributed through social media via endometriosis advocacy groups [11].

The respondents were asked about legal versus illicit *Cannabis* usage, their understanding of access pathways and legal status, and their interactions with health care professionals. Of 237 respondents who reported *Cannabis* use with a medical diagnosis of endometriosis, 186 (72.0%) Australian and 51 (88.2%) N.Z. respondents reported self-administering *Cannabis* illicitly. Only 23.1% of Australian and 5.9% of N.Z. respondents accessed *Cannabis* through a doctor's prescription, with 4.8% of Australian and no N.Z. respondents reporting legally self-administer *Cannabis*.

Substantial substitution effects (>50% reduction) were observed in users of non-opioid analgesia (63.1%), opioid analgesia (66.1%), hormonal therapies (27.5%), antineuropathics (61.7%), antidepressants (28.2%), and antianxiety medications (47.9%). Of Australian respondents, 18.8%, and of N.Z. respondents, 23.5% reported not disclosing their *Cannabis* use to their medical doctor, citing concern over legal repercussions, societal judgment, or their doctors' reaction, and presumed unwillingness to prescribe legal *Cannabis* use.

The respondents self-reported positive outcomes when using *Cannabis* for management of endometriosis, demonstrating a therapeutic potential for *Cannabis* use. Despite this, many are using *Cannabis* without medical supervision. While evidence for a substantial substitution effect by *Cannabis* was demonstrated in the data, of particular concern are the clinical consequences of using *Cannabis* without medical supervision, particularly with regard to drug interactions and the tapering or cessation of certain medications without that supervision. Improving doctor and patient communication about *Cannabis* use may improve levels of medical

oversight, the preference for legal *Cannabis* use adoption over acquisition via illicit supply, and reduce *Cannabis*-associated stigma.

Cannabis and *Cannabis*-derived products' clinical potential relies on their treatment efficacy and potential adverse effects that impact outcomes, including abuse liability and neurocognitive effects. A comprehensive review of placebo-controlled studies investigated *Cannabis* and *Cannabis*-derived products' analgesic effects in 38 studies; 29 assessed *Cannabis* and *Cannabis*-derived products for chronic pain, one for acute pain, and eight for experimental pain tests [12].

The studies ascertained adverse effects through self-report. Few studies specifically probed abuse liability and cognitive and psychomotor effects. Most chronic and experimental pain studies found *Cannabis* and *Cannabis*-derived products to reduce pain. Overall, adverse effects were mild to moderate and dose-related. Studies investigating the impact of *Cannabis* and *Cannabis*-derived products on abuse liability and neurocognitive endpoints were limited to inhaled administration and confirmed dose-related effects.

The *Cannabis* Flower in Pain Management

A dataset in the United States of real-time responses to and side effect reporting from patient-directed *Cannabis* consumption sessions for treating pain under natural conditions was evaluated. The study evaluated how *Cannabis* affects momentary pain intensity levels and which product characteristics are the best predictors of therapeutic pain relief. Between 06/06/2016 and 10/24/2018, 2987 people used the ReleafApp to record 20,513 *Cannabis* administrations measuring *Cannabis*' effects on momentary pain intensity levels across five pain categories: musculoskeletal, gastrointestinal, nerve, headache-related, or nonspecified pain [31]. The average pain reduction was 3.10 points on a 0–10 visual analog scale ($SD = 2.16$, $d = 1.55$, $p < 0.001$).

The entire *Cannabis* flower was associated with greater pain relief than other types of products. Higher tetrahydrocannabinol levels were the strongest predictors of analgesia and side effects prevalence across the five pain categories. The findings suggest benefits from patient-directed, *Cannabis* therapy as a mid-level analgesic treatment. Effectiveness and side effect manifestation vary with the characteristics of the product used.

Cannabis in Capsaicin-Induced Pain

Using a human experimental pain model, a randomized, double-blinded, placebo-controlled, crossover trial in 15 healthy volunteers was conducted to evaluate the concentration-response effects of low-dose (2%), medium-dose (4%), and high-dose (8%) smoked *Cannabis* [32]. The delta-9-tetrahydrocannabinol doses were

evaluated for pain and cutaneous hyperalgesia induced by intradermal capsaicin. Capsaicin was injected into opposite forearms 5 and 45 min after drug exposure, and pain, hyperalgesia, tetrahydrocannabinol plasma levels, and side effects were analyzed.

After 5 min of *Cannabis* exposure, capsaicin-induced pain had no effect at any dose. By 45min after *Cannabis* exposure, there was a significant decrease in capsaicin-induced pain with the medium dose and a significant increase in capsaicin-induced pain with the high dose. There was no effect seen with the low dose, nor was there an effect on the area of hyperalgesia at any dose. After adjusting the overall dose effects, significant negative correlations were found between pain perception and plasma delta-9-tetrahydrocannabinol levels. There was no significant difference in performance on the neuropsychological tests.

***Cannabis* Use Preoperatively**

Cannabis use is potentially associated with increased harm and varied effects on pain control. A retrospective cohort study compared surgical patients reporting *Cannabis* use preoperatively to control patients with no *Cannabis* exposure in a 1–2 ratio. To control for confounding, a propensity score-matched analysis was used to assess the adjusted association between *Cannabis* use and study outcomes. The primary outcome was a composite of respiratory arrest or cardiac arrest, intensive care admission, stroke, myocardial infarction, and mortality during this hospital stay. The effects on pain control, opioid usage, induction agent dose, and nausea-vomiting were also assessed.

Between January 2018 and March 2019, 1818 patients consisting of *Cannabis* users (606) and controls (1212) were evaluated for the study. For propensity score-matched analyses, 524 *Cannabis* patients were compared with 1152 control patients. No difference in the incidence of composite outcomes was observed (OR 1.06, 95% CI: 0.23–3.98). A higher incidence of arrhythmias (2.7% vs. 1.6%) and decreased incidence of nausea-vomiting needing treatment (9.6% vs. 12.6%) were observed with *Cannabis* users vs. controls, the results were not statistically significant, and no significant differences were observed with the other secondary outcomes [30]. The results demonstrate no convincing association between self-reported *Cannabis* use and major surgical outcomes or pain management, and perioperative decisions should be made based on dose, duration, and indication considerations.

***Cannabis* and Postoperative Pain**

A clinical study investigated whether a single oral dose of *Cannabis* plant extract could provide pain relief with minimal side effects for postoperative pain [38]. The patients, aged 18–75 years, were recruited and consented before surgery if

patient-controlled analgesia was planned to provide postoperative pain relief. Each patient received a single dose of 5, 10, or 15 mg of Cannador if he or she had at least moderate pain after stopping patient-controlled analgesia. Starting with 5 mg, the dose escalation was based on the number of patients requesting rescue analgesia and the adverse effects. Pain relief, intensity, and side effects were recorded over 6 h and analyzed using tests for trend with dose.

Rescue analgesia was requested by all 11 patients (100%) receiving 5 mg, 15 of 30 patients (50%) receiving 10 mg, and 6 of 24 patients (25%) receiving 15 mg Cannador (log-rank test for trend in time to rescue analgesia with dose $p < 0.001$). There were significant trends across the escalating dose groups for decreasing pain intensity at rest ($p = 0.01$), increasing sedation ($p = 0.03$), and more adverse events ($p = 0.002$). The number needed to treat to prevent one rescue analgesia request for the 10-mg and 15-mg doses, relative to 5 mg, were 2.0 (95% confidence interval, 1.5–3.1) and 1.3 (95% confidence interval, 1.1–1.7), respectively. The study was terminated because of a serious vasovagal adverse event in a patient receiving 15 mg.

Studies of endogenous cannabinoids (endocannabinoids) have demonstrated that they are present in most tissues and that in some pain states, such as neuropathic pain, levels of endocannabinoids are elevated at key sites involved in pain processing. Cannabinoid CB1 and CB2 receptors are located at key sites involved in the relaying and processing of noxious inputs. CB1 and CB2 receptor agonists have analgesic effects in various inflammatory and neuropathic pain models. Clinical trials of *Cannabis*-based medicines indicate that the preclinical effects of cannabinoid agonists may translate into therapeutic potential in humans [39].

One of the areas of concern with this pharmacological approach is that CB1 receptors have a widespread distribution in the brain and that global activation of CB1 receptors is associated with adverse side effects. An alternative approach that can be used to harness the potential therapeutic effects of cannabinoids is to maximize the effects of the endocannabinoids, the actions of which are terminated by reuptake and metabolism by various enzymes, including fatty acid amide hydrolase, monoacylglycerol lipase and cyclooxygenase type 2. Preventing endocannabinoids' metabolism or uptake elevates the lipid compounds' levels in tissue and produces behavioral analgesia in models of acute pain.

Despite the multiple enzymes involved in the metabolism of ECs, selective inhibition of FAAH increases levels of ECs that are associated with CB1 and CB2 receptor-mediated antinociceptive effects in models of acute and inflammatory pain. Importantly, increased levels of ECs have not been associated with the adverse side effects associated with direct agonists. This is particularly relevant in inflammatory pain models associated with an upregulation of COX2. The consensus of studies in neuropathic rats is that levels of ECs are increased at multiple levels following this type of injury. However, the effects of inhibition of FAAH are less clear-cut. Further studies of the effects of inhibitors of MAGL and transport mechanisms in models of persistent pain are required to identify their potential therapeutic effects.

AKB48 is a designer drug that belongs to the indazole synthetic cannabinoids class. It is sold as an herbal blend, incense, or research chemicals for its

psychoactive *Cannabis*-like effects. The in vivo pharmacological and behavioral effects of AKB48 were investigated in male rats. The pharmacodynamic effects were measured, and its plasma pharmacokinetics was simultaneously determined [40].

AKB48, at low doses preferentially stimulated dopamine release in the nucleus accumbens shell (0.25 mg/kg) and impaired visual sensorimotor responses (0.3 mg/kg) without affecting acoustic and tactile reflexes, which are reduced only to the highest dose tested (3 mg/kg). Increasing doses (0.5 mg/kg) of AKB48 impaired place preference and induced hypolocomotion in rats. At the highest dose (3 mg/kg), AKB48 induced hypothermia, analgesia, and catalepsy; inhibited the startle/prepulse inhibition test; and caused cardiorespiratory changes characterized by bradycardia and mild bradypnea, and SpO₂ reduction.

The study demonstrated the pharmacological and behavioral effects of AKB48 in rats and their correlation to the plasma levels of synthetic cannabinoids. AKB48 plasma concentrations rose linearly with increasing doses and were correlated with changes in the somatosensory, hypothermic, analgesic, and cataleptic responses in rats. All behavioral and neurochemical effects were fully prevented by the selective CB1 receptor antagonist/inverse agonist AM251.

The activation of cannabinoid CB1 receptors (CB1R) by delta-9-tetrahydrocannabinol induces analgesia. CB1R activation also produces cognitive impairment via the serotonin 5HT_{2A} receptor (5HT_{2AR}), a component of a CB1R-5HT_{2AR} heteromer, posing a serious drawback for cannabinoid therapeutic use. It has been shown that peptides reproducing CB1R transmembrane (TM) helices 5 and 6, fused to a cell-penetrating sequence (CPP), can alter the structure of the CB1R-5HT_{2AR} heteromer and avert delta-9-THC cognitive impairment while preserving analgesia. Optimizing the prototypes into drug-like leads to (i) shortening the TM5, TM6, and CPP sequences, without losing the ability to disturb the CB1R-5HT_{2AR} heteromer and (ii) extensive sequence remodeling to achieve protease resistance and blood-brain barrier penetration has been reported. The efforts have culminated in identifying an ideal candidate for *Cannabis*-based pain management, an orally active 16-residue peptide preserving delta-9-THC-induced analgesia.

CB1R is the most-abundant GPCR in the mammalian brain and the main target of delta-9-THC. While originally thought to exist exclusively as a monomer, evidence indicates its ability to form homo- and heterodimer complexes with new pharmacological and functional properties. Of particular relevance is the formation of the CB1R-5HT_{2AR} heteromer, as revealed by: In vitro proximity-based biophysical techniques, specific biochemical signatures of heteromers distinct from those of the protomers, in situ proximity ligation assay to detect protein-protein interactions in native tissues, membrane-permeable peptides altering heteromeric interfaces, and mice expressing heteromerization-deficient receptors established criteria for the existence of CB1R-5HT_{2AR} heteromers in vivo.

Therefore, the CB1R-5HT_{2AR} heteromer is not simply due to receptor overexpression but the consequence of an explicit molecular mechanism of delta-9-THC to dissociate beneficial antinociceptive response and detrimental amnesic effects, hence defining it as a novel therapeutic target [41].

An evaluation was conducted on the efficacy of delta-9-tetrahydrocannabinol in postoperative pain. In a randomized, double-blind, placebo-controlled, single-dose trial, 40 women undergoing elective abdominal hysterectomy were investigated [88]. Randomization occurred when postoperative patient-controlled analgesia was discontinued on the second postoperative day. When patients requested further analgesia, they received a single, identical capsule of either oral delta-9-THC 5 mg ($n = 20$) or placebo ($n = 20$) in a double-blind fashion. The primary outcome measure was summed pain intensity difference (SPID) at 6 h after the administration of the study medication derived from visual analog pain scores on movement and at rest. Secondary outcome measures were time to the rescue medication and the adverse effects of the study medication. Mean (SD) VAS pain scores before medication in the placebo and delta-9-THC groups were 6.3(2.6) and 6.4(1.3) cm on movement, and 3.2(1.9) and 3.3(0.9) on rest, respectively.

There were no significant differences in mean (95% confidence interval of the difference) SPID at 6 h between the groups [placebo 7.9, delta-9-THC 4.3(−1.8 to 9.0) cm hour on movement; placebo 8.8, delta-9-THC 4.9(−0.2 to 8.1) cm hour at rest] and time to rescue analgesia [placebo 217, delta-9-THC 163(−22 to 130) minutes]. Increased awareness of surroundings was reported more frequently in patients receiving delta-9-THC (40 vs. 5%, $p = 0.04$). There were no other significant differences concerning adverse events. The study demonstrates no evidence of an analgesic effect of orally administered delta-9-THC 5 mg in postoperative pain in humans.

A 53-year-old female with a history of congenital lumbar meningocele status post-L4-L5 laminectomy was presented to the Mayo Clinic for treatment of foot pain that began 3 days after her laminectomy. She experienced a 6/10 burning, tingling sensation in the lateral dorsal portion of her right foot and posterolateral calf, in addition to allodynia that prevented her from wearing shoes. She failed gabapentin, amitriptyline, *Cannabis*, transforaminal epidural steroid injections, and two spinal cord stimulator trials. The patient ultimately underwent implantation of a right sural nerve stimulator, resulting in a 50% improvement in pain and functionality at a 2-year follow-up.

The case emphasizes the recent development of design-specific PNS devices and their successful use in this case. Peripheral nerve stimulation technology and applications have diminished the role of spinal cord stimulation devices used for the periphery. Peripheral nerve stimulation should be considered for patients with isolated extremity pain, especially in those with spinal abnormalities (e.g., arachnoid cysts).

Anecdotal reports indicated that *Cannabis* users require increased postoperative analgesia. A prospective randomized study was carried out with 73 patients undergoing elective operations [20]. Postoperatively, all patients were assessed by a blinded investigator, who recorded pain intensity, sedation levels, mood, and hourly analgesia requirements. The mean pain intensity difference at the first postoperative hour (MPID1) and the sum of pain intensity differences (SPID1) was calculated. The endpoints were compared between *Cannabis* users and nonusers using SPSS 16: MPIDI, SPID; patient mood; supplemental analgesia requirements, and global assessment scores.

There were 42 *Cannabis* users and 31 nonusers. *Cannabis* users had significantly higher supplemental pethidine requirements (82.7 mg, SD = 3.4 vs. 51.6 mg, SD = 42.7, $p = 0.003$) and significantly greater MPID1 scores (1.88, SD = 1.09 vs. 1.35, SD = 1.12, $p = 0.001$) compared to nonusers. Female *Cannabis* users required significantly more analgesia than males (93.3 mg, SD = 45.8 vs. 78.3 mg, SD = 44.3, $p = 0.025$). *Cannabis* users require significantly more opioid rescue analgesia in the immediate postoperative period.

The Tetrad Effects – Analgesia, Hypothermia, Catalepsy, and Suppressed Locomotion

Cannabis produced characteristic tetrad effects—analgesia, hypothermia, catalepsy, and suppressed locomotion, which are believed to be mediated by the activation of the cannabinoid CB1 receptors. Recent findings of CB2 and GPR55 receptors in the brain were examined to determine whether they are also involved in cannabinoid action [44]. Delta-9-tetrahydrocannabinol (delta-9-THC)-, WIN55212-2-, or XLR11-induced tetrad effects between wild-type (WT) and each genotype of CB1-, CB2-, or GPR55-knockout (KO) mice, were compared and then the effects of antagonists of the receptors on the tetrad effects in WT mice were examined.

Systemic administration of delta-9-THC, WIN55212-2, or XLR11 produced dose-dependent tetrad effects in WT mice. Genetic deletion or pharmacological blockade of CB1 receptors abolished the tetrad effects produced by the three cannabinoids. Unexpectedly, genetic deletion of the CB2 receptor abolished analgesia and catalepsy produced by delta-9-THC or WIN55212-2 but not by XLR11. Microinjections of delta-9-THC into the lateral ventricles also produced tetrad effects in WT but not in CB1-KO mice. CB2-KO mice displayed a reduction in intraventricular delta-9-THC-induced analgesia and catalepsy.

In contrast to CB1 and CB2 receptors, genetic deletion of GPR55 receptors caused enhanced responses to delta-9-THC or WIN55212-2. Antagonism of CB1, CB2, or GPR55 receptors produced alterations similar to those observed in each genotype mouse line. The findings indicate that in addition to CB1, the CB2 and GPR55 receptors are also involved in the pharmacological effects produced by cannabinoids. CB1/CB2, in contrast to GPR55, receptors appear to play opposite roles in cannabinoid action.

Delta-9-tetrahydrocannabinol exerts its actions through the endocannabinoid system by stimulating the cannabinoid type 1 receptor. The widespread distribution of the receptor in different neuronal cell types and the functions modulated by the endocannabinoid system explains the versatility of the effects of delta-9-THC. Conditional CB1 receptor knockout mice were previously used to identify CB1 receptor subpopulations for the tetrad effects of a high dose of delta-9-THC: hypothermia, hypolocomotion, catalepsy, and analgesia [49].

A mouse model for the conditional CB1 receptor in the dorsal telencephalic glutamatergic and forebrain GABAergic neurons determines which CB1 receptor subpopulations are adequate for the tetrad effects. The glutamatergic CB1 receptor was sufficient for delta-9-THC-induced hypothermia and hypolocomotion. Since no sufficiency was found, analgesic and cataleptic effects of delta-9-THC are largely independent of glutamatergic and GABAergic CB1 receptors. It was also revealed that in animals with CB1 receptors exclusively in forebrain GABAergic neurons, delta-9-THC stimulated rather than reduced locomotion. The cell-type selective and hitherto unsuspected hyper-locomotive effect may be occluded in wild-types and conditional knockouts and only be exposed when CB1 signaling is absent in all other cell types, thus underlining the importance of investigating both necessary and sufficient functions to unravel cell-type specific actions unequivocally.

A goal in cannabinoid research has been the discovery of potent synthetic analogs of the natural substances that might be developed as clinically useful drugs; it requires that they be free of the psychotropic effects that characterize the recreational use of *Cannabis*. An important driving force for the goal is the long history of using *Cannabis* as a medicinal agent, especially in treating pain and inflammation. While few compounds appear to have those properties, ajulemic acid, also known as CT-3 and IP-751, is a potential candidate that could achieve the goal. Its chemical structure was derived from that of the metabolite of delta-9-THC. Preclinical studies displayed many properties of nonsteroidal anti-inflammatory drugs; however, it seems free of undesirable side effects [45]. The short-term trials in healthy human subjects and patients with chronic neuropathic pain demonstrated a complete absence of psychotropic actions.

Moreover, it proved to be more effective than the placebo in reducing this pain as measured by the visual analog scale. Unlike the narcotic analgesics, signs of dependency were not observed after withdrawal of the drug at the end of the 1-week treatment period. Data on its mechanism of action are not complete; however, the activation of PPAR-gamma, and regulation of eicosanoid and cytokine production appears to be important for its potential therapeutic effects.

Phytochemical studies on the liverwort *Radula* genus have identified the bibenzyl (-)-cis-perrottetinene (cis-PET), which is structurally similar to (-)-delta-9-trans-tetrahydrocannabinol (delta-9-trans-THC). *Radula* preparations are sold as cannabinoid-like legal high even though pharmacological data are lacking. Synthesis of (-)-cis-PET and its (-)-trans diastereoisomer demonstrate that both molecules readily penetrate the brain and induce hypothermia, catalepsy, hypolocomotion, and analgesia in a CB1 receptor-dependent manner in mice.

The natural product (-)-cis-PET was profiled on major brain receptors, indicating selective cannabinoid pharmacology. The study also uncovers pharmacological differences between delta-9-THC and PET diastereoisomers. Most notably, (-)-cis-PET and (-)-trans-PET significantly reduced basal brain prostaglandin levels associated with delta-9-trans-THC side effects in a CB1 receptor-dependent manner, thus mimicking the action of the endocannabinoid 2-arachidonoyl glycerol. Therefore, the natural product (-)-cis-PET is a psychoactive cannabinoid from bryophytes, indicating the convergent evolution of bioactive cannabinoids in the plant

kingdom. Our findings may have implications for bio-prospecting and drug discovery and provide a molecular rationale for the reported effects of consuming certain *Radula* preparations as moderately active legal highs [55].

(-)-Trans-delta-9-tetrahydrocannabinol is responsible for the intoxicant activity of *Cannabis*. The length of the side alkyl chain influences the biological activity of this cannabinoid. In particular, synthetic analogs of delta-9-THC with a longer side chain have shown cannabimimetic properties far higher than delta-9-THC. To define the phytocannabinoids profile that characterizes a medicinal *Cannabis* variety, a new phytocannabinoid with the same structure of delta-9-THC but with a seven-term alkyl side chain was identified [60].

The phytocannabinoid (-)-trans-delta-9-tetrahydrocannabiphorol (delta-9-THCP) was isolated, characterized and its stereochemical configuration assigned by the match with the same compound obtained by stereoselective synthesis. Along with delta-9-THCP, the corresponding cannabidiol homolog with a seven-term side alkyl chain (CBDP) was isolated and identified by a match with its synthetic counterpart. The binding activity of delta-9-THCP against human CB1 receptor in vitro ($K_i = 1.2$ nM) resulted similarly to that of CP55940 ($K_i = 0.9$ nM), a potent full CB1 agonist.

In the cannabinoid tetrad pharmacological test, delta-9-THCP induced hypomotility, analgesia, catalepsy, and decreased rectal temperature indicating a delta-9-THC-like cannabimimetic activity. The new phytocannabinoid could account for the pharmacological properties of some *Cannabis* varieties that are difficult to explain by the presence of the sole delta-9-THC.

Cannabis contains unique compounds, and its abuse is linked to an increased risk of developing schizophrenia. The behavioral profiles of the psychotropic *Cannabis* constituent delta-9-tetrahydrocannabinol and the nonpsychotomimetic constituent cannabidiol were investigated with a battery of behavioral tests relevant to anxiety and positive, negative, and cognitive symptoms of schizophrenia. Male adult C57BL/6JArc mice were given 21 daily intraperitoneal injections of vehicle, delta-9-THC (0.3, 1, 3, or 10 mg/kg) or CBD (1, 5, 10, or 50 mg/kg).

Delta-9-THC produced the classic cannabinoid CB1 receptor-mediated tetrad of hypolocomotion, analgesia, catalepsy, and hypothermia, while CBD had modest hyperthermic effects [61]. While delta-9-THC (10 mg/kg) sedative at this dose produced locomotor-independent anxiogenic effects in the open field and light-dark tests, chronic CBD produced moderate anxiolytic-like effects in the open-field test at 50 mg/kg and in the light-dark test at a low dose (1 mg/kg). Acute and chronic delta-9-THC (10 mg/kg) decreased the startle response, while CBD had no effect.

Prepulse inhibition was increased by acute treatment with delta-9-THC (0.3, 3, and 10 mg/kg) or CBD (1, 5, and 50 mg/kg) and by chronic CBD (1 mg/kg). Chronic CBD (50 mg/kg) attenuated dexamphetamine (5 mg/kg)-induced hyperlocomotion, suggesting an antipsychotic-like action for this cannabinoid. Chronic delta-9-THC decreased locomotor activity before and after dexamphetamine administration suggesting functional antagonism of the locomotor stimulant effect. The data provide the first evidence of anxiolytic- and antipsychotic-like effects of chronic but not

acute CBD in C57BL/6JArc mice, extending findings from acute studies in other inbred mouse strains and rats.

Delta-9-tetrahydrocannabinol elicits central nervous system responses, including cognitive alterations and euphoria. The responses account for the abuse potential of *Cannabis*, while other effects, such as analgesia, suggest potential medicinal applications. To study the role of the major known target of cannabinoids in the central nervous system, the CB1 cannabinoid receptor, a mouse strain with a disrupted CB1 gene was produced [82].

CB1 knockout mice appeared healthy and fertile but had a significantly increased mortality rate. They also displayed reduced locomotor activity, increased ring catalepsy, and hypoalgesia in hot-plate and formalin tests. Delta-9-THC-induced ring-catalepsy, hypomobility, and hypothermia were completely absent in CB1 mutant mice. In contrast, delta-9-THC-induced analgesia in the tail-flick test and other behavioral (licking of the abdomen) and physiological (diarrhea) responses after delta-9-THC administration were recognized. Thus, most, but not all, central nervous system effects of delta-9-THC are mediated by the CB1 receptor.

K2 and several similar purported “incense products” spiked with synthetic cannabinoids are abused as *Cannabis* substitutes. It was hypothesized that the metabolism of JWH-073, a prevalent cannabinoid found in K2, contributes to toxicity associated with K2 use. Competition receptor binding studies and G-protein activation assays were performed employing mouse brain homogenates to determine the affinity and intrinsic activity of potential monohydroxylated (M1, M3-M5) and monocarboxylated (M6) metabolites at cannabinoid 1 receptors. Surprisingly, M1, M4, and M5 retain nanomolar affinity for CB1Rs, while M3 displays micromolar affinity and M6 does not bind to CB1Rs. JWH-073 displays equivalent efficacy to the CB1R full agonist CP-55,940, while M1, M3, and M5 act as CB1R partial agonists, and M4 shows little or no intrinsic activity.

The complex mix of metabolically produced CB1R ligands may contribute to the adverse effect profile of JWH-073-containing products. In vitro investigation by Schild analysis revealed that M4 acts as a competitive neutral CB1R antagonist ($K(b) \sim 40$ nM) [89]. In agreement with in vitro studies, M4 also demonstrates CB1R antagonism in vivo by blunting cannabinoid-induced hypothermia in mice. M4 does not block agonist-mediated responses of other measures in the cannabinoid tetrad (e.g., locomotor suppression, catalepsy, or analgesia). Also, as predicted by in vitro results, M1 exhibits agonist activity in vivo by inducing significant hypothermia and suppressing locomotor activity in mice.

Stress-Induced Ulcerogenesis

The activation of cannabinoid CB1 receptors inhibits stress-induced ulcerogenesis due to strengthening the gastric mucosa's antioxidant defense. Nonopioid stress-induced analgesia results from the activation of CB1 receptors by the increased level of 2-arachidonoyl glycerol, anandamide, in the periaqueductal gray matter in

the midbrain. CB1 receptor antagonists promote an increase in ACTH and corticosterone concentrations in the blood of intact animals; the knockout of the gene encoding the CB1 receptor exhibits the same effect. Antagonists of CB1 receptors enhance the stressor elevation of ACTH and corticosterone levels in the blood of experimental animals. Increased stress-induced elevation of corticosterone and ACTH levels were found in the blood of mice with a knockout of the gene encoding the CB1 receptor. An increase in the endogenous anandamide level and a disturbance of the reuptake of endogenous cannabinoids were found after the application of pharmacological agents promoted reducing levels of corticosterone in stressed animals.

The endogenous CB1 receptor agonists play an anxiolytic role and prevent the occurrence of pathological anxiety [46]. Consequently, endogenous cannabinoids inhibit basal and suppress the stress-induced activity of the hypothalamic-pituitary-adrenal axis. The regulation is carried out on the hypothalamus, pituitary, and adrenal cortex levels. Stimulation of central cannabinoid receptors leads to an activation of the sympathetic system. The activation of peripheral CB1 receptors leads to the inhibition of norepinephrine release from sympathetic terminals and the release of epinephrine from the adrenal glands.

The acid metabolites of delta-9-THC were shown to possess analgesic and anti-inflammatory activities in various models. Ajulemic acid (CT3) is a more potent analog of delta-9-THC-11-oic acid in which a dimethylheptyl side chain is substituted for the pentyl side chain of the naturally occurring metabolite [57]. It produces analgesia in the mouse hot plate, the PPQ writhing, the formalin, and the tail clip assays. It was equipotent to morphine; however, it produced a much greater duration of action. The paw edema, subcutaneous air pouch, and rat adjuvant-induced arthritis models of inflammation; produced significant therapeutic activity at a dose of 0.2 mg/kg p.o. The arthritis model greatly reduced permanent damage to joints compared to an indomethacin control, as evidenced by an improved joint soreness over vehicle controls and histopathological examination.

In contrast to the NSAIDs, it was nonulcerogenic at therapeutically relevant doses. Moreover, it does not depress respiration, exhibit dependence, induce body weight loss, or cause mutagenesis. It shows none of the typical actions in models of the psychotropic actions of cannabinoids, indicating that a good separation of desirable from undesirable effects was achieved. The data suggest the existence of a novel receptor for ajulemic acid with possible downstream effects on eicosanoid production, cytokine synthesis, and metalloprotease activity. There is also circumstantial evidence for a putative endogenous ajulemic acid, arachidonoylglycine [57].

Cannabis and the Psychotropic Side Effects in Migraine Pain

Cannabis has a promising analgesic action associated with psychotropic side effects in migraine pain. To overcome the adverse effects of exogenous cannabinoids, migraine pain relief was initiated by activating the endogenous cannabinoid system

by inhibiting enzymes degrading endocannabinoids. To provide a functional platform for such purpose in the peripheral and central parts of the rat's nociceptive system relevant to migraine, the activity-based protein profiling (ABPP), the activity of the main endocannabinoid-hydrolases, monoacylglycerol lipase, and fatty acid amide hydrolase were examined [66]. MAGL and FAAH activities were identified and blocked by the selective and potent inhibitors JKK-048/KML29 and JZP327A, respectively.

In trigeminal ganglia, the MAGL activity was nine-fold higher than that of FAAH. MAGL also exceeded FAAH activity in DRG, spinal cord, and brainstem. Activities of MAGL and FAAH were comparably high in the cerebellum and cerebral cortex implicated in migraine aura. The high MAGL activity in trigeminal ganglia implicated in the generation of nociceptive signals suggests that part of the endogenous cannabinoid system is a priority target for blocking peripheral mechanisms of migraine pain. In the CNS, both MAGL and FAAH represent potential targets for attenuating migraine-related enhanced cortical excitability and pain transmission.

Capsaicin in Cannabinoid Hyperemesis

Cannabinoid hyperemesis syndrome is a clinical disorder that has become more prevalent with the increasing use of *Cannabis* and synthetic cannabinoids, which is difficult to treat. Cannabinoid hyperemesis syndrome patients often report dramatic symptom relief with hot showers, baths, and topical capsaicin. Standard antiemetics commonly fail to alleviate severe nausea and vomiting characteristic of the syndrome. The pharmacokinetics and pharmacodynamics of capsaicin and possible mechanisms for its beneficial effect, including activation of transient receptor potential vanilloid 1 and neurohumoral regulation, were examined in a review. Putative mechanisms responsible for the benefit of hot water hydrotherapy were also investigated.

A review evaluates the relevant mechanisms behind capsaicin and heat activation having potential antiemetic effects. Topical capsaicin is primarily used for treating neuropathic pain, but it has also been used successfully in about 20 cases of cannabinoid hyperemesis syndrome. The pharmacokinetics and pharmacodynamics of capsaicin as a transient receptor potential vanilloid 1 agonist may explain that effect. Topical capsaicin also has a longer half-life than oral administration; thus, its potential benefit duration is longer.

Topical capsaicin binds and activates the transient receptor potential vanilloid 1 receptor, triggering an influx of calcium and sodium and releasing inflammatory neuropeptides leading to transient burning, stinging, and itching that elicits a novel type of desensitization analgesia. Transient receptor potential vanilloid 1 receptors also respond to noxious stimuli, such as heat ($> 43^{\circ}\text{C}$), acids ($\text{pH} < 6$), pain, change in osmolarity, and endovanilloids. The action of topical capsaicin may mimic the effect of heat-activation of transient receptor potential vanilloid 1.

Cannabinoid hyperemesis syndrome may result from a derangement in the endocannabinoid system secondary to chronic exogenous stimulation. The relief of cannabinoid hyperemesis syndrome symptoms from heat and the use of transient receptor potential vanilloid 1 agonist suggest a complex interrelation between the endocannabinoid system and transient receptor potential vanilloid 1.

Hot water hydrotherapy is a mainstay of self-treatment for cannabinoid hyperemesis syndrome patients. This may be explained by heat-induced transient receptor potential vanilloid 1 activation. “Sensocrine” antiemetic effects: Transient receptor potential vanilloid 1 activation by heat or capsaicin results in modulation of tachykinins, somatostatin, pituitary adenylate-cyclase activating polypeptide, and calcitonin gene-related peptide, as well as histaminergic, cholinergic, and serotonergic transmission. The downstream effects represent further possible explanations for transient receptor potential vanilloid 1-associated antiemesis.

The complex interactions between the endocannabinoid systems and transient receptor potential vanilloid 1, in the setting of cannabinoid receptor desensitization, may yield important clues into the pathophysiology and treatment of cannabinoid hyperemesis syndrome [68]. This knowledge can provide clinicians caring for *the* patients with additional treatment options that may reduce the length of stay, avoid unnecessary imaging and laboratory testing, and decrease the use of potentially harmful medications such as opioids.

The Analgesic Effects of MAGL and FAAH Inhibitors

URB937, a peripheral fatty acid amide hydrolase inhibitor, exerts profound analgesic effects in animal models. The pharmacokinetic profile of oral URB937 and its ability to elevate levels of the representative FAAH substrate, oleoylethanolamide, and the compound’s tolerability after oral administration were examined [69]. A liquid chromatography/tandem mass spectrometry (LC/MS-MS) method was used to measure URB937, and a pre-existing LC/MS-MS assay was used to quantify oleoylethanolamide. The fatty acid amide hydrolase activity was measured using a radioactive substrate.

The tolerability of single or repeated (once daily for 2 weeks) oral administration of supramaximal doses of URB937 (100, 300, 1000 mg/kg) was assessed by monitoring food intake, water intake, and body weight, followed by a postmortem evaluation of organ structure. URB937 was orally available in male rats ($F = 36\%$). However, it remained undetectable in the brain when administered at doses that maximally inhibit fatty acid amide hydrolase activity and elevate oleoylethanolamide in plasma and the liver. Acute and subchronic treatment with high doses of URB937 was well-tolerated and resulted in fatty acid amide hydrolase inhibition in the brain. The pharmacokinetic and pharmacodynamic properties of URB937 were favorable in the study.

Monoacylglycerol lipase and fatty acid amide hydrolase inhibitors exert preclinical effects indicative of therapeutic potential (i.e., analgesia). FAAH and MAGL

inhibition was examined separately and together in a delta-9-tetrahydrocannabinol, 5.6 mg/kg i.p. Discrimination assay predictive of subjective effects associated with *Cannabis* use, and the relative contribution of N-arachidonoyl ethanolamine and 2-arachidonoylglycerol in the prefrontal cortex, hippocampus, and caudate putamen to that effect [71].

Delta-9-THC dose-dependently increased delta-9-THC appropriate responses (ED_{50} value = 2.8 mg/kg), whereas the FAAH inhibitors PF-3845 [N-3-pyridinyl-4-[[3-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenyl]methyl]-1-piperidinecarboxamide] and URB597 [(3'-(aminocarbonyl)[1,1'-biphenyl]-3-yl)-cyclohexylcarbamate] or a MAGL inhibitor JZL184 [4-nitrophenyl-4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate] alone did not substitute for the delta-9-THC discriminative stimulus. The nonselective FAAH/MAGL inhibitors SA-57 [4-[2-(4-chlorophenyl)ethyl]-1-piperidinecarboxylic acid 2-(methylamino)-2-oxoethyl ester] and JZL195 [4-nitrophenyl 4-(3-phenoxybenzyl)piperazine-1-carboxylate] fully substituted for delta-9-THC with ED_{50} values equal to 2.4 and 17 mg/kg, respectively. A combination of JZL184 and PF-3845 also produced full substitution for delta-9-THC, but not by combining JZL184 and URB597 (i.e., 52% maximum).

Cannabinoid receptor type 1 antagonist rimonabant attenuated the discriminative stimulus effects of delta-9-THC, SA-57, JZL195, and the combined effects of JZL184 and PF-3845. Full substitution for the delta-9-THC discriminative stimulus occurred only when both 2-AG and AEA were significantly elevated, and the patterns of increased endocannabinoid content were similar among brain regions. The results indicate that increasing both endogenous 2-AG and AEA produces qualitatively unique effects (i.e., the subjective effects of *Cannabis*) that are not obtained from increasing either 2-AG or AEA separately.

The Endocannabinoid System and Glutamate N-Methyl-D-Aspartate

Through the cannabinoid receptor 1, the endocannabinoid system plays a physiological role in maintaining the activity of glutamate N-methyl-D-aspartate (NMDA) receptors within harmless limits. The influence of cannabinoids must be proportional to the stimulus to prevent NMDAR over-activation or exaggerated hypofunction that may precipitate symptoms of psychosis. In this framework, the reported association of CB1 receptors with NMDARs, which mediates the reduction of cannabinoid analgesia promoted by NMDAR antagonism, could also support the precipitation of schizophrenia brought about by the abuse of smoked *Cannabis*, mostly among vulnerable individuals. That possibility was investigated using neuroprotection and analgesia as reporters of the CB1-NMDAR connection.

The Sigma 1 receptor ($\sigma 1R$) acts as a safety switch, releasing NMDARs from the influence of CB1 receptors and thereby avoiding glutamate hypofunction. In

$\sigma 1R(-/-)$ mice, the activity of NMDARs increases and cannot be regulated by cannabinoids, and NMDAR antagonism produces no effect on cannabinoid analgesia. In wild-type mice, ligands of the $\sigma 1R$ did not affect the CB1-NMDAR regulatory association; however, experimental NMDAR hypofunction enabled $\sigma 1R$ antagonists to release NMDARs from the negative control of CB1 receptors [79].

Of the $\sigma 1R$ antagonists tested, the order of activity was: S1RA > BD1047 $\gg$ NE100 = BD1063. Although SKF10047, PRE-084, and (+)pentazocine were inactive, they could abolish the effect of S1RA in the paradigm. Thus, the $\sigma 1R$ controls the extent of CB1-NMDAR interaction. Its failure might constitute a vulnerability factor for *Cannabis* abuse, potentially precipitating schizophrenia that might otherwise be induced later by the endogenous system.

Synthetic Cannabinoids on the Visual, Auditory, and Tactile Responses

JWH-018-Cl, JWH-018-Br, and AM-2201 (JWH-018 halogenated-derivatives, JWH-018-R compounds) are synthetic cannabinoid agonists marketed as “Spice,” “K2,” “herbal blend,” and research chemicals for their *Cannabis*-like psychoactive effects. In rodents, JWH-018 and its halogenated derivatives reproduce the typical effects of delta-9-tetrahydrocannabinol, i.e., hypothermia, analgesia, hypolocomotion, and akinesia. The effects of JWH-018-R compounds on sensorimotor functions were investigated in mice [42]. Acute intraperitoneal (i.p.) administration of JWH-018-R compounds (0.01–6 mg/kg) on sensorimotor functions in mice was compared to the reference compound JWH-018 and delta-9-THC.

A battery of behavioral tests was used to investigate the effects of the synthetic cannabinoids on the visual, auditory, and tactile responses in mice; the prepulse inhibition (PPI) test was used to investigate their effects on sensorimotor gating. A video tracking analysis also measured the effects of the synthetic cannabinoids on spontaneous locomotion to assess potential cannabinoid-induced motor impairment. The results indicated that similarly to JWH-018, systemic administration of JWH-018-R compounds inhibits sensorimotor and PPI responses at lower doses (0.01–0.1 mg/kg) and reduces spontaneous locomotion at intermediate/high doses (1–6 mg/kg). The effects were prevented by administering the selective cannabinoid CB1 receptor antagonist/inverse agonist AM-251, thus confirming a CB1 receptor-mediated action.

Discovering that the lower doses of JWH-018-R compounds selectively impair sensorimotor and PPI responses without affecting locomotion should be considered to understand better the potential danger that halogenated derivatives of JWH-018 may pose to public health, particularly about decreased performance in driving and hazardous works.

Spasticity in Multiple Sclerosis

In 15 clinical trials, cannabinoids were significantly better than placebo in managing spasticity in Multiple sclerosis. The patients self-reported a greater sense of motor improvement in Multiple sclerosis than could be confirmed objectively; there was a significant objective improvement of tics in Tourette's disease and neuropathic pain [51]. A nonpsychotropic cannabinoid also had analgesic activity in neuropathic pain. No significant improvement was found in levodopa-induced dyskinesia in Parkinson's disease or postoperative pain. No difference from the active placebo was found for the management of cachexia.

There were changes in immune system parameters in HIV-1 and Multiple sclerosis patients treated with cannabinoids. Quality-of-life assessments were made in only three of the 15 qualifying clinical trials. Cannabinoids appear useful for conditions without effective treatment, such as spasticity, tics, and neuropathic pain.

Nabiximols oromucosal spray is approved as an add-on therapy for symptomatic relief of spasticity in people with Multiple sclerosis. Most safety data for nabiximols derive from its use in Multiple sclerosis spasticity and some data from its analgesic effects. Aligning with the known safety profile of nabiximols as demonstrated in randomized controlled trials, common adverse events reported under clinical practice conditions were dizziness, fatigue, and somnolence. The serious adverse event rate with nabiximols in Multiple sclerosis spasticity observational studies was 3.1% (137/4351). Thirty-nine treatment-related serious adverse events were reported in 32 patients with spasticity, all of which were resolved. No treatment-related adverse events were recorded in the nabiximols pain studies [54].

Cannabis in Amyotrophic Lateral Sclerosis

The understanding of the molecular mechanisms of amyotrophic lateral sclerosis has improved significantly, yet it has yet to be translated into effective therapies. Some abnormal physiological processes occur simultaneously in the devastating disease. It appears that a multidrug regimen, including glutamate antagonists, antioxidants, a centrally acting anti-inflammatory agent, microglial cell modulators (including tumor necrosis factor- α [TNF- α] inhibitors), an antiapoptotic agent, one or more neurotrophic growth factors, and a mitochondrial function-enhancing agent would be required to address the known pathophysiology of amyotrophic lateral sclerosis comprehensively. *Cannabis* has activities in all of those areas.

The G93A-SOD1 amyotrophic lateral sclerosis mouse translated to prolonged neuronal cell survival, delayed onset, and slower disease progression [22]. *Cannabis* also applies to symptomatic management of amyotrophic lateral sclerosis, including analgesia, muscle relaxation, bronchodilation, saliva reduction, appetite stimulation, and sleep induction. From a disease-modifying and symptomatic management viewpoint, clinical trials with *Cannabis* are the logical step for treating ALS. Based

on the available scientific data, *Cannabis* might significantly slow the progression of amyotrophic lateral sclerosis, potentially extending life expectancy and substantially reducing the overall burden of the disease.

Two subtypes of G-protein coupled cannabinoid receptors, CB1 and CB2, have been cloned, and several putative endogenous ligands (endocannabinoids) have been detected. The main endocannabinoids are arachidonoyl ethanolamide, anandamide, and 2-arachidonoyl glycerol, derivatives of arachidonic acid produced by the cleavage of membrane lipid precursors. Besides phytocannabinoids of the *Cannabis* plant, modulators of the cannabinoid system comprise synthetic agonists and antagonists at the CB receptors and inhibitors of endocannabinoid degradation.

Cannabinoid receptors are distributed in the central nervous system and many peripheral tissues, including the immune system, reproductive and gastrointestinal tracts, sympathetic ganglia, endocrine glands, arteries, lungs, and heart. There is evidence for some nonreceptor-dependent mechanisms of cannabinoids and endocannabinoid effects mediated by vanilloid receptors. Properties of CB receptor agonists that are of therapeutic interest include analgesia, muscle relaxation, immunosuppression, anti-inflammation, antiallergic effects, mood improvement, appetite stimulation, antiemesis, and intraocular pressure, bronchodilation, neuroprotection, and antineoplastic effects. The main focus of clinical research is their efficacy in chronic pain and neurological disorders. CB receptor antagonists are investigated for medical use in obesity and nicotine addiction. Additional potential has been proposed for treating alcohol and heroin dependency, schizophrenia, conditions with lowered blood pressure, Parkinson's disease, and memory impairment in Alzheimer's [23].

***Cannabis* in Takotsubo Cardiomyopathy**

A case was reported in which a 31-year-old man with 10 years of cocaine and *Cannabis* dependence developed reverse Takotsubo cardiomyopathy (rTC), a rare variant of Takotsubo cardiomyopathy [13]. The patient presented with severe left temporal headache and vomiting that began while smoking *Cannabis* and several hours after smoking methamphetamine and using cocaine via insufflation. Computed tomography and brain angiography were normal, and the headache was resolved with analgesia. Urine drug screen was positive for benzodiazepines, cannabinoids, cocaine, opiates (attributed to morphine administered in E.D.), and amphetamines. Three hours later, he had a seizure and, within 10 min, developed cardiogenic shock with anteroinferior S.T. segment depression on electrocardiogram, and troponin-T rose to 126 ng/L.

Coronary angiography indicated normal coronary arteries. A transthoracic echocardiogram indicated severely impaired left ventricular (LV) systolic function with an ejection fraction of 15–20% and hypokinesis sparing the apex. Thyrotoxicosis, nutritional, vasculitic, autoimmune, and viral screens were negative. Cardiac

magnetic resonance imaging demonstrated severe LV functional impairment with dilated hypocontractile basal segments and T2 hyperintensity consistent with myocardial edema and rTC. The patient received supportive management. Proposed mechanisms of rTC include catecholamine cardiotoxicity and coronary artery vasospasm. In this case, multiple insults, including severe headache, *Cannabis* hyperemesis, and cocaine and methamphetamine-induced serotonin toxicity, culminated in a drug-induced seizure, which led to catecholamine cardiotoxicity resulting in rTC.

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Chapter 8

Cannabis and Perinatal Development



Evidence supports the endocannabinoid (eCB) system's role in the central nervous system patterning in structures relevant to mood, cognition, and reward, such as the mesocorticolimbic system. Exposure to *Cannabis* during early ontogeny is not benign, and potential compensatory mechanisms that might be expected to occur during neurodevelopment appear insufficient to eliminate vulnerability to neuropsychiatric disorders in certain individuals [1].

An overview of the endocannabinoid system discusses the neurobiological consequences of *Cannabis* exposure in pregnancy and early life by addressing its impact on the development of neurotransmitter systems relevant to neuropsychiatric disorders and its association with the disorders later in life. Both human longitudinal cohort studies and animal models strongly emphasize the long-term influence of prenatal cannabinoid exposure on behavior and mental health. Studying in utero *Cannabis* exposure in association with genetic mutations of neural systems that have strong relationships to endocannabinoid function, such as dopamine, opioid, glutamate, and gamma-aminobutyric acid (GABA), helps to identify individuals at risk.

Cannabis preparations have grown potently in recent years, potentially creating global clinical, public health, and workforce concerns. As more countries legalize medical and adult recreational *Cannabis*, the incidences of prenatal *Cannabis* exposure (PCE) will likely increase. Unlike fetal alcohol spectrum disorder, no phenotypic syndrome is associated with PCE. There is also no preponderance of evidence that PCE causes lifelong cognitive, behavioral, or functional abnormalities and susceptibility to subsequent addiction. However, there is compelling evidence, based on the principles of teratology and fetal malprogramming, suggesting that pregnant women should refrain from smoking *Cannabis*. The usage of *Cannabis* during pregnancy disturbs the fetal endogenous cannabinoid signaling system (ECSS), which is present and active from the early embryonic stage, modulating neurodevelopment and continuing this role into adulthood.

The ECSS is present in virtually every brain structure and organ system. There is also evidence that this system is important in regulating cardiovascular processes.

Endocannabinoids (eCBs) support a broad spectrum of processes, including the early stages of fetal neurodevelopment and uterine implantation. Delta-9-tetrahydrocannabinol (THC) enters maternal circulation and readily crosses the placental membrane. Delta-9-THC binds to CB receptors of the fetal ECSS, altering neurodevelopment and possibly rewiring ECSS circuitry.

Prenatal *Cannabis* exposure, similar to a neurodevelopmental teratogen, delivers the first hit to the ECSS, which is compromised in such a way that a second hit (i.e., postnatal stressors) will precipitate the emergence of a specific phenotype. It is concluded that perturbations of the intrauterine milieu via the introduction of exogenous CBs alter the fetal ECSS, predisposing the offspring to abnormalities in cognition and altered emotionality [2]. Based on experimental evidence, it can be argued that young women who become pregnant should immediately stop using *Cannabis*.

The use of *Cannabis* to self-medicate depression and anxiety during pregnancy is a growing medical concern. Clinical reports of *Cannabis* use prevalence during pregnancy vary widely from 3% to upwards of 35% in North America; the disparity is likely due to underestimates from self-reporting. A lack of evidence-based policy and guidelines for prenatal *Cannabis* use compounds the trends. This has led to inconsistent messaging by healthcare providers and medically licensed *Cannabis* dispensaries regarding prenatal *Cannabis* use for treating symptoms like nausea.

The Health Effects of *Cannabis* and Cannabinoids

The National Academies of Sciences, Engineering, and Medicine (NASEM) conducted a rapid turn-around comprehensive review of medical literature on The Health Effects of *Cannabis* and Cannabinoids [78]. The 16-member committee adopted the key features of a systematic review process, conducting an extensive search and considering 10,000 recent abstracts to determine their relevance. Primacy was given to recently published systematic reviews and primary research that studied one of the committee's 11 prioritized health endpoints: therapeutic effects, cancer incidence, cardiometabolic risk, respiratory disease, immune function, injury and death, prenatal outcomes, perinatal outcomes, postnatal outcomes, psychosocial outcomes, mental health problem, *Cannabis* use, and *Cannabis* use and abuse of other substances.

The committee developed standard language to categorize the weight of evidence regarding whether *Cannabis* or cannabinoids used for therapeutic purposes are an effective or ineffective treatment for the prioritized health endpoints of interest. In the therapeutics chapter, the report concluded that there was conclusive and substantial evidence that *Cannabis* or cannabinoids are effective for treating pain in adults, chemotherapy-induced nausea and vomiting, and spasticity associated with multiple sclerosis.

A chapter of the NASEM report enumerated multiple barriers to conducting research on *Cannabis* in the United States that may explain the paucity of positive

therapeutic benefits in the published literature. The evidence supporting improvement in appetite, Tourette syndrome, anxiety, posttraumatic stress disorder, cancer, irritable bowel syndrome, epilepsy, and a variety of neurodegenerative disorders was described as limited, insufficient, or absent. Moderate evidence was found for secondary sleep disturbances.

Physiological and Neurodevelopmental Outcomes of *Cannabis* Exposure

Clinical and preclinical data on neonatal outcomes and long-term physiological and neurodevelopmental outcomes of prenatal *Cannabis* exposure were evaluated. Although many of the outcomes under investigation have produced mixed results, it does consider the data in light of the unique challenges facing *Cannabis* research. The limited longitudinal clinical studies available have not previously accounted for the exponential increase in (-)-delta-9-tetrahydrocannabinol concentrations found in *Cannabis* over the past two decades. Multi-drug use and the long-term effects of individual *Cannabis* constituents (delta-9-THC vs. cannabidiol) are not fully studied, along with sex-dependent outcomes. Despite the limitations, prenatal *Cannabis* exposure has been linked to low birth weight (LBW).

Prenatal exposure to delta-9-THC crosses the placenta, impacts placental development, and may have wide-ranging physiological and neurodevelopmental consequences. Reports indicated that delta-9-THC increases the risk of cognitive impairment and neuropsychiatric disease, including psychosis, depression, anxiety, and sleep disorders; the long-term effects of the changes require continued rigorous investigation [3]. In light of the current trends in the perception and use of *Cannabis* during pregnancy, it is emphasized that it is socially and medically imperative for a more rigorous investigation of the long-term consequences of prenatal *Cannabis* exposure.

More women planning pregnancy, pregnant, or breastfeeding will be exposed to *Cannabis*. The potential effects on pregnancy and lactation are important for the obstetrical care provider to provide optimal counseling. A review of *Cannabis* for the obstetrical care provider indicated that no amount of *Cannabis* and its metabolites used in pregnancy and lactation is safe. *Cannabis* and its products have the potential for adverse maternal, fetal, and long-term childhood development. Its use should be discouraged during pregnancy and lactation [4].

The maternal risks of *Cannabis* are related to the method of ingestion, associated risk behaviors, polysubstance abuse, and associated mental health complications. *Cannabis* use disorder (CUD) is defined by the Diagnostic and Statistical Manual of Mental Disorders as impaired control, social difficulties, risky use, tolerance, and withdrawal [4]. Studies have shown an association with depression, anxiety, and other psychiatric conditions. Such associated mental health conditions can lead to addiction as *Cannabis* is used to treat the underlying psychiatric illness [5].

Using the provincial birth registry containing information on *Cannabis* use during pregnancy, a retrospective analysis of all live births in Ontario, Canada, between April 1, 2007, and March 31, 2012, was performed. Pregnancy and birth data were linked to provincial health administrative databases to ascertain child neurodevelopmental outcomes [29]. Matching techniques to control for confounding and Cox proportional hazards regression models were used to examine associations between prenatal *Cannabis* use and child neurodevelopment.

The study reveals an association between maternal *Cannabis* use in pregnancy and the incidence of autism spectrum disorder in the offspring. The incidence of intellectual disability and learning disorders was higher among offspring of mothers who used *Cannabis* during pregnancy, although less statistically robust. The incidence of autism spectrum disorder diagnosis was 4.00 per 1000 person-years among children with exposure compared to 2.42 among unexposed children, and the fully adjusted hazard ratio (HR) was 1.51 (95% confidence interval [CI] = 1.17–1.96) in the matched cohort.

The liberalization of *Cannabis* use globally has raised concern regarding its impact on vulnerable populations, such as pregnant women. Pregnant women frequently use *Cannabis* for its anecdotal use as a treatment for morning sickness and its acceptance as a harmless drug. Epidemiological studies have demonstrated a relationship between developmental *Cannabis* exposure and later mental health symptoms. The relationship is strong in people with particular genetic polymorphisms, indicating that *Cannabis* use interacts with genotype to increase mental health risk.

Exposure to *Cannabis* during critical stages of neurodevelopment can alter the endogenous cannabinoid system critical for the developing brain. Seminal animal research directly linked prenatal and adolescent exposure to delta-9-tetrahydrocannabinol with protracted effects on adult neural systems relevant to psychiatric and substance disorders. Nursing mothers often use *Cannabis* without realizing that delta-9-tetrahydrocannabinol is transferred into breast milk, posing risks to infants.

The risks are not limited to the prenatal and perinatal periods; the onset of *Cannabis* use typically occurs during adolescence, a period of continued brain maturation. Maternal *Cannabis* use likely affects the fetal development of numerous circuits, but effects on the mesocorticolimbic system may have particularly strong impacts on the future psychiatric health of the offspring. The system's functioning is regulated by dopamine, a neurotransmitter highly implicated in various psychiatric and substance use disorders. Its receptors are involved in developmental differentiation and circuit formation of the forebrain.

Human fetal studies have revealed alterations in dopamine D2 receptor gene expression in mesocorticolimbic brain structures after in utero *Cannabis* exposure. Animal models have substantiated dopamine receptor alterations in forebrain regions similar to those observed in humans; those brain regions play key roles in motivation, emotional regulation, reward, and cognition [18]. Therefore, *Cannabis*-induced perturbation of the dopaminergic system during early development may influence the onset and trajectory of psychiatric disorders.

Cannabis during pregnancy may increase adverse outcomes for women and their neonates. To assess the effects of the use of *Cannabis* during pregnancy on maternal and fetal outcomes, databases were reviewed to determine the effects of the use of *Cannabis* during pregnancy on maternal and fetal outcomes [19]. Maternal, fetal, and neonatal outcomes to 6 weeks postpartum after exposure to *Cannabis* were meta-analyzed on variables that had three or more studies that measured an outcome consistently. Outcomes for which meta-analyses were conducted included anemia, birth weight, low birth weight, neonatal length, and placement in the neonatal intensive care unit, gestational age, head circumference, and preterm birth. Twenty-four studies were included in the review.

The results demonstrated that women who used *Cannabis* during pregnancy had an increase in the odds of anemia (pooled odds ratio [pOR] = 1.36; 95% CI = 1.10–1.69) compared with women who did not use *Cannabis* during pregnancy. Infants exposed to *Cannabis* in utero had a decrease in birth weight (low birth weight pOR = 1.77; 95% CI = 1.04–3.01; the pooled mean difference for birth weight = 109.42 g: 38.72–180.12) compared with infants whose mothers did not use *Cannabis* during pregnancy. Infants exposed to *Cannabis* in utero were also more likely to need placement in the neonatal intensive care unit than infants whose mothers did not use *Cannabis* during pregnancy (pOR = 2.02; 1.27–3.21). As the use of *Cannabis* gains social acceptance, pregnant women and their medical providers should be aware of the potential adverse effects of the use of *Cannabis* during pregnancy.

The US Surgeon General issued an advisory against the use of *Cannabis* during pregnancy [20]. To evaluate whether *Cannabis* use during pregnancy is associated with adverse outcomes among offspring, data were obtained from the baseline session of a longitudinal Adolescent Brain and Cognitive Development Study, which recruited 11,875 children aged 9–11 years, as well as a parent or caregiver, from 22 sites across the United States between June 1, 2016, and October 15, 2018.

Symptoms of psychopathology in children (i.e., psychotic-like experiences [PLEs] and internalizing, externalizing, attention, thought, and social problems), cognition, sleep, birth weight, gestational age at birth, body mass index (BMI), and brain structure (i.e., total intracranial volume, white matter volume, and gray matter volume) were measured. Covariates included familial (e.g., income and familial psychopathology), pregnancy (e.g., prenatal exposure to alcohol and tobacco), and child (e.g., substance use) variables. *Cannabis* exposure was accepted prior to and after maternal knowledge of pregnancy.

Among 11,489 children (5997 boys [52.2%]; mean [SD] age, 9.9 [0.6] years) with nonmissing prenatal *Cannabis* exposure data, 655 (5.7%) were exposed to *Cannabis* prenatally. Relative to no exposure, *Cannabis* exposure only before (413 [3.6%]) and after (242 [2.1%]) maternal knowledge of pregnancy was associated with greater offspring psychopathology characteristics (i.e., PLEs and internalizing, externalizing, attention, thought, and social problems), sleep problems, and body mass index, as well as lower cognition and gray matter volume ($|\beta| > 0.02$; all false discovery rate [FDR]-corrected $p < 0.03$).

Exposure after knowledge of pregnancy was associated with lower birth weight as well as total intracranial volume and white matter volumes relative to no exposure and exposure only before knowledge ($|\beta| > 0.02$; FDR-corrected $p < 0.04$). When including potentially confounding covariates, exposure after maternal knowledge of pregnancy remained associated with greater PLEs and externalizing, attention, thought, and social problems ($\beta > 0.02$; FDR-corrected $p < 0.02$). Exposure only prior to maternal knowledge of pregnancy did not differ from no-exposure on any outcomes when considering potentially confounding variables ($|\beta| < 0.02$; FDR-corrected $p > 0.70$). The study indicates that prenatal *Cannabis* exposure and its correlated factors are associated with greater risk for psychopathology during middle childhood, and *Cannabis* use during pregnancy should be discouraged.

In recent years, there has been a transformation in the perception of the health risks and benefits of *Cannabis*. There have been unintended neurodevelopmental implications given the increased use of *Cannabis* and the potent levels of delta-9-tetrahydrocannabinol consumed by pregnant women, young mothers, and teens. A review was conducted to provide an overview of the neurobiological effects of cannabinoid exposure during prenatal/perinatal and adolescent periods, in which the endogenous cannabinoid system plays a fundamental role in neurodevelopmental processes. The brain's development is a dynamic process that extends well beyond the prenatal period, where full maturity is not achieved until early adulthood.

The endocannabinoid system, which mediates the actions of delta-9-THC, plays a critical regulatory role throughout all developmental stages, from determining cell fate and neuronal migration to regulating signaling pathways and synaptic transmission in the mature central nervous system. Therefore, the supraphysiological impact on the endocannabinoid system by *Cannabis* exposure during critical periods of development could change the normal trajectory of cellular processing and neuro-circuitry, leading to behavioral disturbances later in life.

Endocannabinoids regulate brain development via modulating neural proliferation, migration, and the differentiation of lineage-committed cells. In the fetal nervous system, endocannabinoid-sensing receptors and the enzymatic machinery of endocannabinoid metabolism exhibit a cellular distribution map different from that in adults, implying distinct functions. Cannabinoid receptors serve as molecular targets for the psychotropic delta-9-tetrahydrocannabinol and synthetic derivatives.

Recreational *Cannabis* is recognized as a niche drug for adolescents and young adults [13]. Data from human and experimental studies indicated that long-term and heavy *Cannabis* use during pregnancy could impair brain maturation and predispose the offspring to neurodevelopmental disorders.

A study using state-level data on women, who delivered live-born infants in the United States from the Pregnancy Risk Assessment Monitoring System (PRAMS), indicated that *Cannabis* use was associated with changes in maternal *Cannabis* use before and after pregnancy [14]. Data were collected from two states that had legalized recreational *Cannabis* (Alaska and Maine) and two states that had not legalized recreational *Cannabis* (New Hampshire and Vermont). The women completed surveys 2–6 months after delivery, reporting preconception, prenatal, and postpartum *Cannabis* use.

The three primary outcomes were self-reported during the 12 months before, during, and 2–6 months after pregnancy. A difference-in-differences analysis was used to compare changes in the prevalence of maternal *Cannabis* use during each period before and after state legalization of recreational *Cannabis*, controlling for maternal characteristics (age, race/ethnicity, educational level, income, cigarette smoking, and breastfeeding) and state fixed effects. State-specific survey weights were used.

The study included 23,082 women in the preconception period, 23,859 in the prenatal period, and 26,610 in the postpartum period. In each analysis, most women were married (range among all groups, 63.9–64.8%), aged 25–34 years (preconception, 55.4%; prenatal, 55.9%; postpartum, 56.1%), and had an annual household income less than \$50,000 (preconception, 55.7%; prenatal, 56.3%; postpartum, 55.5%). In adjusted analyses, preconception and postpartum *Cannabis* use increased significantly in states that had legalized recreational *Cannabis* compared with states that had not legalized it (preconception risk difference [RD], 0.0457 [95% CI = 0.0013–0.0900]; $p = 0.04$; postpartum risk difference, 0.0539 [95% CI = 0.0259–0.0818]; $p < 0.001$). The risk difference for prenatal *Cannabis* use was insignificant (0.0070; 95% CI = -0.0120 to 0.0260 ; $p = 0.47$).

Prenatal infection is associated with brain structural and functional abnormalities and may increase the risk for psychosis by directly affecting neurodevelopment. The longitudinal Northern Finland Birth Cohort 1986 study examined the associations of maternal prenatal C-reactive protein levels with psychosis risk factors in adolescent offspring [70]. C-reactive protein was measured in maternal sera collected during pregnancy. In offspring, school performance was measured at 7, while school performance, psychotic experiences, and *Cannabis* use were measured at 16.

Associations of C-reactive protein with offspring measures using regression analysis controlling for offspring sex, maternal education level, prenatal maternal body mass index, smoking, and alcohol use in pregnancy, place of birth, maternal psychiatric admission, paternal psychiatric admission, mothers age at birth, and gestational week of C-reactive protein sample were tested. Tests were also performed to see if adolescent *Cannabis* use mediated the associations between maternal C-reactive protein and offspring outcomes.

Controlling for covariates, maternal C-reactive protein was associated with academic performance at age 16 years ($\beta = 0.062$, 95% CI = 0.036 – 0.088) but not with possible psychotic experiences at 16 years (odds ratio [OR] = 1.09, 95% CI = 0.96 – 1.24). Maternal C-reactive protein was also associated with adolescent *Cannabis* use (OR = 1.24, 95% CI = 1.07 – 1.43). The findings suggest that prenatal inflammation may influence later mental illness risk by affecting neurodevelopment and also indirectly by increasing the risk of exposure to *Cannabis*.

Cannabinoid receptors serve as molecular targets for delta-9-tetrahydrocannabinol and its synthetic derivatives in designer drugs. Recreational *Cannabis* is recognized as a niche drug for adolescents and young adults. Data from human and experimental studies revealed that long-term and heavy *Cannabis* use during pregnancy could impair brain maturation and predispose the offspring to neurodevelopmental disorders. Exploration of the mechanisms of cannabinoid receptor-mediated signaling

events at critical stages of fetal brain development revealed organized histopathological, biochemical, molecular, and behavioral findings into a logical hypothesis predicting neuronal vulnerability to and attenuated adaptation toward environmental challenges (stress, drug exposure, medication) in children affected by in utero cannabinoid exposure. Conversely, it has been indicated that endocannabinoid signaling can be an appealing target to dampen neuronal activity in pre-existing pathologies associated with circuit hyperexcitability [85].

Behavioral Consequences

Trends of the most recent 50 years indicate an increase in popularity in childbearing women and a constant increase in *Cannabis* potency. In addition, potent herbal “legal” highs containing synthetic cannabinoids that mimic the effects of *Cannabis* with unknown pharmacological and toxicological effects have gained rapid popularity among young adults.

Despite the surge in *Cannabis* use during pregnancy, little is known about the neurobiological and psychological consequences in the exposed offspring. The importance of maternal programming, defined as the intrauterine presentation of maternal stimuli to the fetus, in neurodevelopment has been emphasized. The focus was on *Cannabis*-mediated maternal adverse effects, resulting in direct central nervous system alteration or sensitization to late-onset chronic and neuropsychiatric disorders.

A comparison of clinical and preclinical experimental studies on the effects of fetal *Cannabis* exposure until early adulthood was conducted to emphasize the importance of animal models that permit the fine control of environmental variables and allow the dissection of *Cannabis*-mediated molecular cascades in the developing central nervous system [38]. It was concluded that preclinical, experimental models confirm clinical studies and that *Cannabis* exposure evokes significant molecular modifications to neurodevelopmental programs leading to neurophysiological and behavioral abnormalities.

Gestational *Cannabis* exposure has been associated with various adverse outcomes. *Cannabis* use during pregnancy increases the risk for offspring psychotic-like experiences. In a prospective cohort, the relationship between parental *Cannabis* use during pregnancy and offspring psychotic-like experiences was examined. Comparisons were made between maternal and paternal *Cannabis* use during pregnancy to investigate causal influences of intrauterine *Cannabis* exposure during fetal neurodevelopmental. The study was embedded in the Generation R birth cohort and included 3692 participants.

Maternal *Cannabis* exposure was determined using self-reports and *Cannabis* metabolite levels from urine. Paternal *Cannabis* use during pregnancy was obtained by maternal report. Maternal *Cannabis* use increased the risk of psychotic-like experiences in the offspring (OR_{adjusted} = 1.38, 95% CI = 1.03–1.85). Estimates

were comparable for maternal *Cannabis* use exclusively before pregnancy versus continued *Cannabis* use during pregnancy.

It was demonstrated that maternal and paternal *Cannabis* use was associated with more offspring psychotic-like experiences at age 10 years. Paternal *Cannabis* use was similarly associated with offspring psychotic-like experiences (OR_{adjusted} = 1.44, 95% CI = 1.14–1.82) [66]. It may suggest that those common etiologies, rather than solely causal intrauterine mechanisms, underlie the association between parental *Cannabis* use and offspring psychotic-like experiences. The common backgrounds most likely reflect genetic vulnerabilities and shared familial mechanisms, shedding potentially new light on the debated causal path from *Cannabis* use to psychotic-like phenomena. The findings indicate that diagnostic screening and preventative measures must be adapted for young people at risk for severe mental illness.

The broad-based legalization of *Cannabis* use has created a need to understand its impact on human health and behavior. The risks associated with *Cannabis* use, particularly for sensitive subgroups such as pregnant women, are difficult to define because of a paucity of dose–response data and the variations in *Cannabis* potency. Although the mode of action of delta-9-tetrahydrocannabinol in adults is well known, not much attention has been given to understanding how *Cannabis* use during pregnancy may impact the development of the fetal nervous system and whether additional plant-derived cannabinoids might participate. The historical and contemporary literature focused on the mode of action of delta-9-THC in the developing brain, comparative pharmacokinetics in pregnant and non-pregnant model systems, and neurodevelopmental outcomes in exposed offspring.

Despite public health concerns, pharmacokinetic studies of delta-9-THC have focused on non-pregnant adult subjects. Preclinical data indicate that delta-9-THC readily crosses the placenta, although fetal exposures appear lower than maternal exposures [26]. The neurodevelopmental data in humans and animals indicate that prenatal exposure to delta-9-THC may lead to subtle, persistent changes in targeted aspects of higher-level cognition and psychological well-being.

In an Adolescent Brain Cognitive Development study, a cohort of approximately 12,000 children with and without prenatal *Cannabis* exposure after knowledge of pregnancy was measured by caregivers' reports. The participants with prenatal *Cannabis* exposure ($n = 224$) were included and compared to two control groups: those matched on tobacco and alcohol exposure and those without prenatal tobacco or alcohol exposure. The outcomes were measured with the Adolescent Brain Cognitive Development baseline assessment when the participants were 9–10 years of age and included attention, internalizing, externalizing, and total problems scales on the Child Behavior Checklist (CBCL). Teacher reports were used when available.

Mixed effects modeling assessed the association between prenatal *Cannabis* exposure and outcomes controlling for parental psychopathology, prematurity, and socioeconomic status. For participants with available data, patterns of brain activity during three fMRI tasks: the Stop Signal Task measuring response inhibition, the Monetary Incentive Delay task measuring reward processing, and the EN-Back task

measuring working memory were analyzed using Permutation Analyses of the Linear Model.

Compared to both control groups, participants with prenatal *Cannabis* exposure had significantly higher attention problems, externalizing, and total problem scores. Prenatal *Cannabis* exposure did not impact cognitive performance or patterns of brain activation during fMRI tasks [27]. There are long-term associations between prenatal *Cannabis* exposure and early adolescent attention and behavioral problems that are not reflected in cognitive performance or task fMRI measures, a finding consistent with reports that fewer than half of children with ADHD have any specific cognitive deficit [28]. The young age of the sample may also relate to the finding.

A study explored the relationship between prenatal, perinatal, and adolescent exposure to *Cannabis* and aggressive behavior, including physical aggression. Areas of inquiry include animal research and human research on prenatal exposure and *Cannabis* use during adolescence. Potential psychosocial and psychopharmacological mechanisms and relevant confounds were identified. The prenatal *Cannabis* exposure literature provides minimal support for a direct relationship with aggressive behavior in childhood.

The adolescent use literature indicates a marginal association between acute intoxication and aggressive behavior, and an association between chronic use and aggressive behavior heavily influenced by demographic variables rather than direct psychopharmacological mechanisms. *Cannabis* withdrawal symptoms also may include aggression and anger, but there is little evidence to suggest that the effects are large or specific to withdrawal from *Cannabis* compared to other substances [56].

Early identification of neonates exposed to drug abuse during pregnancy permits more precise clinical management. A study described the clinical characteristics and identified the risk factors associated with the early detection of neonates exposed to drugs abuse in a Neonatal Intermediate and Intensive Care Unit [57].

Genetic Involvement in *Cannabis* Exposure

The epigenome is a critical molecular machinery that can maintain persistent alterations of gene expression and behaviors induced by cannabinoids that have been observed across the individual's lifespan and even into the subsequent generation. Though mechanistic investigations regarding the consequences of developmental *Cannabis* exposure remain sparse, human and animal studies have revealed specific epigenetic disruptions in the brain and the periphery. There have been long-term disturbances in epigenetic regulation in relation to prenatal, adolescent, and parental germline cannabinoid exposure. Expanding knowledge about protracted molecular memory could help identify novel targets and develop preventive strategies and treatments for behaviors relevant to neuropsychiatric risks associated with developmental *Cannabis* exposure.

Cannabis use by teenagers and young adults often predates the abuse of harder drugs (known as the classic “gateway” concept). However, the neurobiological underpinnings of such vulnerability are beginning to be unraveled. Converging evidence obtained from animal and human brain studies has shown that similarly to *Drd2*, early *Cannabis* exposure selectively alters the expression of opioid neuropeptide proenkephalin in the mesocorticolimbic system. The disturbances persist into adulthood and modulate drug-seeking behavior later in life.

A direct causal link between the upregulation of the proenkephalin gene in the nucleus accumbens (NAc) due to delta-9-THC exposure during adolescence and enhanced behavioral susceptibility to heroin seeking in adulthood has been reported. On the chromatin level, persistent changes in repressive H3K9me2 and H3K9me3 were observed at the proenkephalin locus in the NAc of adult rats following adolescent delta-9-THC exposure, in line with enduring upregulation of proenkephalin mRNA expression.

This epigenetic effect represents a profound pathologic departure from the distinct developmental pattern of histone H3 methylation that normally occurs at proenkephalin in the NAc across the transition from adolescence to adulthood. The chromatin landscape is highly complex, but trimethylation of H3K9 (a transcriptionally repressive mark) may account for the developmental transcriptional instability of NAc proenkephalin due to adolescent delta-9-THC exposure, allowing the proenkephalin gene to be “primed” to respond to environmental cues later in life.

Cannabis and synthetic cannabinoids have become increasingly popular among young people. While short-term effects on cognition and psychosis are normally observed in those individuals, questions remain as to whether there may be enduring effects of adolescent synthetic cannabinoid use on the adult brain and behavior.

Preclinical studies have started to address this issue primarily with the synthetic cannabinoid receptor agonist WIN55212.2, which acts similarly to delta-9-THC but with much greater efficacy at the cannabinoid receptors. Adult rats with a history of adolescent WIN55212.2 (self-administered or experimenter-administered) failed to show any long-term cognitive dysfunction measured using working memory and spatial recognition tasks [67]. Although prefrontal GABAergic and glutamatergic signaling were altered in the animals’ prefrontal cortex, epigenetic alterations were not examined.

Another study that investigated the chronic administration of WIN55212.2 during adolescence in young adult mice addressed epigenetic consequences. Animals that received the drug during adolescence showed spatial memory disturbances in the Morris water maze and dose-dependent memory impairment in fear conditioning.

RGS proteins are important regulators of striatal G protein-coupled receptor signaling. Moreover, adolescent WIN55212.2 exposure increased adult hippocampal eCB levels and promoted DNA hypermethylation at the intragenic region of the intracellular signaling modulator *Rgs7*, which was accompanied by a lower rate of mRNA transcription of the gene, suggesting a potential causal relationship.

Although the concrete mechanisms underlying the behavioral observations remain to be elucidated, the study demonstrates that exposure to a synthetic

cannabinoid during adolescence could lead to epigenetic gene regulation abnormalities in adulthood. Nevertheless, the equivocal behavioral findings in the few papers published to date emphasize the need for more research focused on the effects of synthetic cannabinoids.

Of the different components of the eCB system, several investigations have focused on the epigenetic regulation of the *Cnr1* gene, which encodes the CB1R. Specific genomic elements of the *Cnr1* locus have been shown to interact with transcription factors, some of which are implicated in the methylation of CpG sites in the DNA and histone posttranslational modifications. CB1R expression has been reported to increase in peripheral blood lymphocytes of human schizophrenic patients with *Cannabis* abuse and is inversely correlated with methylation of the CNR1 gene promoter.

Interestingly, CNR1 mRNA expression levels and promoter DNA methylation detected in the blood were reported to relate to the intensity of *Cannabis* craving and the severity of nicotine, *Cannabis*, and alcohol consumption, suggesting the relevance of CNR1 epigenetic status to brain function and behavior. The findings indicated that cannabinoid exposure during adolescence and young adulthood could imprint on the epigenetic landscape of postnatal development and augment behavioral responses via the dysregulation of genes that have important neurobiological functions related to addiction risk.

Of the known epigenetic mechanisms, information relevant to the neurodevelopmental effects of cannabinoids has been accrued regarding histone modifications; distinct alterations in the histone modification profile of the NAC in adult rats with prenatal THC exposure have been revealed [21].

The disturbances are characterized by decreased levels of the trimethylation of lysine 4 on histone H3 (H3K4me3; a transcriptionally permissive mark), increased levels of dimethylation of lysine 9 on histone H3 (H3K9me2; a repressive mark), and decreased RNA polymerase II association with the promoter and coding regions of the dopamine D2 receptor gene (*Drd2*) in the NAC. Delta-9-THC-related chromatin modifications, which would be predictive of reduced transcription, were linked to disturbances of the *Drd2* mRNA expression in humans and rodents and persisted into adulthood. The reduction of the dopamine D2 receptor function and mRNA levels is characteristic of individuals with substance use disorders, indicating that early cannabinoid exposure potentially enhances addiction vulnerability through altering the epigenetic processes at the *Drd2* locus.

Cannabis is the most commonly used illicit drug among adolescents and young adults, including pregnant women. There is substantial evidence for a significant association between prenatal *Cannabis* exposure and lower birth weight in offspring and mixed results regarding later behavioral outcomes in the offspring [22]. Adolescent *Cannabis* use, especially heavy use, has been associated with altered executive function, depression, psychosis, and use of other drugs later in life.

Human studies have limitations due to several confounding factors and have provided scarce information about sex differences. Animal studies support behavioral alterations reported in humans and have revealed diverse sex differences and

potential underlying mechanisms, altered mesolimbic dopaminergic and hippocampal glutamatergic systems, and interference with prefrontal cortex maturation.

A study examined the associations between prenatal *Cannabis* use disorder and adverse neonatal outcomes and heterogeneities in the associations by mothers' tobacco use status and race/ethnicity. A population-based, retrospective cohort study evaluated a total of 4.83 million mothers who delivered a live singleton birth from 2001 to 2012 and their paired infants. Data were obtained from mother–infant-linked hospital discharge records and birth and death certificates. Identified by ICD-9 codes recorded at delivery, 20,237 mothers had prenatal *Cannabis* use disorder. Neonatal outcomes included length of gestation, preterm birth, birth weight, neonatal intensive care unit admission, hospitalization within 1 year of birth, and death within 1 year of birth. Propensity score matching was used to balance maternal, paternal, and infant characteristics in comparing infants exposed and unexposed to prenatal *Cannabis* use disorder.

Cannabis use disorder increased from 2.8 to 6.9 per 1000 deliveries from 2001 to 2012 [23]. Multivariable regressions in matched samples estimated that prenatal *Cannabis* use disorder was associated with greater odds of being small for gestational age (OR = 1.13, 95% CI = 1.08, 1.18), preterm birth (OR = 1.06, 95% CI = 1.01, 1.12), low birth weight (OR = 1.13, 95% CI = 1.07, 1.20), and death within 1 year of birth (OR = 1.35, 95% CI = 1.12, 1.62). Compared with infants whose mothers were tobacco non-users, infants whose mothers were tobacco users had greater odds of preterm birth, low birth weight, hospitalization, and death associated with prenatal *Cannabis* use disorder. Compared with infants whose mothers were non-Hispanic White, infants whose mothers were Hispanic had greater odds of hospitalization and death, and infants whose mothers were non-Hispanic Black had greater odds of being small for gestational age associated with prenatal *Cannabis* use disorder.

Cannabis exposure during critical windows of development may have inter-generational physiological consequences disrupting epigenetic programming and marks [24]. A review examines pre-gestational and prenatal cannabinoid exposure and its effect on genes and molecular pathways related to the development of psychiatric disease. Developmental *Cannabis* exposure alters epigenetic processes with functional gene consequences, including potentially heritable alterations in genes and molecular pathways critical for brain development and associated with autism spectrum disorder, attention deficit hyperactivity disorder, schizophrenia, addiction, and other psychiatric diseases. *Cannabis* consumption and mental health illness in adolescents and young adults are increasing. Recent studies suggest that *Cannabis* consumption during critical periods of brain development could contribute to mental health illness through epigenetic mechanisms.

A cohort of male offspring was studied in adulthood (PND62) to evaluate long-term impairment induced by prenatal delta-9-THC exposure [25]. Pregnant rats were exposed to delta-9-THC, and changes in NAc *Drd2* gene expression in offspring were evaluated at postnatal day 2 (PND2), a comparable point in brain development as in humans during gestation weeks 18–22. Consistent with the *Cannabis*-exposed human fetuses, rats exposed to delta-9-THC had about a 40%

reduction in NAc *Drd2* mRNA levels at PND2 compared to unexposed control rats. This decrease persisted into adulthood, with about a 30% reduction in NAc *Drd2* mRNA levels observed at PND2 in prenatally exposed rats compared to unexposed controls. There was no difference in *Drd2* mRNA levels, between the exposed and unexposed groups, in the dorsal striatum.

Genetic Mutations

Evidence supports that the endocannabinoid system plays a major role in the central nervous system patterning in structures relevant to mood, cognition, and reward, such as the mesocorticolimbic system. Exposure to *Cannabis* during early ontogeny is not benign, and potential compensatory mechanisms that might be expected to occur during neurodevelopment appear insufficient to eliminate vulnerability to neuropsychiatric disorders in certain individuals. Both human longitudinal cohort studies and animal models emphasized the long-term influence of prenatal cannabinoid exposure on behavior and mental health, the endocannabinoid system and the neurobiological consequences of *Cannabis* exposure in pregnancy and early life, and its impact on the development of neurotransmitters systems relevant to neuropsychiatric disorders and its association with the disorders later in life [92].

Investigating in utero *Cannabis* exposure in association with genetic mutations of neural systems that have strong relationships to endocannabinoid function, such as dopamine, opioid, glutamate, and GABA, might help to identify individuals at risk. Such data could add to the existing knowledge to guide public health platforms regarding the use of *Cannabis* and its derivatives during pregnancy.

Prenatal *Cannabis* exposure influences human brain development, but it is challenging to model prenatal *Cannabis* exposure using animals and current cell culture techniques. A one-stop microfluidic platform was developed to assemble and culture human cerebral organoids from human embryonic stem cells to investigate the effect of prenatal *Cannabis* exposure on early human brain development [93].

By incorporating perfusable culture chambers, an air–liquid interface, and a one-stop protocol, the microfluidic platform can simplify the fabrication procedure and produce many organoids (169 organoids per 3.5×3.5 cm device area) without fusion, as compared with conventional fabrication methods. The one-stop microfluidic assembled cerebral organoids recapitulate not only early human brain structure, biology, and electrophysiology but also have minimal size variation and hypoxia. Under on-chip exposure to the psychoactive cannabinoid delta-9-tetrahydrocannabinol, cerebral organoids exhibited reduced neuronal maturation, downregulation of cannabinoid receptor type 1 receptors, and impaired neurite outgrowth.

Moreover, the transient on-chip delta-9-THC treatment decreased spontaneous firing in the organoids. The one-stop microfluidic technique enables a simple, scalable, and repeatable organoid culture method that can use not only for human brain organoids but also for many other human organoids, including liver, kidney, retina,

and tumor organoids. The technology could be widely used in modeling brain and additional organ development, developmental disorders, developmental pharmacology and toxicology, and drug screening.

Dynamic relations between genetic, hormone, and prenatal and postnatal environments are theorized as critically important for adolescent substance use. A study assessed the impact of interactions of genetic risk and cortisol reactivity with prenatal and parenting influences and frequency of adolescent substance use [94]. Data are from the TRacking Adolescents' Individual Lives Survey, a prospective longitudinal, multi-rater study of 2230 adolescents. Genetic risk was assessed via three substance-specific polygenic scores. Mothers retrospectively reported prenatal risk when adolescents were 11 years old. Adolescents rated their parents' warmth and hostility at age 11. At age 16, salivary cortisol reactivity was measured in response to a social stress task.

Adolescents' self-reported cigarette, alcohol, and *Cannabis* use frequency at age 16. A multivariate hurdle regression model showed that polygenic risk for smoking, alcohol, and *Cannabis* predicted any use of each substance but predicted more frequent use only for smoking. Blunted cortisol reactivity predicted any use and more frequent use for all three outcomes. There were two interactions: blunted cortisol reactivity exacerbated the association of polygenic risk with any smoking and the association of prenatal risk with any alcohol use. The polygenic risk seems important for early use but less so for frequency of use.

In contrast, blunted cortisol reactivity was correlated with both. Blunted cortisol reactivity may also catalyze early risks for substance use, though to a limited degree. Gene–environment interactions play no role in the context of this multifactorial model.

Cannabinoids and the Immune System

The critical stages in perinatal development and the administration of cannabinoids would compromise the function of the immune system. Gestational exposure to delta-9-THC using SR141716A and AM630 as CB1 and CB2 antagonists reduces thymic cellularity in fetuses and produces higher apoptosis rates in thymocytes. The activation of CB1 and CB2 mediates the effects. Immune function, tested with the HIV-1 p17/p24/gp120 protein in adult mice exposed prenatally to delta-9-THC, revealed that the animals exhibited a lower T cell proliferation rate as well as lower amounts of specific antibodies against HIV-1 p17/p24/gp120, indicating that prenatal *Cannabis* exposure can lead to a less efficient immune system in adulthood, increasing the risk of infectious diseases [7].

Studies on male macaques infected with Simian Immunodeficiency Virus (SIV) have reported that chronic delta-9-THC use did not affect the viral load in the plasma but reduced levels of CB1 and CB2 in the hippocampus, correlated with the decreased expression of the proinflammatory cytokine, monocyte chemoattractant protein-1. Treatment with the cannabinoid decreased early mortality [8, 9]. The

effect is gender dependent, as female macaques in the same condition showed that delta-9-THC treatment did not protect them from early mortality [10].

Cannabinoids play an important role in treating certain HIV-related pathologies, such as vision problems, due to an increased inflammatory response associated with the HIV-1 Tat protein. Using the eCBs, AEA, and 2-AG to control Tat-induced cytotoxicity in retinal cells resulted in the suppression of proinflammatory cytokines such as TNF- α , IFN- γ , IL-6, and IL-12p. The increased production of anti-inflammatory cytokines such as TGF- β and IL-10 has been suggested. The effects were associated with the MAPK pathway [11].

***Cannabis* Use and the Perception of the User During Pregnancy**

Observational studies have found associations between *Cannabis* use in pregnancy and low birth weight, preterm labor, and admission to the intensive care unit. A study was conducted to evaluate women's perceptions about the transmission of *Cannabis* to the fetus and whether receiving information from healthcare providers influenced their decision to stop using *Cannabis* during pregnancy [88]. Pregnant women at obstetrical, midwifery, and family practice clinics were asked to complete an anonymous survey. Chi-square tests were used to investigate whether patient knowledge was influenced by healthcare providers or self-directed learning and whether this information influenced their decision to discontinue *Cannabis* use.

Of the 478 women surveyed, the majority perceived that *Cannabis* is transmitted to the fetus during pregnancy and to the infant while breastfeeding (94.3% and 91.2%, respectively). Most women (99%) indicated that the advent of *Cannabis* legalization did not influence their choice to use *Cannabis* during pregnancy. Women who continued to use *Cannabis* during pregnancy were more likely to report receiving information on *Cannabis* from a healthcare provider (52%) than those who chose to discontinue use during pregnancy (35%) ($p = 0.035$). The proportion of pregnant women who understood *Cannabis* could be transmitted to the fetus in utero and the infant via breastmilk was high. Despite this, 4.2% of women reported that they continued to use *Cannabis* during pregnancy despite being informed of its risks.

Maternal Age and *Cannabis* Use

In a prospective study to identify trajectories of maternal *Cannabis* use and to determine if maternal age was associated with different trajectories of use, 456 pregnant women were recruited at a prenatal clinic, ranging in age from 13 to 42 years [89]. The women were interviewed about their *Cannabis* use 1 year before pregnancy, during each trimester of pregnancy, and at 6, 10, 14, and 16 years postpartum. A

growth mixture model of *Cannabis* use reported at each time point delineated four groups: non/unlikely to use, decreasing likelihood of use, late desistance, and increasing likelihood/chronic use (Lo–Mendell–Rubin-adjusted LRT test statistic = 35.7, $p < 0.001$).

The youngest mothers were least likely to be in the “non/unlikely to use” group. Younger maternal age also differentiated between late desistance and increasing likelihood/chronic use versus decreasing likelihood of use postpartum. The study demonstrated that younger mothers are more likely to use *Cannabis* across 17 years, including later desistance postpartum and increasing/chronic use. Other substance uses and chronic depressive symptoms were associated with more frequent use.

The consumption of drugs of abuse among women of childbearing age has experienced a significant increase. The results from analyses of surveys concerning maternal intake of psychoactive prescription drugs during pregnancy indicate that the intake rates are increasing each year. Studies of biological matrices such as maternal hair and neonatal meconium have assessed gestational consumption and consequent prenatal drug abuse exposure in high-risk women groups.

Validated chromatographic-mass spectrometric methodologies analyzed maternal hair and neonatal meconium to disclose the gestational use of drugs of abuse and psychoactive prescription drugs and consequent prenatal exposure in a cohort of 513 mother–newborn dyads at the Sant Joan de Déu Barcelona Hospital, Spain [94]. A total of 3.9% of the women reported drugs of abuse or prescription psychoactive drug consumption at any time during pregnancy. The prevalence of gestational consumption and the consequent prenatal exposure to drugs of abuse (e.g., *Cannabis*, cocaine, and MDMA) was 1.2% in maternal hair and 0.4% in meconium, and of psychoactive prescription drugs (e.g., venlafaxine, citalopram, fluoxetine, clomipramine) was 1.7% in maternal hair and 1.2% in meconium.

The prevalence of drug abuse and prescription psychoactive drug consumption was lower in the specific cohort of Spanish pregnant women than in other cohorts such as those from the United States or Denmark. The analysis of materno-fetal matrices provides a viable alternative to studying prenatal substance exposure and developing specific social and health intervention recommendations.

Pregnancy Risk Assessment

As *Cannabis* legalization expands and *Cannabis* use is increasingly perceived as safe, the prevalence and correlates of the perceived risk of weekly *Cannabis* use, past 30-day *Cannabis* use, and frequency of past 30-day *Cannabis* use among US pregnant women were investigated. Data from 2247 pregnant women, 14–44 years of age, surveyed in a cross-sectional National Survey on Drug Use and Health were analyzed. Primary outcomes included perceived risk of weekly *Cannabis* use, past 30-day *Cannabis* use, and frequency of *Cannabis* use.

Among US pregnant women, 21.6% (95% CI = 19.4, 23.8) did not perceive any risk associated with weekly *Cannabis* use, 5.3% (95% CI = 4.2, 6.5) used *Cannabis*

in the past 30 days, and among past-month users, the average number of days of use was 15.6 (95% CI = 13.5, 17.7). Pregnant women living below the poverty line were both more likely to perceive no risk of weekly *Cannabis* use (adjusted OR [aOR] = 1.8; 95% CI = 1.3, 2.5) and use *Cannabis* more often in the past 30 days (aOR = 2.9; 95% CI = 1.5, 5.7) than pregnant women within an income bracket of more than two times the federal poverty threshold. Age, race, trimester of pregnancy, and co-use of tobacco and alcohol were also associated with the outcomes. Younger age, living in poverty, early trimester of pregnancy, and co-use of tobacco and alcohol increased the odds of *Cannabis* use among pregnant women [84].

Cannabis in Nausea and Vomiting During Pregnancy

Stigmatization has been identified as a barrier to therapeutic *Cannabis* use and related physician communication. The stigma may be particularly salient in the context of prenatal *Cannabis* use. One hundred and three women who reported current or past pregnancies were recruited online. Participants completed a survey querying prenatal experiences, substance use, and attitudes toward prenatal *Cannabis* use. Thirty-five (34%) respondents reported prenatal *Cannabis* use [74].

Treating nausea and vomiting during pregnancy was the most frequently reported reason for prenatal *Cannabis* use (89%), and 24 (69%) reported substituting *Cannabis* for pharmaceutical drugs. Sixty-two percent of prenatal *Cannabis* use participants and 31% of non-prenatal *Cannabis* use participants indicated discomfort discussing prenatal *Cannabis* use with their physician, and 74% of prenatal *Cannabis* use participants and 27% of non-prenatal *Cannabis* use participants indicated they would not disclose prenatal *Cannabis* use to their physician if it occurred in future pregnancies.

The findings suggest that prenatal *Cannabis* use may reflect primarily therapeutic motives of relieving symptoms of morning sickness, nausea, low appetite, and pain and substituting for other prescription medications. Respondents reported discomfort discussing prenatal *Cannabis* use with physicians, which was more pronounced among respondents with lived experience of prenatal *Cannabis* use. The findings suggest prenatal *Cannabis* use might best be evaluated within a therapeutic framework and highlight the importance of efforts to enhance patient–caregiver communication regarding prenatal *Cannabis* use.

Parental Cannabis Use on Child Development

The long-term consequences of maternal *Cannabis* use on child development beyond the neonatal period are adequately understood. A multi-information approach was conducted to assess the association between prenatal *Cannabis* exposure and child behavioral and emotional functioning. To explore the possible causal nature of

the association, an investigation was conducted on whether maternal tobacco and paternal *Cannabis* use during pregnancy were also associated with child problems [37]. The study population included children of a population-based birth cohort in The Netherlands ($n = 5903$). Information on parental *Cannabis* use was collected using questionnaires; the urine of mothers was analyzed for the presence of *Cannabis* metabolites. Child behavioral and emotional problems at approximately 7–10 years of age were measured using validated teacher, child, and mother reports.

The findings indicate associations of maternal *Cannabis* use during pregnancy with offspring externalizing problems ($B = 0.53$; 95% CI = 0.29, 0.77) but not with internalizing problems ($B = -0.10$; 95% CI = -0.31 , -0.11). However, maternal *Cannabis* use before pregnancy was also associated with offspring externalizing problems ($B = 0.27$; 95% CI = 0.02, -0.52). *Cannabis* use by the father was associated with child externalizing problems ($B = 0.36$; 95% CI = 0.22, 0.49) but not internalizing problems.

Prenatal exposure to maternal *Cannabis* use is specifically associated with offspring behavioral problems but not emotional problems. This association is probably not due to an effect of intrauterine *Cannabis* exposure on fetal development because maternal and paternal *Cannabis* exposure during pregnancy was related to offspring externalizing problems. The findings suggest that the association can be explained through residual confounding, most likely through shared genetic vulnerabilities for parental *Cannabis* use and offspring behavioral problems.

Exploration of the therapeutic potential of *Cannabis*-based medicines and synthetic cannabinoid compounds has given us much insight into the physiological roles of endogenous ligands (endocannabinoids) and their receptors. Human epidemiological and animal studies have found that prenatal *Cannabis* exposure influences brain development and can have long-lasting impacts on cognitive functions. Human longitudinal cohort studies that document the long-term influence of prenatal exposure to *Cannabis*, followed by an overview of the molecular composition of the endocannabinoid system and the temporal and spatial changes in their expression during brain development, were examined. Endocannabinoid signaling modulates fundamental developmental processes such as cell proliferation, neurogenesis, migration, and axonal pathfinding.

Prenatal *Cannabis* exposure is associated with fetal growth restriction, learning disabilities, and memory impairment in exposed offspring [75]. The mean potency of *Cannabis* preparations, in terms of its psychoactive constituent, delta-9-THC, has increased from 3.4% in 1993 to 8.8% in 2008 and can reach as high as 30% in certain hashish preparations. It is important since delta-9-THC effects are dose related and classical studies carried out in the 1970s used doses that reflected *Cannabis* intake. Most information is derived from two longitudinal cohort studies, the Ottawa Prenatal Prospective Study (OPPS) and the Maternal Health Practices and Child Development Study (MHPCD). Ottawa Prenatal Prospective Study, initiated in 1978, focused on assessing prenatal exposure effects of tobacco and *Cannabis* in a low-risk, predominantly middle-class Canadian cohort.

In 1982, the MHPCD focused mainly on prenatal alcohol and *Cannabis* exposure in a group of women from Pittsburgh, Pennsylvania. The women were

generally of low socioeconomic status and comprised approximately half Caucasian and half African American ethnicity. In both the OPPS and MHPCD studies, *Cannabis* use during pregnancy was not associated with increased miscarriage rates, premature deliveries, or any other. Physically, *Cannabis* exposure was not correlated with any changes in head circumference at the mid-gestational stage (17–22 weeks). However, a significant foot length and body weight reduction during that gestational period was reported. The changes in body weight and foot length were not present at birth, although head circumference was reportedly larger in the exposed cohort at 8 months. The anthropometric measurements indicated normal fetal development, which correlates with brain development.

Prenatal Tobacco and Co-exposure to Tobacco and *Cannabis*

Substance use during pregnancy and the short- and long-term impacts of different substances on maternal, fetal, and longer-term health outcomes of individuals prenatally exposed has been examined. The study examined prenatal tobacco or co-exposure to tobacco and *Cannabis* and children's cortisol reactivity at kindergarten age and the role of child sex, maternal negative mood (depression/perceived stress), and parenting behavior during play interactions as moderators of the association. The study consisted of 238 mother–child dyads, 67 tobacco users, 83 co-users of tobacco and *Cannabis*, and 88 non-users. Data were obtained from pregnancy and six postnatal assessments at 2, 9, 16, 24, and 36 months and kindergarten age. Infant cortisol was measured in response to two laboratory stress paradigms.

Co-exposed children had a significantly greater decrease from pre-stressor to post-stressor and a lower cortisol response than non-exposed children. The association was moderated by maternal harshness during play interactions across early childhood. Co-exposed children had flatter cortisol responses regardless of the mother's harshness or stress/depression level [31]. However, non-exposed children who experienced low harshness had the normative cortisol peak 20 min post-stressor, while non-exposed children with high maternal harshness had a flatter cortisol pattern.

Similarly, mothers of non-exposed children with more depression and stress had higher pre-stressor cortisol levels. In contrast, those who experienced low maternal depression/stress had lower pre-stressor cortisol but peaked post-stress. The results suggest that prenatal polysubstance exposure is associated with a greater risk for lower cortisol response in children and highlight the role of parenting behavior for non-exposed but not co-exposed children.

Although tobacco and *Cannabis* are often co-used in pregnancy, little is known regarding the joint impact of *Cannabis* + tobacco on offspring development, including the developing neuroendocrine stress system. There is evidence of sex-specific impacts of prenatal exposures in preclinical models; the sex-specific impact of prenatal *Cannabis* + tobacco exposure on offspring neuroendocrine regulation in humans was also investigated [64].

The overall and sex-specific influences of *Cannabis* + tobacco co-use on offspring cortisol regulation were investigated over the first postnatal month. One hundred eleven mother–infant pairs from a racially and ethnically diverse sampling population participated. Based on Timeline Followback data with biochemical verification, three groups were identified: (1) prenatal *Cannabis* + tobacco, (2) tobacco only, and (3) control. Baseline cortisol and cortisol stress response were assessed at seven points over the first postnatal month using a handling paradigm in which saliva cortisol was assessed before, during, and following a standard neurobehavioral assessment (NICU Network Neurobehavioral Scale).

A significant exposure group by offspring sex interaction emerged for baseline cortisol over the first postnatal month ($p = 0.043$); *Cannabis* + tobacco-exposed males showed 35–36% attenuation of baseline cortisol levels versus unexposed and tobacco-exposed males ($p \leq 0.003$), while no effects of exposure emerged for females. *Cannabis* + tobacco and tobacco-exposed infants showed a 22% attenuation of cortisol stress response over the first postnatal month versus. Unexposed infants ($p < 0.03$), with evidence for sex-specific effects in exploratory analyses.

A retrospective cohort study examined the association between prenatal *Cannabis* use and adverse infant outcomes in a nationally representative cohort and the impact of concurrent tobacco exposure on 32,583 new mothers. *Cannabis* use was evaluated as a binary variable (use or no use) and ordinal categories (no, light, moderate, heavy use). Multivariable logistic regression was used to examine the relationship between prenatal *Cannabis* exposure and low birth weight (LBW), preterm birth, and small gestational age [50].

Prenatal *Cannabis* use was associated with significantly greater odds of LBW (aOR, 1.27; 95% CI = 1.05–1.54) and small for gestational age (aOR, 1.35; 95% CI = 1.09–1.68) but not preterm birth. Compared with non-users, heavy users (weekly or more) were twice as likely to deliver an LBW infant (aOR, 2.07; 95% CI = 1.46–2.94) or small for gestational age infant (aOR, 2.14; 95% CI = 1.38–3.30). When examining combined *Cannabis* and cigarette use, prenatal exposure to both substances increased the likelihood of LBW (aOR, 2.27; 95% CI = 1.71–3.01), preterm birth (aOR, 1.61; 95% CI = 1.12–2.31), and small for gestational age (aOR, 3.29; 95% CI = 2.39–4.55) compared with no use, and the increased odds were greater than for either substance alone. The results suggest that *Cannabis* use during pregnancy may harm fetal development.

Cannabis is the most frequently used illicit drug among pregnant women. However, data describing the effects of prenatal *Cannabis* exposure and concurrent nicotine and *Cannabis* exposures on neonatal growth are inconsistent. Testing of meconium, the first neonatal feces, offers objective evidence of prenatal *Cannabis* exposure. However, the relative ability of meconium testing and maternal self-report to identify affected neonates remains unclear.

Eighty-six pregnant women provided detailed self-reports of daily *Cannabis* and tobacco consumption throughout pregnancy. Cannabinoids and tobacco biomarkers were identified in oral fluid samples collected each trimester and quantified in meconium at birth. *Cannabis*-using women were significantly more likely to

consume tobacco and smoked similar numbers of cigarettes as non-*Cannabis*-using tobacco smokers.

As the pregnancy progressed, fewer women smoked *Cannabis*, and those who continued to use *Cannabis* reported smoking fewer *Cannabis* joints. However, positive maternal oral fluid tests cast doubt on the veracity of some maternal self-reports. More neonates were identified as *Cannabis* exposed by maternal self-report than meconium analysis because many women quit *Cannabis* use after the first or second trimester; meconium was more likely to be positive if *Cannabis* use continued into the third trimester. *Cannabis* exposure was associated with decreased birth weight, reduced length, and smaller head circumference, even after data were controlled for tobacco co-exposure [65]. Prenatal *Cannabis* exposure was associated with fetal growth reduction. Meconium testing primarily identifies prenatal *Cannabis* exposure in the third trimester of gestation.

A study to determine the prevalence and correlates of co-use of *Cannabis* and tobacco cigarettes was conducted among adult pregnant women utilizing secondary data from a larger study that compared and validated screeners for illicit and prescription drug use during pregnancy [71]. Pregnant women ($n = 500$), average age of 28 years, were recruited from two urban university obstetric clinics. The participants self-reported demographic, *Cannabis*, and tobacco cigarette use characteristics and provided urine and hair samples for drug testing. The research staff reviewed the participants' electronic medical records and gathered birth outcome data within 2 weeks after the due date.

The results indicated that 9.0% reported co-use of *Cannabis* and tobacco, 12.1% reported *Cannabis* only use, 7.8% reported tobacco cigarette-only use, and 71.1% reported no *Cannabis* or tobacco cigarette use in the past month. The birth outcomes to emerge as significant correlates of co-use of *Cannabis* and tobacco cigarettes were small head circumference and the occurrence of congenital disabilities, with the co-use group having the highest odds of a small head circumference [aOR: 5.7 (1.1–28.9)] and congenital disabilities [aOR: 3.1 (1.2–8.3)] compared with other user groups. The *Cannabis* only group had 12 times higher odds of a stillbirth or miscarriage (aOR = 12.1). Screening and interventions to address concurrent *Cannabis* and tobacco use during pregnancy are needed, particularly among subpopulations with higher co-use rates. It is imperative to explore further and highlight the possible health implications of maternal co-use, given the high prevalence rates in this study sample.

The combination of *Cannabis* and tobacco is increasingly common among women and is associated with tobacco and *Cannabis* dependence and poorer cessation outcomes [90]. Pregnant women (mean age = 23, range = 18–42) were asked about substance use during each trimester of pregnancy and at 8 and 18 months, 3, 6, 10, 14, 16, and 22 years postpartum. Patterns of maternal cigarette and *Cannabis* use during pregnancy and postpartum were examined. As young adults (mean age = 22.8 years, range = 21–26), 603 offspring completed the Diagnostic Interview Schedule (DIS). Growth mixture modeling (GMM) was used to identify four maternal trajectories through 16 years postpartum: no co-use (66%), decreasing co-use (16%), postpartum-only co-use (11%), and chronic co-use (7%). Offspring whose

mothers were in the decreasing co-use group (co-users primarily during prenatal and preschool periods) were more likely to be co-users than the offspring of non-co-users. Offspring whose mothers were chronic co-users of cigarettes and *Cannabis* were more than twice likely to have a drug use disorder than young adults whose mothers were not co-users. The study's results highlight the heterogeneity in maternal co-use of tobacco and *Cannabis* over time, with some women quitting during pregnancy but resuming co-use postpartum and other women co-using during pregnancy but desisting co-use over time. Maternal trajectories of co-use were associated with the inter-generational transfer of risk for substance use and dependence in adult offspring. Prenatal use of *Cannabis* and opioids is increasing and very concerning. Engagement and retention in comprehensive, perinatal substance use disorder care are associated with better outcomes for mothers and babies. The characteristics and engagement in care among women with opioid use disorder who used *Cannabis* late in pregnancy versus those who did not use were compared. The primary outcome, "overall engagement and retention in perinatal substance use disorder care," included utilization of substance use treatment prenatally, negative screening/toxicology at delivery (excluding *Cannabis*), and attendance at routine prenatal and postpartum visits.

Prenatal Exposure to Alcohol and Delta-9-THC

A 2 (ethanol, air) $\times$ 2 (delta-9-THC, vehicle) design was used to expose pregnant Sprague–Dawley rats to either EtOH or air, in addition to either delta-9-THC or the inhalant vehicle solution, during gestational days 5–20. Immunohistochemistry was performed to detect parvalbumin (PV) interneurons in one male and one female pup from each litter at postnatal day 70. Significant between-group and subregion-specific effects were found in the dorsal cornu ammonis 1 (CA1) subfield and the ventral dentate gyrus [32]. In the dorsal CA1 subfield, there was an increase in the number of PV interneurons in the EtOH and EtOH + delta-9-THC groups but a decrease with delta-9-THC alone. There were fewer changes in interneuron numbers overall in the DG, though there was a sex difference, with a decrease in the number of PV interneurons in the delta-9-THC-exposed group in males. There was also a greater cell layer volume in the DG in the EtOH + delta-9-THC group than in the control group and in the CA1 region in the EtOH group compared to the control and delta-9-THC groups.

Prenatal exposure to alcohol and delta-9-THC differentially affects parvalbumin-positive interneuron numbers in the hippocampus, indicating that both separate and combined exposure can impact the balance of excitation and inhibition in a structure critically involved in learning and memory processes.

Alcohol has been recognized as harmful during pregnancy and has been recognized as a neurobehavioral teratogen, and the pattern of effects has been termed fetal alcohol spectrum disorder. The effects of prenatal *Cannabis* exposure have been vigorously explored as a priority research area following the decriminalization

or legalization of *Cannabis* [33]. The data revealed that *Cannabis* during pregnancy can negatively affect maternal and fetal outcomes and longer-term neurodevelopmental and cognitive functions.

Prenatal Tobacco, Alcohol, and *Cannabis* Exposures

Combustible cigarette use is common among *Cannabis* users, and dual use of cigarettes and *Cannabis* is associated with detrimental outcomes. A study was conducted to examine data from the prenatal and adolescent phases of a prospective, longitudinal study to predict adult daily dual, including prenatal tobacco, alcohol, and *Cannabis* exposures, and ages at the initiation of use. Young adult offspring (mean age = 22.8 years, 53% female) from a prenatal cohort reported combustible cigarette and *Cannabis* use ($n = 500$, 58% Black, 42% White). Pathways to daily dual use were modeled using variables from the gestational and adolescent phases of the study, cigarettes and *Cannabis*, and adolescent learning/memory, impulsivity, and behavior problems.

Prenatal *Cannabis* and tobacco use were not directly linked to adult daily dual use of *Cannabis* and tobacco. However, structural equation modeling revealed three significant indirect pathways from prenatal cigarette and *Cannabis* exposures to adult daily dual use of cigarettes and *Cannabis* via early cigarette initiation, early *Cannabis* initiation, and adolescent behavior problems. The study identified pathways from prenatal *Cannabis* and tobacco exposure to adult daily dual use and clarified adolescent outcomes that may be part of the pathways [41]. In an environment of a growing acceptance of *Cannabis* use and increasing legalization of recreational use, the findings warn that early exposure to *Cannabis* may have an important role in shaping long-term dual use of tobacco and *Cannabis*.

With the increased prevalence, potency, and acceptability of *Cannabis* use during pregnancy, understanding the developmental effects of prenatal *Cannabis* exposure is vital. There is evidence of the associations between PCE and restricted growth at birth but not for long-term effects on growth. A review discussed the methodological considerations for studies of PCE, including the assessment of exposures, covariates, and outcomes, and reviews findings from prospective, longitudinal studies of PCE.

Prenatal *Cannabis* exposure appears to have subtle yet enduring effects on memory and achievement in children and adolescents. Despite differences in sample demographics and measurement, PCE has remarkably consistent effects on externalizing behaviors, such as delinquency and substance use, which persist into adulthood. Longitudinal analyses demonstrate the importance of early *Cannabis* initiation for pathways between PCE and adult functioning, including substance use and abuse, memory deficits, and psychotic symptoms. Animal studies demonstrate direct effects on the development of the brain via the activation of endogenous endocannabinoid systems [42, 44, 46]. *Cannabis*-induced activation of the endocannabinoid system causes alterations in the release of neurotransmitters and the

modulation of brain plasticity in neural pathways that underlie cognition, motivation, and behavior regulation.

A study examined the association between prenatal alcohol and tobacco exposures and offspring *Cannabis* use [53]. Data were from the Raine Study, a longitudinal prospective birth cohort. *Cannabis* use at 17 years of age was measured with a self-reported questionnaire developed to capture risky behaviors in adolescents. Associations between prenatal alcohol and tobacco exposures and the risk of *Cannabis* use in offspring were examined using log-binomial regression models, computing relative risk (RR). The E-values (E) were also computed to estimate the extent of unmeasured confounding.

After adjusting for potential confounders, an increased risk of *Cannabis* use in offspring exposed to first-trimester prenatal alcohol use (RR = 1.38, 95% CI = 1.09–1.75; E = 2.10, CI:1.40) and tobacco use (RR = 1.42, 95% CI = 1.08–1.86; E = 2.19, CI:1.37) as well as third-trimester prenatal alcohol use (RR = 1.39, 95% CI = 1.09–1.79; E = 2.13, CI:1.40) and tobacco use (RR = 1.39, 95% CI = 1.09–1.79; E = 2.21, CI:1.34) was observed. A dose–response association in which risk estimates in offspring increased with exposure to prenatal alcohol and tobacco use was also noted.

The association of tobacco and *Cannabis* use in the first trimester with maternal immune response and psychosocial well-being relative to tobacco use only was examined in 138 pregnant women with biologically verified tobacco use, 38 of whom (28%) also tested positive for recent *Cannabis* use [53]. Maternal perceived stress (Perceived Stress Scale), depressive symptoms (Edinburgh Postnatal Depression Scale), and serum immune markers (IL-1 β , IL-2, IL-6, IL-8, IL-10, TNF α , CRP, MMP8) were analyzed. However, cytokine data were only available for 122 women. The participants' average age was 29.1 years, approximately half had a high school education or less, and half were unemployed. In the adjusted linear regression models, TNF- α level was significantly lower among co-users relative to tobacco-only users after adjusting for age, race/ethnicity, body mass index, and tobacco use group (tobacco cigarettes, electronic nicotine delivery devices [ENDS], or both). TNF- α was the only immune marker found to be significant in the analysis.

Measured stress levels (M = 5.9, SD = 3.3; potential range 0–16) and depression scores (M = 7.8, SD = 5.8; potential range 0–30) were low across all participants and did not differ as a function of co-use. The results indicated that women co-using during the first-trimester exhibit decreased proinflammatory immune responsivity on one out of eight markers.

Prenatal Exposures to Adult Electronic Cigarette

The pathways from the prenatal exposures of adult to electronic cigarette use were evaluated by a test to determine whether there were indirect effects of the prenatal exposures via childhood behavior dysregulation, early tobacco use, and adolescent tobacco dependence [42, 44]. Telephone interviews were conducted with 427 adult

offspring (22–33 years old) from three prenatal cohorts with trimester-specific data on tobacco, alcohol, and *Cannabis* exposures. Prenatal exposures included mothers' quantity/frequency of tobacco, alcohol, and *Cannabis* use during the first trimester.

Using logistic regression and structural equation modeling, the effects of gestational exposures on adult electronic cigarette use via early cigarette use (prior to age 14), controlling for covariates of combustible and electronic cigarette use, were examined. There were no effects of childhood behavioral dysregulation on electronic cigarette use. However, prenatal exposures to tobacco and *Cannabis* were significantly indirect to electronic cigarette use via early adolescent combustible cigarette use and adolescent risk for tobacco dependence.

One implication of the findings is that the inter-generational risk for tobacco use conferred via gestational exposures to tobacco and *Cannabis* generalizes to novel products such as electronic cigarettes. The results have implications for public health, as more women use *Cannabis* and co-use cigarettes and *Cannabis* during pregnancy.

Prenatal *Cannabis* Exposure on Emotional, Social, and Cognitive Endpoints

Studies examining the effects of prenatal *Cannabis* exposure typically employ injections of synthetic cannabinoids or isolated *Cannabis* constituents that may not accurately model *Cannabis* use in human populations. A novel e-cigarette technology-based system was developed to deliver vaporized *Cannabis* extracts to pregnant Long Evans rats. The model was used to determine the effects of prenatal *Cannabis* exposure on emotional, social, and cognitive endpoints of male and female offspring during early development and into adulthood.

Dams were exposed to *Cannabis* vapor (CAN-THC: 400 mg/mL), vehicle vapor (VEH), or no vapor (AIR) twice daily during mating and gestation [95]. The offspring exposed to CAN-THC and VEH showed reduced weight gain relative to offspring exposed to AIR before weaning. CAN-THC offspring made more isolation-induced ultrasonic vocalizations on a postnatal day 6 close to VEH-exposed offspring, which indicates increased emotional reactivity. Male CAN-THC offspring engaged in fewer social investigation behaviors than VEH-exposed male offspring during a social play test on day 6. In adulthood, CAN-THC-exposed offspring spent less time exploring the open arms of the elevated plus maze. They exhibited dose-dependent deficits in behavioral flexibility in an attentional set-shifting task relative to AIR controls. The data indicate that prenatal *Cannabis* exposure may cause enduring effects on the behavioral profile of offspring.

***Cannabis* and Prenatal Choline on Fetal Brain Development**

Prenatal choline is a key nutrient for fetal brain development and subsequent mental function, like folic acid and vitamin D. A study was conducted to determine whether the effects of higher maternal plasma choline concentrations on childhood attention and social problems, found in an initial clinical trial of choline supplementation, are observed in a second cohort.

One hundred eighty-three mothers were enrolled in an urban safety net hospital clinic. One hundred and sixty-two complied with gestational assessments and brought their newborns for study at 1 month; 83 continued assessments through 4 years of age. At 16 weeks of gestation, the effects of maternal plasma choline concentrations $\geq 7.07 \mu\text{M}$, 1 SD below the mean level obtained with supplementation in the previous trial, were compared to lower levels. The principal outcomes were the principal outcomes of Attention Problems and Withdrawn Syndrome scales on Child Behavior Checklist 1½–5.

Higher maternal plasma choline was associated with lower mean Attention Problems percentiles in children, and male children had lower withdrawn percentiles [35]. Higher plasma choline concentrations also reduced Attention Problems percentiles for children of mothers who used *Cannabis* during gestation and children of mothers who had a gestational infection. Prenatal choline's positive associations with early childhood behaviors are found in a second, more diverse cohort. Increases in attention problems and social withdrawal in early childhood are associated with later mental illnesses, including attention deficit disorder and schizophrenia. Choline concentrations in the pregnant women in the study replicate other research findings indicating that most women may not have adequate choline in their diets.

***In Utero* Exposure to *Cannabis*, Tobacco, and Cocaine**

The potential consequences for the newborn, child, and adolescent after exposure to tobacco, *Cannabis*, and cocaine in utero were examined in retrospective and prospective studies on the somatic or psychiatric consequences of alcohol, tobacco, and drug consumption by pregnant women on newborns and children [34]. Consumption during pregnancy was assessed in the studies using simple questionnaires, biomarkers analysis, or both. The pregnancies were at high risk for both the mother and the fetus: for example, an increased risk of miscarriage or reduced length of gestation, an increased risk of uterine apoplexy and placenta previa, more premature births, and hypotrophy were reported.

The occurrence of a newborn's withdrawal syndrome may be misdiagnosed. Many consequences on child development may be observed, such as growth disorders, learning or motor disorders, language disorders, cognitive disorders (attention, memory, executive functions), attention deficit disorders with impulsivity or

hyperactivity (ADHD), and memory disorders. The prevalence of depressive or anxiety disorders may also be increased in children. The risk of addictive disorders or schizophrenia in children exposed in utero to illicit drugs or tobacco is still not fully understood.

The combined use of different substances increases; consequently, it is difficult to disentangle the consequences on child development of each drug used during pregnancy owing to potential interactions between the drugs. The consequences on child development will also depend on the dose and on the time of drug use during pregnancy. The National Institute of Drug Abuse reported that 75% of the infants exposed in utero to one or more substances would present medical problems during childhood, compared to only 27% of the non-exposed infants. The main limitations are methodological biases, such as using different rating scales among studies and the populations' heterogeneity.

***Cannabis* Use Late in Pregnancy**

Cannabis use late in pregnancy was objectively assessed at delivery via maternal urine drug screen (UDS) and neonatal meconium/cord toxicology; between-group comparisons utilized chi-square, t-test, or Mann–Whitney [91]. Associations were assessed using Spearman Rho and two multivariate, binary logistic regressions for *Cannabis* use and the primary outcome. Eighteen percent (85/472) consumed *Cannabis* late in pregnancy. Younger women and those diagnosed with concurrent *Cannabis* use disorder were more likely to consume *Cannabis*. Engagement and retention in perinatal substance use disorder care were not associated with *Cannabis* use but with prescribed pharmacotherapy for psychiatric disorders.

The use of prescribed buprenorphine and naloxone was associated with *Cannabis* avoidance late in pregnancy. *Cannabis* use late in pregnancy, compared to none, did not impact engagement and retention in the perinatal substance use disorder program. Adjunctive psychotropic medication and buprenorphine and naloxone prescription were associated with *Cannabis* avoidance.

Infant Neurobehavioral Development

Cannabis use during pregnancy has been associated with negative behavioral outcomes and psychopathology in offspring. The association between prenatal *Cannabis* exposure and brain morphology in young children has been investigated in 96 children prenatally exposed to tobacco only (without *Cannabis*). One hundred and thirteen unexposed control subjects were matched based on age and gender, and 54 children were exposed to prenatal *Cannabis*, mostly combined with tobacco. The children, aged 6–8 years, were part of a population-based study followed from pregnancy onward. The brain volumetric measures and cortical thickness in magnetic

resonance imaging scans using FreeSurfer were assessed. Vertexwise analyses in FreeSurfer and linear regression analyses adjusting for relevant covariates using Statistical Package for the Social Sciences were performed [51].

Prenatal *Cannabis* exposure was not associated with global brain volumes, such as total brain volume, gray matter, or white matter. However, prenatal *Cannabis* exposure was associated with differences in cortical thickness. Compared with non-exposed control subjects, *Cannabis*-exposed children had thicker frontal cortices. Compared with non-exposed control subjects, prenatal tobacco exposure was associated with cortical thinning, primarily in the superior frontal and parietal cortices.

The impact of prenatal exposure to *Cannabis* and tobacco on infant neurobehavioral development over the first postnatal month was investigated [62]. The participants were 111 mother–infant pairs from a low-income, diverse sample (mean age = 25 ± 5). Tobacco and *Cannabis* use were assessed by Timeline Followback interview with biochemical confirmation.

Newborn neurobehavior was assessed at seven-time points over the first postnatal month using the NICU Network Neurobehavioral Scale. Three groups were identified: (a) prenatal *Cannabis* + tobacco, (b) prenatal tobacco only, and (c) controls. *Cannabis* + tobacco-exposed infants showed decreased ability to self-soothe (self-regulation). They attended to stimuli (attention) and increased need for examiner soothing (handling) and low motor activity (lethargy) versus unexposed infants.

Despite low levels of *Cannabis* use in *Cannabis* + tobacco co-users, co-exposure was associated with nearly double the impact on infant self-soothing and need for examiner soothing versus tobacco exposure alone. The effects of *Cannabis* + tobacco co-exposure appeared more pronounced for daughters than for sons. Although the results are preliminary, they highlight additional risks from a double exposure to *Cannabis* + tobacco versus tobacco exposure alone, particularly for daughters. The results also highlight the importance of investigating prenatal exposures in concert and the need for intervention efforts to address *Cannabis* co-use in pregnant tobacco users.

Prenatal *Cannabis* exposure negatively impacts child development and behavior; however, few studies have examined the associations at early ages among children exposed to *Cannabis*. Using a prospective prenatal cohort, prenatal *Cannabis* exposure was determined from maternal self-report, medical chart abstraction, and urine toxicology from prenatal visits and delivery [63]. At age 3.5 years, 63 offspring completed tasks assessing executive function, visual–spatial ability, emotion regulation, and aggressive behavior. Caregivers reported on children's executive function and problem behaviors. Logistic regressions and analyses of covariance controlling for key variables were used to examine associations between prenatal *Cannabis* exposure and child outcomes.

The findings indicate that behavioral problems associated with prenatal *Cannabis* exposure may manifest by age 3.5 years. Compared to non-exposed children, children with prenatal *Cannabis* exposure had more sleep-related problems, withdrawal symptoms, and externalizing problems, including aggressive and oppositional defiant behaviors. Children with and without prenatal *Cannabis* exposure did not differ in executive functioning.

Prenatal neurodevelopment depends on the precise functioning of multiple signaling pathways in the brain, including those mobilized by glucocorticoids and endocannabinoids. Prenatal exposure to drugs, including opioids, alcohol, cocaine, and *Cannabis*, has been shown to impact glucocorticoid signaling and alter the functioning of the hypothalamic–pituitary–adrenal axis [52]. The exposures can have long-lasting neurobehavioral consequences, including alterations in the stress response in the offspring. *Cannabis* contains cannabinoids that signal via the endocannabinoid pathway, which is linked to components of glucocorticoid signaling in the adult brain.

Tobacco and *Cannabis* Exposure on Child Behavior Problems

The prospective associations between pre- and postnatal tobacco and *Cannabis* exposure on child behavior problems from 2–3 years of age, sex differences in the associations, and bidirectional associations between maternal postnatal substance use and child behavior problems across time were examined [80].

The sampled population consisted of 247 primarily young, unmarried, low-income mothers, and their children (97 prenatally exposed to tobacco and *Cannabis*, 81 exposed to tobacco only, and 69 non-exposed). The mothers were assessed during each trimester of pregnancy, at 2, 9, and 16 months and 2 and 3 years of age.

Bivariate results indicated significant differences, mainly for girls. Girls in the prenatal tobacco exposure group had higher internalizing problems than the other two groups and greater attention and sleep problems at 3 years compared to the control group. The higher number of cigarettes per day during pregnancy was significantly associated with higher anxiety/depression and attention problems at 3 years of age. The associations were stronger for girls compared to boys.

In model testing controlling for prenatal exposure, the results indicated bidirectional associations between behavioral problems at 2 years of age and maternal postnatal *Cannabis* use, such that higher *Cannabis* use across the infant–toddler period predicted greater behavior problems at 2 years of age, which in turn predicted higher *Cannabis* use a year later. The results complemented the literature on the combined effects of tobacco and *Cannabis*, highlighted the importance of considering bidirectional associations between maternal substance use and child behavior problems, and indicated generally stronger prenatal tobacco exposure effects for girls.

The Hypothalamic/Pituitary/Adrenal Axis

Glucocorticoids are frequently used in pregnancy to prevent complications of prematurity. With the increasing use of *Cannabis* during pregnancy, the likelihood of fetal co-exposure to cannabinoids may have additional implications for long-term

neurodevelopment. Studies have found that rates of *Cannabis* use during pregnancy are rising and approaching nearly 4% in all groups. However, when prevalence measures do not rely on self-report and are based on testing of urine or umbilical cord blood, rates frequently exceed 20%. The ability of lipophilic cannabinoids to readily cross the placenta is a concern for the potentially detrimental effects of in utero exposure.

Cannabinoids signal primarily through established endocannabinoid signaling pathways within the brain. Studies have demonstrated the significant role of endocannabinoids as effectors of glucocorticoids-mediated negative feedback on the hypothalamic–pituitary–adrenal axis and highlight the complexity of the relationship between the two signaling systems, despite the establishment of a scientific premise for the endocannabinoid pathway interactions at multiple points with glucocorticoids signaling. In humans, *Cannabis* use typically has been shown to have activating effects on the hypothalamic–pituitary–adrenal axis, including increased levels of cortisol after administration; it reduces response in cortisol elevation after a stressor in chronic compared to non-users.

Studies that evaluated the lasting effects of prenatal cannabinoids exposure on the hypothalamic–pituitary–adrenal axis revealed sexually dimorphic responses within hypothalamic–pituitary–adrenal axis effectors in rats exposed to delta-9-THC in utero. Female rats demonstrated increased expression of corticotrophin-releasing hormone in the medial basal hypothalamus and increased plasma corticosterone levels as adults, whereas male rats demonstrated decreased levels.

Following low-dose delta-9-THC exposure, females showed elevated basal levels of both adrenocorticotrophic hormone and corticosterone compared to controls, and males demonstrated no significant change or lower levels. Adrenocorticotrophic hormone increased in both groups after place preference testing, more significantly in males. Corticosterone levels were also increased in females while decreased in males, and place preference testing led to a significant increase in males but a blunted response in females.

It is hypothesized that because a similar hormonal and response pattern was seen in rats exposed to prenatal stress, delta-9-THC exposure may have led to maternal hypothalamic–pituitary–adrenal axis elevation. While a precise mechanism has not been described, fetal exposure to *Cannabis*, as with other drugs of abuse, can impact gene expression in select brain regions triggered most likely by select alterations in select histone modifications leading to long-lasting changes in gene expression, which thus could represent a potential mechanism for the persistent effects of exposure.

Somatometric Alterations and Risk Factors

In a prospective observational study of neonates with and without clinical suspicion of prenatal exposure to drugs of abuse and the analysis of meconium using standard chromatographic techniques, univariate and multivariate statistical analyses were

performed. Three hundred and seventy-two neonates were involved. Exposure to drugs was detected in 49 (13.2%) cases: in 41 (83.7%) cases, one drug and in 8 (16.3%) cases, more than one.

Somatometric alterations and risk factors can help in the early detection of neonates exposed to drugs. The somatometric alterations identified can be useful to extend the differential diagnosis of the alterations and to study their causes. Somatometry at birth revealed: (a) lower length percentile in those exposed to some drug, more than one and *Cannabis* and (b) lower weight percentile in those exposed to *Cannabis* than those exposed to alcohol. In neonates older than 34 weeks of pregnancy: (a) lower length percentile in those exposed to any substance and (b) lower percentile of length and weight in those exposed to more than one. The most clinically relevant independent risk factors useful to detect cases of prenatal exposure to drugs were (odds ratio (95% CI)): the reason for admission other than prematurity (5.52 (2.55–1.93)), length percentile less than 33 (1.95 (1.05–3.60) and 2.14 (1.04–3.40)) in older than 34 weeks of pregnancy and social dystocia/uncontrolled pregnancy in older than 34 weeks pregnancy (4.47 (1.03–19.29)).

Child Maltreatment and Its Association with *Cannabis* Use

To investigate whether child maltreatment is associated with lifetime *Cannabis* use, early-onset *Cannabis* use, daily *Cannabis* use, and DSM-IV *Cannabis* abuse in young adulthood; or behavioral problems, tobacco use, and alcohol use at the age of 14 years are associated with *Cannabis* use, a birth cohort, using linked government agency child protection data to define exposure to child maltreatment, was conducted [76].

Of the original cohort of 7223 mother and child pairs obtained from consecutive presentations for prenatal care at a hospital serving a cross-section of the community, 3778 (52.3%) of the young people participated at the age of 21. Substantiated government agency reports established exposure to child maltreatment. *Cannabis* outcomes were by self-report questionnaire and Composite International Diagnostic Interview-Auto at age 21. Associations were adjusted for a range of potential confounders. An additional adjustment was carried out for variables measured at age 14—youth behavioral problems [Achenbach Child Behavior Checklist (CBCL)], tobacco use, and alcohol use.

After adjustment, substantiated child maltreatment was associated with any lifetime *Cannabis* use [odds ratio (OR) = 1.60, 95% confidence interval (CI) = 1.08–2.39], *Cannabis* use prior to age 17 (OR = 2.47, 95% CI = 1.67–3.65), daily *Cannabis* use (OR = 2.68, 95% CI = 1.49–4.81), and DSM-IV *Cannabis* abuse/dependence (OR = 1.72, 95% CI = 1.07–2.77). Children who are documented as having been maltreated are more likely to become *Cannabis* users before the age of 17, use *Cannabis* as an adult, use *Cannabis* daily, and meet DSM-IV criteria for *Cannabis* dependence. Externalizing behavior and tobacco and alcohol use at age 14 was associated significantly with almost all *Cannabis* outcomes ($p < 0.05$), with

internalizing behavior associated inversely ($p < 0.05$). Externalizing behavior in adolescence appears partly to mediate the association with adult *Cannabis* use.

Prenatal *Cannabis* Exposure and Transport-Related Injuries

The extent to which traffic injuries may be attributed to parents who use *Cannabis* before driving and whether prenatal *Cannabis* use disorders may predict future road traffic injuries in children were examined. A cohort study of 792,082 children in Quebec, Canada, with 6,280,663 follow-up between 2006 and 2019, was conducted. The main exposure measure was maternal *Cannabis* use disorder before or during pregnancy [30].

The main outcome measure was future hospitalizations for transport-related injuries in children after birth. Using Cox proportional hazards regression models adjusted for potential confounders, the hazard ratios and 95% confidence intervals (CIs) for the association of prenatal *Cannabis* use disorders with transport-related injuries in children were estimated. Maternal *Cannabis* use disorders before birth may be an early predictor of childhood injuries from motor vehicle crashes. Maternal *Cannabis* use disorders before birth were associated with 5.64 times the risk of hospitalization for future motor vehicle crash injuries in children (95% CI = 2.61–12.21). The risk increased with the child's age. Prenatal cocaine, opioid, and other drug use disorders were not associated with pediatric transport-related injuries.

Postpartum Exposure to *Cannabis* and Tobacco on Immune Function

Secretory Immunoglobulin A (SIgA) plays a critical role in immune functioning by preventing pathogens from adhering to epithelial mucosa. Most infectious agents enter the body via mucosal surfaces; thus, SIgA defends against respiratory, intestinal, and urogenital infections, periodontal disease, and caries. A study examined the possibility that pre- and postnatal exposure to cigarettes and *Cannabis* is associated with individual differences in Secretory Immunoglobulin A levels in early childhood [43, 45]. The participants were 50 mother/infant (29 boys) dyads recruited at their first prenatal appointment at a northeastern community hospital in the United States. Repeated assessments of pre- and postnatal cigarettes and *Cannabis* were conducted beginning in the first trimester of pregnancy, using multiple methods (i.e., saliva, meconium, self-report). Infants were grouped into those prenatally exposed to either cigarette only ($n = 19$), cigarette and *Cannabis* ($n = 19$), or with no prenatal substance exposure ($n = 12$). At age 5, the children's saliva was collected and assayed for SIgA.

There were group differences in SIgA levels as a function of prenatal exposure to cigarettes and *Cannabis*. Children in the cigarette-only and the cigarette and *Cannabis* groups had higher SIgA levels than the non-exposed children. Children who experienced the combination of postnatal exposure to cigarettes and *Cannabis* had higher levels of SIgA, even after accounting for prenatal exposures and other covariates relevant to immune system functioning. Prenatal and postnatal exposure to cigarettes and *Cannabis* may be associated with hyperactivity of mucosal immunity in early childhood. Prenatal and postnatal exposure-related changes in mucosal immunity may partially explain links between cigarette and *Cannabis* exposure and health problems in early childhood.

Fetal exposure to *Cannabis* and tobacco during pregnancy leads to adverse fetal and childhood outcomes. The associations of parental *Cannabis* and tobacco use during pregnancy with childhood hair cortisol and cortisone concentrations at 6 years as biomarkers of long-term HPA-axis functioning were examined in a population-based prospective birth cohort among 2577 mothers and their children [46]. Cortisol and cortisone were measured in hair samples at 6 years. Questionnaires collected information on parental *Cannabis* and tobacco use, and maternal urine samples were analyzed to detect *Cannabis* metabolite concentrations. Linear regression analysis with adjustment for several confounders was used to test the hypothesis.

Compared to non-exposed children, offspring exposed to *Cannabis* during pregnancy (in combination with tobacco) had higher childhood cortisol concentrations (log-10 transformed difference 0.16, 95% CI = 0.04–0.28). This association was not mediated by birth weight. No differences in cortisone concentrations among *Cannabis*-exposed children were observed. Maternal tobacco use during pregnancy was not associated with childhood cortisol or cortisone concentrations. Further, paternal *Cannabis* or tobacco use was not associated with childhood cortisol or cortisone concentrations. The findings suggest that maternal *Cannabis* use, combined with tobacco, during pregnancy is associated with alterations in offspring HPA-axis functioning.

The association between a *Cannabis*-related diagnosis (CRD) in pregnancy and adverse maternal and infant outcomes was evaluated [47]. An administrative birth cohort of singleton deliveries linked to maternal and infant hospital discharge records was queried. The pregnancies were classified with CRD from the International Classification of Disease codes. Nicotine and other substance-related diagnosis (SRD) in the same manner were identified. Outcomes of interest included maternal (hypertensive disorders) and infant (prematurity, small for gestational age, NICU admission, major structural malformations) adverse outcomes.

From 3,067,069 pregnancies resulting in live births, 29,112 (1.0%) had a CRD. The *Cannabis*-related diagnosis was associated with an increased risk of all outcomes studied; the strongest risks observed were for very preterm birth (aRR 1.4, 95% CI = 1.3, 1.6) and small for gestational age (aRR 1.4, 95% CI = 1.3, 1.4). When analyzed with or without co-exposure diagnoses, CRD alone conferred an increased risk for all outcomes compared to no use. The strongest effects were seen for CRD with other SRD (preterm birth aRR 2.3, 95% CI = 2.2, 2.5; very preterm birth aRR 2.6, 95% CI = 2.3, 3.0; gastrointestinal malformations aRR 2.0, 95%

CI = 1.6, 2.6). The findings were generally robust to unmeasured confounding and misclassification analyses. *Cannabis*-related diagnosis in pregnancy was associated with an increased risk of adverse maternal and infant outcomes.

Cannabis use is increasing among women of reproductive age, warranting an understanding of the impact of prenatal *Cannabis* use on offspring developmental outcomes. Tests were conducted to determine the potential relationship between prenatal *Cannabis* use disorders (CUDs) and offspring educational outcomes across primary and secondary school. Data were drawn from the New South Wales (NSW) Perinatal Data Collection, which included all live births in the Australian state of NSW. The data were linked with the NSW Admitted Patient Data Collection for mothers and offspring and the NSW National Assessment Program-Literacy and Numeracy (NAPLAN).

The study population was 189,558 offspring who completed the NAPLAN in grades 3, 5, and 7, resulting in 568,674 examination periods [48]. The exposure variable was ICD-10 *Cannabis* use disorders (CUD = F13.0–F13.9). The study included five outcome variables measured at three time points as not meeting the minimum national standards for numeracy, reading, spelling, writing, grammar, and punctuation.

In unadjusted analyses, prenatal CUD was associated with an increased risk of not meeting the national minimum standard of all outcomes [odds ratios (OR) ranging from 3.42 (95% confidence interval (CI) = 2.94, 3.99) to 4.17 (95% CI = 3.55, 4.91)], with no evidence for interaction across time. However, the associations attenuated greatly after exact matching by covariates, with reading and numeracy no longer associated with prenatal CUD, while the increased risk of the other outcomes ranged from OR = 1.31 (95% CI = 1.09, 1.57) to OR = 1.40 (95% CI = 1.17, 1.68). Socioeconomic status appears to confound the association between prenatal *Cannabis* use disorder and poor educational performance in offspring.

The rise in *Cannabis* use is mirrored by increasing global legalization and the overall perceptions of safety, even during pregnancy. Clinical reports of *Cannabis* use prevalence during pregnancy vary widely from 3% to upwards of 35% in North America; the disparity is likely owing to underestimates from self-reporting. The trends are further compounded by a lack of evidence-based policy and guidelines for prenatal *Cannabis* use, which has led to inconsistent messaging by healthcare providers and medically licensed *Cannabis* dispensaries regarding prenatal *Cannabis* use for treating symptoms such as nausea.

Additionally, the use of *Cannabis* to self-medicate depression and anxiety during pregnancy is a growing medical concern. A review summarized findings of clinical and preclinical data on neonatal outcomes and long-term physiological and neurodevelopmental outcomes of prenatal *Cannabis* exposure [49].

Although many of the outcomes under investigation have produced mixed results, we consider the data in light of the unique challenges facing *Cannabis* research. In particular, the limited longitudinal clinical studies available have not previously accounted for the exponential increase in (-)-delta-9-tetrahydrocannabinol concentrations found in *Cannabis* over the past two decades. Polydrug use and the long-term effects of individual *Cannabis* constituents, delta-9-THC versus

cannabidiol, are also understudied, along with sex-dependent outcomes. Despite the limitations, prenatal *Cannabis* exposure has been linked to low birth weight.

Emerging evidence suggests that prenatal exposure to delta-9-THC, which crosses the placenta and impacts placental development, may have wide-ranging physiological and neurodevelopmental consequences. The long-term effects of the changes require more rigorous investigation, though early reports suggest delta-9-THC increases the risk of cognitive impairment and neuropsychiatric disease, including psychosis, depression, anxiety, and sleep disorders.

Eye Disorders in Infants Prenatally Exposed to Opioids, Cocaine, and *Cannabis*

Prenatal substance exposure is associated with abnormal visual evoked potentials in offspring, but whether ocular abnormalities are present or past infancy is unclear. The association between prenatal substance exposure and hospitalizations for eye disorders in childhood has been examined. A longitudinal cohort study of 794,099 infants born between 2006 and 2016 in all hospital centers in Quebec, Canada, was conducted [45].

Infants prenatally exposed to opioids, cocaine, *Cannabis*, and other illicit substances were followed over time to assess eye disorders that required in-hospital treatment, including retinal detachment and breaks, strabismus, and other ocular pathologies. Incidence rates and hazard ratios (HR) with 95% confidence intervals (CIs) for the association of prenatal substance exposure with the risk of eye disorders, adjusted for patient characteristics, were calculated. Infants exposed to substances prenatally had a higher incidence of hospitalizations for eye disorders than unexposed infants (47.0 vs. 32.0 per 10,000 person-years). Prenatal substance exposure was 1.23 times the risk of hospital admission for any eye disorder during childhood compared with no exposure (95% CI = 1.04–1.45). Risks were greatest for strabismus (HR 1.55, 95% CI = 1.16–2.07) and binocular movement disorders (HR 1.96, 95% CI = 1.00–3.83). Opioid use was strongly associated with the risk of ocular muscle disorders (HR 3.15, 95% CI = 1.98–5.01). Prenatal substance exposure is significantly associated with future hospitalizations for eye disorders in childhood.

Retinal Development

Although the retina is part of the central nervous system, the impact of maternal *Cannabis* use on retinal development and its postnatal consequences can be of concern. The prenatal period is potentially sensitive to the normal development of the retina. Therefore, it hypothesized that the recreational use of *Cannabis* during pregnancy might alter the structure of the retina in the offspring [68].

A murine model mimics human exposure in terms of dose and use was developed. Pregnant BalbC mice were exposed daily for 5 min to *Cannabis* smoke (0.2 g of *Cannabis*) or filtered air from gestational day 5–18 ($n = 10/\text{group}$). After the weaning period, the pups were separated and examined weekly. On days 60, 120, 200, and 360 after birth, ten pups from each group were randomly selected for Spectral Domain Optical Coherence Tomography analysis of the retina. The retinal layers were measured, and inner, outer, and total retina thickness were calculated. Other 37 mice from both groups were sacrificed on days 20, 60, and 360 for retinal stereology (total volume of the retina and volume fraction of each retinal layer) and light microscopy. Means and standard deviations were calculated, and multivariate analysis of variance was performed.

Gestational exposure to *Cannabis* smoke may cause structural changes in the retina of the offspring that return to normal in mice adulthood. The retina of the animals whose mothers were exposed to *Cannabis* during gestation was 17% thinner on day 120 (young adult) than controls ($p = 0.003$) due to 21% thinning of the outer retina ($p = 0.001$). The offspring of mice from the exposed group presented thickening of the IS/OS compared to controls on day 200 ($p < 0.001$). In the volumetric analysis by retinal stereology, the exposed mice presented a transitory increase of the IS/OS total volume and volume fraction on day 60 (young adult) compared to controls ($p = 0.008$ and 0.035 , respectively). On light microscopy, exposed mice presented thickening of the IS/OS on day 360 (adult) compared to controls ($p = 0.03$) [69]. The experimental evidence indicates that children and young adults whose mothers smoked *Cannabis* during pregnancy may require earlier and more frequent clinical care than the non-exposed population.

Adverse Neonatal Outcomes

A study was conducted with 3207 respondents from the Colorado Pregnancy Risk Assessment Monitoring System with state-developed questions on *Cannabis* use. The study evaluated the prevalence of prenatal and early postnatal *Cannabis* use. Differences in perinatal *Cannabis* use were evaluated according to maternal characteristics, breastfeeding patterns, and pregnancy intendedness. Multiple logistic regression models evaluated the relationship between prenatal *Cannabis* use and adverse neonatal outcomes, including low birth weight, small for gestational age, preterm birth, and admission to the neonatal intensive care unit.

The self-reported prevalence of *Cannabis* use at any time during pregnancy was $5.7 \pm 0.5\%$, and the prevalence of early postnatal *Cannabis* use among women who breastfed was 5.0% . Prenatal *Cannabis* use was associated with a 50% increased likelihood of low birth weight, independent of maternal age, race/ethnicity, level of education, and tobacco use during pregnancy [12]. Small for gestational age, preterm birth, and neonatal intensive care unit admission were not associated with prenatal *Cannabis* use, independent of prenatal tobacco use.

Birth Weight

To investigate the association between *Cannabis* use during pregnancy and birth outcomes, data from the Norwegian Mother and Child Cohort Study were used in a prospective pregnancy cohort [54, 55]. Nine thousand, three hundred, and twelve women with 10,373 pregnancies who reported *Cannabis* use before or during pregnancy were recruited. The women reported their illegal drug use before pregnancy, at weeks 17, 18, and 30, and 6 months postpartum. Linear regression was used to estimate crude and adjusted effects of prenatal *Cannabis* exposure on birth outcomes. In 10,101 pregnancies, *Cannabis* was used before but not during pregnancy. In 272 pregnancies, *Cannabis* was used during pregnancy; among them, in 63 pregnancies, the women had used *Cannabis* in at least two periods.

In adjusted analyses for potential confounders, only *Cannabis* use during at least two periods of pregnancy showed statistically significant effects on birth weight. The effect was observed in the complete cohort ($B = -228$ g, 95% CI = -354 to -102 , $p < 0.001$) and for the subgroup where information about the child's father was available ($B = -225$ g, 95% CI = -387 to -63 , $p = 0.01$). The results indicate that prolonged use causes more harm, whereas short-term use did not indicate adverse effects on birth outcomes. There was a statistically significant and clinically relevant association between the use of *Cannabis* during pregnancy and reduced birth weight. Clinicians should screen not only for *Cannabis* use but also for the length and intensity of use as part of a comprehensive substance use screening.

A study estimated the association between *Cannabis* use during pregnancy and preterm birth. *Cannabis* use was assessed by questionnaire, record abstraction, and urine toxicology [16]. The women received ante-natal care from 2010 to 2015 and were observed through the end of pregnancy. Relative risks were assessed with Poisson regression and time to delivery with proportional hazard models. Of the 363 eligible women, 119 (33%) used *Cannabis* in pregnancy by at least one measure. In the cohort study, preterm birth occurred in 36.0% of the users and 34.6% of the non-users ($p = 0.81$), indicating that preterm birth was not significantly reduced before 0.52 (95% CI = 0.22–1.23) and after 0.75 (95% CI = 0.29–2.15) adjustment.

The unadjusted relative risk of all preterm birth was 1.06 (95% confidence interval [CI] = 0.76–1.47); the adjusted relative risk was similar at 1.04 (95% CI = 0.72–1.50). Spontaneous preterm birth was not significantly elevated among users before 1.32 (95% CI = 0.89–1.96) and after 1.21 (95% CI = 0.76–1.94) adjustment. The unadjusted hazard ratio (HR) for time to preterm birth was 1.26 (95% CI = 0.84–2.00); the adjusted HR was 1.32 (95% CI = 0.80–2.07). Both unadjusted 1.77 (95% CI = 1.06–2.93) and adjusted 2.16 (95% CI = 1.16–4.02) HRs for spontaneous preterm birth were significantly elevated, primarily due to an increased risk of spontaneous birth <28 weeks among users.

The unadjusted and adjusted HRs for time to indicate preterm birth were 0.69 (95% CI = 0.33–1.43) and 0.58 (95% CI = 0.23–1.46). *Cannabis* use was not associated with total preterm birth in the study, indicating that among women already at high risk of preterm birth, *Cannabis* does not increase the risk further. However,

there was an indication that pregnant women who use *Cannabis* may deliver earlier, particularly from spontaneous preterm birth, than women who do not use *Cannabis*.

The effects of *Cannabis* on preterm birth have found mixed results, in part of the lack of attention to the role of maternal tobacco smoking during pregnancy. An investigation was conducted to determine whether maternal *Cannabis* use was independently associated with gestational age, preterm birth, and two preterm birth subtypes (spontaneous vs. clinician-initiated) [17].

A study of 8261 mother–newborn pairs was assessed through a questionnaire after birth. Information on gestational age was collected from electronic medical records. *Cannabis* use and tobacco smoking during pregnancy were evaluated. Linear and log-linear regression models were used to assess associations between *Cannabis* use with and without tobacco smoking during pregnancy and the outcomes. Of the 8261 mothers, 27.5% had preterm births. About 3.5% of mothers with term deliveries and 5.2% with preterm births used *Cannabis* during pregnancy.

Cannabis use and cigarette smoking were independently associated with a decrease in gestational age by 0.50 weeks (95% confidence interval [CI] = −0.87, −0.13) and 0.52 weeks (95% CI = −0.76, −0.28), respectively. *Cannabis* use during early or late pregnancy was associated with a similar decrease in gestational age by 0.50 weeks. When the effects on the preterm birth subtypes were examined, simultaneous *Cannabis* use and tobacco smoking were associated with a higher risk of spontaneous preterm birth (RR 1.64, 95% CI = 1.23, 2.18). The elevated risk was not observed with clinician-initiated preterm birth. In the high-risk US population, maternal *Cannabis* use and cigarette smoking during pregnancy were independently associated with shorter gestational age. The effects on preterm birth subtypes indicated that the elevated risk was only observed with spontaneous preterm birth.

A population-based retrospective cohort study was conducted to determine whether there are associations between self-reported prenatal *Cannabis* use and adverse maternal and perinatal outcomes. The study includes live births and stillbirths among women aged 15 years and older. Self-reported *Cannabis* exposure in pregnancy was ascertained through routine perinatal care.

The primary outcome was preterm birth before 37 weeks gestation. Indicators were defined for birth occurring at 34–36 6/7 weeks gestation (late preterm), 32–33 6/7 weeks gestation, 28–31 6/7 weeks gestation, and less than 28 weeks preterm birth. Ten secondary outcomes were examined, including small for gestational age, placental abruption, transfer to neonatal intensive care, and 5-min Apgar score. Coarsened matching techniques and Poisson regression models were used to estimate the risk difference (RD) and relative risk (RR) of outcomes associated with *Cannabis* exposure and control for confounding.

In a cohort of 661,617 women, the mean gestational age was 39.3 weeks, and 51% of the infants were male [39]. The mothers had a mean age of 30.4 years, and 9427 (1.4%) reported *Cannabis* use during pregnancy. Imbalance in measured maternal obstetrical and sociodemographic characteristics between reported *Cannabis* users and non-users was attenuated using matching, yielding a sample of 5639 reported users and 92,873 non-users.

The crude rate of preterm birth less than 37 weeks gestation was 6.1% among women who did not report *Cannabis* use and 12.0% among those reporting use in the unmatched cohort (RD, 5.88% [95% CI = 5.22%–6.54%]). In the matched cohort, reported *Cannabis* exposure was significantly associated with an RD of 2.98% (95% CI = 2.63%–3.34%) and an RR of 1.41 (95% CI = 1.36–1.47) for preterm birth. Compared with no reported use, *Cannabis* exposure was significantly associated with greater frequency of small for gestational age (third percentile, 6.1% vs. 4.0%; RR, 1.53 [95% CI = 1.45–1.61]), placental abruption (1.6% vs. 0.9%; RR, 1.72 [95% CI = 1.54–1.92]), transfer to neonatal intensive care (19.3% vs. 13.8%; RR, 1.40 [95% CI = 1.36–1.44]), and 5-min Apgar score less than 4 (1.1% vs. 0.9%; RR, 1.28 [95% CI = 1.13–1.45]). Pregnant women reported that *Cannabis* use was significantly associated with an increased risk of preterm birth. The findings may be limited by residual confounding.

Neonatal Withdrawal Syndrome

Neonatal abstinence syndrome is defined as drug withdrawal symptoms experienced by a neonate upon birth due to maternal drug use during pregnancy. Symptoms can include irritability, poor sucking, hypertonia, low birth weight, and seizures [73]. Neonatal abstinence syndrome occurs most commonly with maternal opioid use but can occur with other substances such as cocaine, *Cannabis*, and amphetamines (including crystal methamphetamine).

Maternal drug abuse, both licit and illicit, has been growing. Pregnant persons who abuse drugs and their children are often vulnerable and require extra care and treatment. Mothers who use methamphetamines during pregnancy tend to be younger, have lower levels of education, and lower socioeconomic status, are more likely to drink and smoke during pregnancy, and have less prenatal care before childbirth. Care for neonatal abstinence syndrome due to opioids usually includes non-pharmacological and pharmacological treatments, but standardization of care is difficult as withdrawal symptoms may differ between neonates.

Cannabis During Lactation

The prevalence of *Cannabis* use during pregnancy and postpartum has been estimated at 5% of the population [15]. Women who use *Cannabis* during lactation expose their infants to the risk of *Cannabis* and its metabolites in breast milk. A study of women's infant outcomes associated with *Cannabis* use during lactation followed by clinical recommendations was conducted to address the impact of postpartum *Cannabis* use by lactating women and developmental outcomes for infants.

The results indicated conflicting outcomes regarding the risk of exposure to *Cannabis* in breast milk and recommended that women be advised to abstain from

Cannabis use during lactation or reduce consumption if abstinence is impossible. Furthermore, women should avoid breastfeeding within 1 h of inhaled use to reduce exposure to the highest concentration of *Cannabis* in breast milk, despite some evidence regarding the health risks of postnatal exposure to *Cannabis*.

Adult Working Memory

The associations between developmental patterns of *Cannabis* use and adult working memory processes were examined in 75 adults with longitudinal assessments of *Cannabis* use (60 with reported use, 15 with no reported use) and prenatal drug exposure assessment completed a spatial working memory task during fMRI at age 28. All participants passed a multi-drug urine screen on the day of testing and denied recreational drug use in the past week. A fast event-related design with partial trials was used to separate the BOLD response associated with the working memory task's encoding, maintenance, and retrieval periods [72].

Behavioral results indicated that the participants who began using *Cannabis* earlier in adolescence had longer reaction times than those with later initiation. *Cannabis* age of onset was further associated with reduced posterior parietal cortex encoding BOLD activation, which significantly mediated age of onset working memory reaction times associations. However, *Cannabis* age-of-onset brain-behavior associations did not differ between groups with single reported use and those with repeated use, indicating the age-of-onset effects may reflect substance use risk characteristics rather than a developmentally timed *Cannabis* exposure effect.

Within repeated *Cannabis* users, greater levels of total *Cannabis* use were associated with performance-related increases in dorsolateral prefrontal cortex activation during maintenance. The pattern of significant results remained unchanged with the inclusion of demographic and prenatal measures as covariates. However, at the group level, *Cannabis* users generally performed better than participants who reported never using *Cannabis* (faster reaction times, higher accuracy). Previous investigations were extended by identifying that working memory associations with *Cannabis* age of onset may be primary to posterior parietal cortex stimulus encoding activity. At the same time, the amount of *Cannabis* use is associated with dorsolateral prefrontal cortex maintenance processes.

Cannabinoid Hyperemesis Syndrome

The obstetrical care provider should be aware of cannabinoid hyperemesis syndrome. Cannabinoid hyperemesis syndrome can be challenging to differentiate from hyperemesis gravidarum. Cannabinoid hyperemesis syndrome should be considered in patients with hyperemesis gravidarum and a history of chronic *Cannabis* use [6]. A history of chronic *Cannabis* use characterizes cannabinoid hyperemesis

syndrome, acute-onset nausea, vomiting, and abdominal pain; symptoms alleviated by hot showers and episodes lasting 24–48 h.

***Cannabis*, Tobacco, Alcohol, and Cocaine**

Cannabis, tobacco, alcohol, and cocaine are the most consumed psychoactive drugs, and prenatal exposure could alter normal fetal development and threaten future welfare. The main changes observed in prenatal exposure to tobacco are caused by nicotine and carbon monoxide, which can impede nutrient and oxygen exchange between mother and fetus, restricting fetal growth. Memory, learning processes, hearing, and behavior can also be affected. Alcohol may cause physical and cognitive alterations in prenatally exposed infants, fundamentally caused by altered NMDAR and GABAR activity. Delta-9-tetrahydrocannabinol can activate CB1R, inducing connectivity deficits during fetal brain development, which can be linked to behavioral and cognitive deficits [58]. The effects of prenatal cocaine exposure are caused by altered cell proliferation, migration, differentiation, and dendritic growth processes. Cocaine causes long-term behavioral and cognitive alterations and affects the uteroplacental unit.

Research on prenatal cocaine exposure indicates that exposed children experienced behavioral dysregulation resulting in risky adolescent behavior, including earlier initiation of *Cannabis* use and sexual intercourse. A study examined the long-term effects of prenatal cocaine exposure on adult sexual behavior. The prospective cohort study of the association between prenatal cocaine exposure and risky adult sexual behavior and sexually transmitted infections (STIs) in 202 young adults (mean age = 27, SD = 0.98 years) was examined [60]. Data from the study's prenatal, childhood, and adolescent phases were used to delineate pathways from prenatal cocaine exposure to adult sexual behavior. The most common risky sexual behavior was having sex while drunk or high (63%).

Evidence for an indirect pathway from prenatal cocaine exposure to adult sex while drunk or high via early *Cannabis* initiation was revealed. One-third of the sampled population reported that they “almost always” had sex while drunk or high. There were no other effects of prenatal cocaine exposure on adult risky sexual behavior or on risk for STIs after controlling for sex assigned at birth, race, age at sexual initiation, and family history of drug and alcohol problems.

Although prenatal cocaine exposure has been associated with earlier initiation of sex in prior studies, prenatal cocaine exposure was not directly associated with risky adult sex or a history of STI. Exposed individuals were at greater risk of sex under the influence of alcohol or drugs via earlier initiation of *Cannabis* use during adolescence.

Prenatal cocaine/polydrug exposure (PCE) may increase vulnerability to substance use disorders due to associated cognitive deficits. Whether neurocognitive deficits in executive functions and attention observed in PCE children persisted to adolescence compared to non-cocaine/polydrug (NCE) children and whether

adolescent substance use (tobacco, alcohol, marijuana) was also associated with neurocognitive deficits were examined [61].

Three hundred and fifty-four (180 PCE, 174 NCE) adolescents in a longitudinal study from birth were administered the Wechsler Intelligence Scales for Children – IV and the Integrated Visual and Auditory Continuous Performance Test at age 15.5. Assessments of prenatal exposure to cocaine, alcohol, *Cannabis*, and tobacco and measures of use at age 15.5 years were taken. Confounding factors measured included lead, the caregiving environment, and violence exposure.

Relationships between drug use and prenatal exposures on outcomes were assessed through multiple regressions. Adolescents with PCE had deficits in perceptual reasoning, IQ, and visual attention. Prenatal alcohol exposure predicted verbal and working memory IQ and visual and auditory attention deficits. Adolescent tobacco, alcohol, and *Cannabis* use predicted attention in addition to PCE, lead, and the caregiving environment. Prenatal cocaine and alcohol exposure and adolescent use of substances are associated with neurocognitive deficits known to increase vulnerability to SUDs.

In two Copenhagen University hospitals, 12,885 pregnant women answered questionnaires regarding the consumption of alcohol, tobacco, *Cannabis*, and other drugs [77]. The prevalence of *Cannabis* use was 0.8%. Women using *Cannabis* but no other illicit drugs were each retrospectively matched with four randomly chosen pregnant women in the same period and the same age group and with the same parity. Eighty-four *Cannabis* users were included. The women were socioeconomically disadvantaged and had a higher prevalence of present and past use of alcohol, tobacco, and other drugs.

No significant difference in pregnancy, delivery, or puerperal outcome was found. Children of women using *Cannabis* were 150 g lighter, 1.2 cm shorter, and had 0.2 cm smaller head circumference than the control infants. Controlling for the child's sex and maternal use of alcohol did not eliminate the significant differences in birth weight and length; however, they were eliminated by controlling for maternal tobacco smoking. It is concluded that the use of *Cannabis* is not a major prognostic factor regarding the outcome of pregnancy but is an indicator of low socioeconomic status and the use of other substances.

Modes of *Cannabis* Administration

A study examined self-reported modes of *Cannabis* administration among women the year before and during pregnancy and their association with self-reported *Cannabis* use frequency [68]. Data from 585 women were screened for *Cannabis* use during standard prenatal care in two medical centers in California. *Cannabis* use was 12% before pregnancy and 3% during pregnancy.

Among the 71 women who reported *Cannabis* use before pregnancy and the 19 women who reported *Cannabis* use during pregnancy, smoking was the most common mode of administration (58% and 42%), followed by edibles (27% and 16%),

vaping (23% and 16%), lotions (11% and 5%), and other (10% and 0%). In the year before pregnancy and during pregnancy, monthly or less use was most common (56% and 58%), followed by weekly use (24% and 26%) and daily use (20% and 16%).

The results indicate that while smoking is the most common mode of *Cannabis* administration during the perinatal period, there is variation in the use and co-use of alternative modes. Among *Cannabis* users, 43% used more than one mode before pregnancy compared to 15% during pregnancy. Daily *Cannabis* use was most common among women who reported smoking only or in combination with other modes.

Stressful Life Events and *Cannabis* Use

Using data from the Pregnancy Risk Assessment Monitoring System (PRAMS), a cross-sectional, population-based surveillance system and mailed and telephoned surveys were conducted in five states – Alaska, Colorado, Maine, Michigan, and Washington – in the United States to determine the association between stressful life events (SLEs) in the year prior to childbirth with pre-pregnancy *Cannabis* use, cessation of *Cannabis* use during pregnancy, and postpartum relapse to *Cannabis* use. The participants were 6061 women who delivered a live infant within the last 6 months and had data on *Cannabis* use.

Self-reported data included SLEs (yes/no response for 14 individual events in the 12 months prior to childbirth) and *Cannabis* use [yes/no prior to pregnancy, during pregnancy, and at the time of the survey (approximately 2–6 months postpartum)]. The associations between SLEs and *Cannabis* use (primary outcomes) were examined in logistic regression models adjusted for maternal demographics (e.g., age and education), geography (i.e., state of residence), and cigarette smoking [79].

For pre-pregnancy, 16.4% (997/6061) of the respondents endorsed using *Cannabis*, with 36.4% (363/997) continuing *Cannabis* use during pregnancy. Among the 63.6% (634/997) did not report use during pregnancy, 23.2% (147/634) relapsed to *Cannabis* use postpartum. Nine of the 14 possible SLEs were associated with increased odds of pre-pregnancy *Cannabis* use [e.g., husband/partner or mother went to jail, adjusted odds ratio (aOR) = 2.16, 95% confidence interval (CI) = 1.30–3.62] and four were associated with increased odds of continued *Cannabis* use during pregnancy (e.g., husband/partner lost job, aOR = 2.19, 95% CI = 1.21–3.96).

The odds of postpartum relapse to *Cannabis* were significantly associated with two SLEs (husband/partner said they did not want pregnancy, aOR = 2.86, CI = 1.10–7.72; husband/partner or mother went to jail, aOR = 0.37, 95% CI = 0.13–1.00). Stressful life events during the year before childbirth appear to be linked to greater odds of women's *Cannabis* use during the perinatal period, especially during pre-pregnancy.

The Demographics of *Cannabis* Consumption

A study determined the prevalence and correlation of *Cannabis* consumption among pregnant individuals following the national legalization of *Cannabis*. For May to October 2019, an anonymous cross-sectional survey was distributed to pregnant patients attending family practice, midwifery, and low-risk and high-risk obstetrics clinics in Hamilton, Ontario [36]. Eligibility was based on English literacy and pregnancy status.

The survey included questions regarding lifetime and in-pregnancy *Cannabis* use, intent for postpartum use, and patterns of use. Demographic information was also collected. Descriptive statistics and logistic regression analyses were conducted to explore the relations between *Cannabis* consumption and demographic characteristics. Of 531 pregnant individuals approached, 478 agreed and were able to participate, for a 90% participation rate. Among the 478 respondents, 54 (11%) reported consuming *Cannabis* at some point during the pregnancy, and 20 (4%) reported consuming *Cannabis* at the time of the survey. Among the 460 respondents who intended to breastfeed, 23 (5%) planned to consume *Cannabis* during the postpartum period. Of 20 current users, 13 (65%) reported consuming *Cannabis* at least weekly, and 19 (95%) reported nausea, sleep problems, or anxiety as reasons for use.

The respondents without postsecondary education had 10.0-fold (95% confidence interval [CI] = 4.6–23.5) greater odds of prenatal *Cannabis* consumption than university-educated respondents. In addition, respondents who reported that their partners used *Cannabis* had 3.9-fold (95% CI = 2.2–7.3) greater odds of prenatal *Cannabis* consumption than those who reported that their partners did not use *Cannabis*. Lower educational attainment and partners' *Cannabis* consumption were associated with greater odds of *Cannabis* use during pregnancy. The results may help to inform early intervention strategies to decrease *Cannabis* consumption during this vulnerable period of fetal and neonatal development.

Cannabis use is common among individuals of reproductive age. Publicly posted questions about perinatal *Cannabis* use and licensed US healthcare provider responses were examined. Data are medical questions on perinatal *Cannabis* use posted online on an anonymous digital health platform. Posters could “thank” healthcare providers for their responses, and providers could “agree” with other providers’ responses. The discussion includes 364 user questions and 596 responses from 277 unique providers and examines endorsement of responses through provider “agrees” and user “thanks.” The most frequent questions concerned prenatal *Cannabis* use detection (24.7%), effects on fertility (22.6%), harms of prenatal use to the fetus (21.3%), and risks of baby exposure to *Cannabis* through breast milk (14.4%).

Provider sentiment regarding perinatal *Cannabis* use was coded as 55.6% harmful, 8.8% safe, 8.8% mixed/unsure, and 26.8% safety unaddressed. Half of the providers (49.6%) discouraged perinatal *Cannabis* use, 0.5% encouraged to use, and 49.9% neither encouraged nor discouraged use. Provider response received 1004 providers “agrees” and 583 users “thanks.” Provider response indicating that

perinatal *Cannabis* use is unsafe received more provider “agrees” than responses indicating that use is safe ($B = 0.42$, 95% CI = 0.02–0.82, $p = 0.04$). User “thanks” did not differ by provider response regarding the safety or dis/encouragement. The data indicate public interest in *Cannabis* use effects before, during, and after pregnancy [59]. While most healthcare providers indicated that *Cannabis* use during pregnancy and breastfeeding is unsafe, many did not address safety or discourage use, indicating a missed educational opportunity.

Using cross-sectional data from the 2016 Pregnancy Risk Assessment Monitoring System, the associations between state *Cannabis* legalization and self-reported maternal *Cannabis* use were examined [40]. Using logistic regression, the adjusted prevalence ratio (PR) of *Cannabis* use during the preconception, prenatal, and postpartum period for women delivering a live-born infant in three states that had legalized recreational *Cannabis* (Alaska, Colorado, and Washington) and three states that had not legalized (Maine, Michigan, and New Hampshire) by 2016 was estimated. The sample size was 7258 women. A 95% confidence interval and a significance level of $\alpha = 0.05$ were utilized.

After adjustment for potential confounders, women who resided in states with legalized recreational *Cannabis* were significantly more likely to use *Cannabis* during the preconception (PR 1.52; 95% CI ranging from 1.28 to 1.80; $p < 0.001$), prenatal (PR 2.21; 95% CI ranging from 1.67 to 2.94; $p < 0.001$), and postpartum (PR 1.73; 95% CI ranging from 1.30 to 2.30; $p < 0.001$) periods, compared to women who resided in states without legalized recreational *Cannabis*. Although evidence about the effect of *Cannabis* use during the periods is nascent, the findings show potential for an increased incidence of child exposure to *Cannabis*.

The Association of Prenatal Substance Use with Prenatal and Postpartum Care

Data from a Pregnancy Risk Assessment Monitoring System for nine states were used to estimate the association of prenatal substance use with prenatal and postpartum care [87]. Adjusted linear regression was conducted to compare the month of gestation of the first prenatal visit and adjusted logistic regression to compare receipt of adequate prenatal care and a postpartum healthcare visit among women who reported prenatal use of any opioids, prescription opioids, *Cannabis*, and illicit drugs to those who did not report the use of each substance.

Women who reported prenatal use of any opioids (RD = 0.16; 95% CI = 0.04, 0.28), prescription opioids (RD = 0.16; 95% CI = 0.04, 0.28), *Cannabis* (RD = 0.25; 95% CI = 0.10, 0.41), and illicit drugs (RD = 0.52; 95% CI = 0.09, 0.95) initiated prenatal care later than those who did not report the use of each substance.

Women who reported prenatal use of any opioid (OR = 0.82; 95% CI = 0.68, 1.00), prescription opioids (OR = 0.84; 95% CI = 0.69, 1.02), *Cannabis* (OR = 0.59; 95% CI = 0.48, 0.73), and illicit drugs (OR = 0.29; 95% CI = 0.20, 0.42) were less

likely to receive adequate prenatal care. Women who reported prenatal use of any opioid (OR = 0.82, 95% CI = 0.65, 1.04), prescription opioids (OR = 0.83; 95% CI = 0.66, 1.05), *Cannabis* (OR = 0.65; 95% CI = 0.51, 0.82), and illicit drugs (OR = 0.47; 95% CI = 0.30, 0.72) had a lower likelihood of a postpartum visit than those who did not report the use of each substance. The results indicate the potential intervention points for pregnant women who use substances.

Patterns of Maternal *Cannabis* Use Predicted Offspring Substance Use

A study was conducted to model maternal patterns of *Cannabis* use from 1-year pre-pregnancy to 16 years postpartum and to determine if different patterns of maternal *Cannabis* use predicted offspring substance use at age 22 [82]. Women were recruited from a prenatal clinic between 1982 and 1984. Maternal *Cannabis* use was assessed by trained interviewers twice during pregnancy, at delivery, at 8 and 18 months, 3, 6, 10, 14, and 16 years postpartum. At age 22, substance use and dependence were measured in offspring. Growth mixture models of maternal *Cannabis* use were calculated, and adult offspring substance use outcomes were regressed onto maternal *Cannabis* trajectory classes ($n = 551$).

There were five distinct patterns of maternal *Cannabis* use. Offspring of mothers who were chronic *Cannabis* users were more likely to use *Cannabis* ($p < 0.001$) and develop CUD ($p < 0.05$) than offspring whose mothers did not use *Cannabis*. Offspring of chronic *Cannabis* users were also more likely to be nicotine dependent by age 22 than offspring whose mothers did not use *Cannabis* ($p < 0.01$) and more than offspring whose mothers were decreasingly likely to use over time ($p < 0.01$). Integrated variable- and person-centered analyses revealed long-term and meaningful patterns of *Cannabis* use and resistance. Chronic maternal *Cannabis* use is a risk factor for regular and dependent *Cannabis* use and dependent tobacco use among young adult offspring. The findings have implications for maternal-child health, given the increasing prevalence of *Cannabis* use among women.

Encephalograms in Prenatal Exposure to *Cannabis*

A prospective cohort study of prenatally delta-9-THC-exposed newborns determined that prenatal exposure to *Cannabis* results in abnormal amplitude-integrated encephalograms [83]. Maternal and infant demographics, urine, and umbilical cord drug screening were recorded. A limited channel continuous aEEG was obtained within 48 h of birth. Statistical analysis included univariate, multivariate, and logistical regression.

Thirty mother/infant dyads were enrolled. Sixty percent (18/30) of the neonates had abnormal aEEGs with sleep-wake cycle disturbances ($p < 0.001$). Umbilical cord drug screening carboxy-delta-9-THC pg/g levels were similar in infants with abnormal [1758 (296,2838)] and normal aEEG [1589 (332,2794)], $p = 0.82$. The absence of sleep-wake cycles on aEEG is associated with prenatal delta-9-THC exposure. Delta-9-THC umbilical cord drug screening levels did not correlate with aEEG results; longitudinal studies are necessary to obtain a detailed history of delta-9-THC use and to evaluate its association with abnormal aEEG and neurodevelopmental outcomes.

***Cannabis* and Children's Dental Health**

A longitudinal cohort study of 790,758 infants, born between 2006 and 2016 in Quebec, Canada, was examined for the children's dental health, including the risk of caries [86]. Women with substance use disorders before or during pregnancy were identified. The main outcome measure was hospitalization for dental caries in offspring up to 12 years after birth. Children exposed to maternal substance use had a higher incidence of hospitalization for dental caries than unexposed children (105.2 vs. 27.0 per 10,000 person-years).

Maternal substance use was associated with 1.96 times the risk of childhood dental caries (95% CI = 1.80–2.14), including a greater risk of caries of enamel, dentin, or cementum (HR, 2.00; 95% CI = 1.82–2.19) and dental pulp (HR, 2.36; 95% CI = 2.07–2.70), relative to no substance use. Associations were elevated for alcohol (HR, 2.31; 95% CI = 2.03–2.64) but were also present for cocaine, *Cannabis*, opioids, and other substances. Substance use during pregnancy was more strongly associated with dental caries hospitalization than pre-pregnancy substance use. Associations were stronger in early childhood.

***Cannabis* and Maternal Choline**

Prenatal *Cannabis* use adversely affects fetal brain development and subsequent behavioral self-regulation, a precursor to later, more serious problems in childhood. Maternal choline levels correlated with the children's improved duration of attention, cuddliness, and bonding with parents. Higher maternal choline mitigates some of *Cannabis*' adverse effects on the fetus [88]. Discontinuing *Cannabis* use before 10 weeks of gestational age prevented those effects. Mothers refuse to cease *Cannabis* use mainly because of familiarity with *Cannabis* and belief in its safety.

Cannabis use was assessed during pregnancy in women who included their newborns in the study. The mothers were informed about choline and other nutrients, but not specifically for *Cannabis* use. Choline transported into the amniotic fluid from the mother activates $\alpha 7$ -nicotinic acetylcholine receptors on the fetal

cerebrocortical inhibitory neurons, whose development is impeded by *Cannabis* blockade of their cannabinoid-1 receptors.

Maternal serum choline was measured at 16 weeks gestation. *Cannabis* use for the first 10 weeks of gestation or more by 15% of the mothers decreased newborns' inhibition of evoked potentials to repeated sounds ($d' = 0.55$, $p < 0.05$). The effect was ameliorated in women with higher gestational choline ($rs = -0.50$, $p = 0.011$). At 3 months of age, children whose mothers continued *Cannabis* use through their tenth gestational week or more had poorer self-regulation ($d' = -0.79$, $p < 0.05$). The effect was ameliorated if mothers had higher gestational choline ($rs = 0.54$, $p = 0.013$).

***Cannabis* and Tobacco Use**

The combination of *Cannabis* and tobacco is increasingly common among women and is associated with tobacco and *Cannabis* dependence and poorer cessation outcomes [90]. Pregnant women (mean age = 23, range = 18–42) were asked about substance use during each trimester of pregnancy and at 8 and 18 months, 3, 6, 10, 14, 16, and 22 years postpartum. Patterns of maternal cigarette and *Cannabis* use during pregnancy and postpartum were examined.

As young adults (mean age = 22.8 years, range = 21–26), 603 offspring completed the Diagnostic Interview Schedule (DIS). Growth mixture modeling (GMM) was used to identify four maternal trajectories through 16 years postpartum: no co-use (66%), decreasing co-use (16%), postpartum-only co-use (11%), and chronic co-use (7%). Offspring whose mothers were in the decreasing co-use group (co-users primarily during prenatal and preschool periods) were more likely to be co-users than the offspring of non-co-users. Offspring whose mothers were chronic co-users of cigarettes and *Cannabis* were more than twice likely to have a drug use disorder than young adults whose mothers were not co-users.

The study's results highlight the heterogeneity in maternal co-use of tobacco and *Cannabis* over time, with some women quitting during pregnancy but resuming co-use postpartum and other women co-using during pregnancy but desisting co-use over time. Maternal trajectories of co-use were associated with the inter-generational transfer of risk for substance use and dependence in adult offspring.

Prenatal use of *Cannabis* and opioids is increasing and very concerning. Engagement and retention in comprehensive, perinatal substance use disorder care are associated with better outcomes for mothers and babies. The characteristics and engagement in care among women with opioid use disorder who used *Cannabis* late in pregnancy versus those who did not use were compared. The primary outcome, "overall engagement and retention in perinatal substance use disorder care," included utilization of substance use treatment prenatally, negative screening/toxicology at delivery (excluding *Cannabis*), and attendance at routine prenatal and postpartum visits.

Maternal and Paternal Tobacco and *Cannabis* Use

The associations of maternal and paternal tobacco and *Cannabis* use during pregnancy with offspring body fat and cardiometabolic outcomes were examined in a population-based prospective cohort study among 4792 mothers, fathers, and children. Childhood outcomes were assessed at 10 years by body mass index (BMI), body fat, blood pressure, and lipid, glucose, and insulin concentrations. Questionnaires were collected for parental substance use.

Children exposed to maternal tobacco use during pregnancy had a higher android/gynoid fat mass ratio (difference 0.22 SDS, 95% confidence interval = 0.13, 0.30), fat mass index (difference 0.20 SDS, 95% CI = 0.12, 0.28), triglyceride concentrations (difference 0.15 SDS, 95% CI = 0.04, 0.26), and a higher risk of overweight (odds ratio 1.35, 95% CI = 1.07, 1.71), compared to non-exposed. Children exposed to maternal *Cannabis* during pregnancy had a higher BMI (difference 0.26 SDS, 95% CI = 0.08, 0.44), android/gynoid fat mass ratio (difference 0.21 SDS, 95% CI = 0.04, 0.39), and fat-free mass index (difference 0.24 SDS, 95% CI = 0.06, 0.41), compared to non-exposed.

The associations between paternal substance use and child cardiometabolic health outcomes were similar to maternal use. Similar associations for maternal and paternal substance use during pregnancy suggest that the findings may be explained by shared family-based social and lifestyle factors rather than by direct fetal programming.

Cannabis Use and Fetal Birth Weight

A retrospective medical record review of singleton births with urine drug screens (UDS) at the initiation of prenatal care and delivery was conducted at a large tertiary academic referral center [96, 97]. Patients who used drugs other than *Cannabis* were excluded.

The prevalence of *Cannabis* use in pregnancies not complicated by other medications, as evidenced by tetrahydrocannabinol in the urine of 2173 patients, was 22.6%. Infants born to mothers who tested positive for only tetrahydrocannabinol in urine at both presentation for prenatal care and delivery were of lower median birth weight compared with those who tested negative [2925 g (IQR 2522–3265) vs. 3235 g (IQR 2900–3591), $p < 0.001$].

There was no clinically relevant difference in gestational age at birth [39.0 weeks (IQR 37.1–40.0) vs. 39.3 weeks (IQR 38.3–40.0), $p = 0.012$] between those who tested positive for tetrahydrocannabinol and those who tested negative. Concomitant tobacco use during pregnancy was not noted to impact infant birth weight using the analysis of covariance. Higher perinatal mortality was observed among those who used *Cannabis*, with an adjusted odds ratio of 4.2 (95% CI = 1.53–11.49). *Cannabis* use is negatively correlated with fetal birth weight (up to 450 g less) in patients who tested positive for delta-9-THC compared to those who did not, as documented in the urine drug screens.

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Prenatal *Cannabis* Exposure

Due to the negative effects of prenatal *Cannabis* exposure, it is necessary to develop an objective, sensitive, and specific method to determine cannabinoid use during pregnancy. Four different biological samples, maternal hair, meconium, umbilical cord, and placenta, were compared to detect in utero *Cannabis* exposure [98].

The biological samples were collected from 627 mother–newborn dyads. Hair and meconium samples were analyzed. The umbilical cord and placenta were analyzed only if hair and meconium were positive for cannabinoids. Meconium and hair appear to complement each other, with an agreement between hair and

meconium results of 96.7% but only 34.3% if just positive results were considered. Umbilical cord and placenta results showed a better agreement with meconium (91.3% and 92.6%, respectively) than with hair (39.1% and 34.6%, respectively).

The predominant metabolites in meconium were 11-nor-carboxy-THC (THCCOOH) and 8,11-dihydroxy-THC (diOHTHC), and the predominant metabolite in umbilical cord and placenta was THCCOOH-glucuronide. Cannabidiol and cannabinol were detected in the meconium but not in any umbilical cord or placenta. Hair and meconium positivity rates were similar, but a more sensitive and specific analytical method for the hair may resolve discrepancies between the matrices. The umbilical cord and placenta may be considered suitable alternative matrices to meconium by determining THCCOOH-glucuronide as a biomarker of *Cannabis* exposure.

Development and Infancy Study of Prenatal Drug Use

One hundred and twenty-one participants from a wide range of backgrounds were recruited to participate in a longitudinal development and infancy study of prenatal drug use and outcomes [99]. Eighty-six women were interviewed prospectively while pregnant and soon after their infants were born. The participants reported use immediately before and during pregnancy and over their lifetime. The levels of lifetime drug use of the women recruited were high, with women reporting having used at least four illegal drugs over their lifetime. Most of the users of cocaine, 3,4-methylenedioxy-N-methylamphetamine, and other stimulants stopped using them by the second trimester, and levels of use were low. However, during pregnancy, 64% of the participants continued to use alcohol, 46% tobacco, and 48% *Cannabis*. The level of alcohol use was reduced substantially. Still, the average tobacco and *Cannabis* levels were sustained at pre-pregnancy levels even into the third trimester (50 cigarettes and 11 joints per week).

Whether *Cannabis* use relates to altered human brain development before birth and, specifically, whether maternal prenatal *Cannabis* use relates to the connectivity of fetal functional brain systems are still unclear. The link between maternal prenatal *Cannabis* use and altered neural and physiological development of the child has been revealed in research.

Prenatal drug toxicology and fetal fMRI data were available in 115 fetuses [43% female; mean age 32.2 weeks (SD = 4.3)]. Voxelwise hippocampal connectivity analysis in a subset of age and sex-matched fetuses revealed that prenatal *Cannabis* exposure was associated with alterations in fetal dorsolateral, medial, and superior frontal, insula, anterior temporal, and posterior cingulate connectivity [100]. Classification of group differences by age 5 suggests that compared to the non-prenatal *Cannabis* exposure group, the prenatal *Cannabis* exposure group is more likely to have increased connectivity to regions associated with less favorable outcomes and decreased connectivity to regions associated with more favorable outcomes. This alteration of fetal neural connectome may contribute to neurobehavioral vulnerability observed in children exposed to *Cannabis* in utero.

The Genetics of *Cannabis* Use Behavior

The heritability of ever using *Cannabis* or becoming dependent on it is moderately high (between 17 and 76%), suggesting that *Cannabis* use behaviors have genetic underpinnings. The cannabinoid receptor 1 (Human Gene Nomenclature Committee [HUGO approved symbol CNR1]), alternatively known as CB1, has a cytogenetic location at chromosome 6q15 and is found throughout the brain, where it is highly expressed. The cannabinoid receptor 2 (CNR2), also known as CB2, appears confined to the periphery. Type I cannabinoid receptors have been localized to multiple brain regions, including the prefrontal cortex, amygdala, hippocampus, striatum, and cerebellum. The cannabinoid gene CNR1 encodes for the CB1 receptor making it an ideal candidate for studying *Cannabis* exposure effects.

Cannabis use has been associated with reduced volume in some brain regions, particularly those rich in cannabinoid CB1 receptors, including the medial temporal cortex, parahippocampal gyrus, insula, orbitofrontal cortex, hippocampus and amygdala, and cingulum, with users showing reduced volume relative to non-users. Although results of studies assessing the effects of *Cannabis* on brain structure have been mixed, what is clear is that regions that appear to show a reduction in volume in association with *Cannabis* use are regions that have an abundance of CB1 receptors. Variation in the CNR1 gene appears to confer important variation that should be considered in understanding the effects of *Cannabis* exposure. *Cannabis* users had smaller bilateral hippocampi and amygdale associated with variation in the G allele of rs2023239 of the CNR1 gene. Similarly, CNR1 genetic polymorphisms and *Cannabis* use are associated with white matter changes and cognitive deficits in participants with schizophrenia.

Measures of *Cannabis* use and co-occurring exposures were obtained from a longitudinal cohort for an average of 13 years from when they received a structural MRI scan [101]. MRI scans were analyzed for 88 participants (mean age = 25.9 years), 34 of whom were regular users of *Cannabis*. Whole brain voxel-based morphometry analyses (SPM8) were conducted using 50 voxel clusters at $p = 0.005$. Controlling for age, familial risk, and gender, there was a reduced volume of regular users compared to non-users, in the lingual gyrus, anterior cingulum (right and left), and the rolandic operculum (right). The right anterior cingulum reached family-wise error statistical significance at $p = 0.001$, controlling for personal lifetime use of alcohol and cigarettes and any prenatal exposures. CNR1 haplotypes were formed from four CNR1 SNPs (rs806368, rs1049353, rs2023239, and rs6454674) and tested with a level of *Cannabis* exposure to assess their interactive effects on the lingual gyrus and cingulum (right and left) and rolandic operculum, regions showing *Cannabis* exposure effects in the SPM8 analyses. The analyses used mixed model analyses to control for multiple potentially confounding variables. The level of *Cannabis* exposure was associated with decreased volume of the right anterior cingulum and showed interaction effects with haplotype variation.

Prenatal cannabinoid exposure leads to long-lasting cognitive, motor, and behavioral deficits in the offspring and alterations in neural circuitry through various

mechanisms. The cerebellum plays an important role in balance, motor control, and cognitive functions such as attention, language, and procedural memories. The effect of prenatal cannabinoid exposure on the cerebellum of adolescent offspring was investigated [102].

Pregnant rats were treated with the synthetic cannabinoid agonist, WIN55212-2. The offspring were evaluated for various cerebellar oxidative stress markers, mitochondrial function, and apoptosis. Signaling proteins associated with glutamate-dependent synaptic plasticity were also examined. Administration of WIN55212-2 during pregnancy altered oxidative stress markers by significantly reducing oxidative stress and nitrite content. Mitochondrial Complex I and Complex IV activities were enhanced following prenatal cannabinoid exposure.

With regard to apoptosis, pP38 levels were significantly increased, and proapoptotic factor caspase-3 activity, pERK, and pJNK levels were significantly decreased. CB1R and GluA1 levels remained unchanged; however, GluN2A was reduced considerably. There was a significant decrease in MAO activity, although tyrosine hydroxylase activity was unaltered. The study indicates that the effects of prenatal cannabinoid exposure on the cerebellum are unique compared to other brain regions by enhancing mitochondrial function and promoting neuronal survival.

The Impact of *Cannabis* Laws on *Cannabis* Use

Cannabis laws may impact *Cannabis* use and the use of other substances. The association is of particular concern in pregnant women, where exposure to substances can cause harm to both the pregnant woman and the fetus. A cross-sectional analysis was conducted using combined survey data from PRAMS collected from 36,391 women [81]. Logistic regression was used to estimate the impact of state legalization, adequacy of prenatal care, and other substance use on *Cannabis* use during the preconception, prenatal, and postpartum periods.

In the preconception model, residence in a recreationally legal state (OR: 2.37; 95% CI = 2.04–2.75) or medically legal state (OR: 3.32; 95% CI = 2.90–3.80) compared to a non-legal state was associated with higher odds of *Cannabis* use. In the prenatal model, residence in a recreationally legal state was associated with higher odds of *Cannabis* use (OR: 1.51; 95% CI = 1.29–1.79), whereas there was no association with residence in a medically legal state.

Recreational *Cannabis* legalization is associated with the use of *Cannabis* prior to, during, and after pregnancy. Tobacco use, including e-cigarettes and moderate prenatal alcohol use, was also significantly associated with *Cannabis* use. Renewed clinical and policy efforts may be warranted to update prenatal substance use prevention programs, educational campaigns, and provider education as *Cannabis* legalization expands.

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Chapter 9

Cannabis and Multiple Sclerosis



Multiple sclerosis (MS) is a chronic demyelinating disease of the central and peripheral nervous systems. The symptoms, such as vision problems, muscle weakness, pain, balance problems, and paralysis, are due to the uncontrolled or inappropriate neural transmission that gradually worsens as the disease progresses. During the early stage of the disease, patients may experience long symptom-free periods, and the periods are interrupted by flare-ups that last days to weeks. It has been indicated that *Cannabis*, delta-9-tetrahydrocannabinol (THC), nabiximols, and oral *Cannabis* extract (OCE) may diminish spasticity associated with MS [19]. Nabiximols is a mucosal spray containing delta-9-THC and cannabidiol (CBD) in the 1:1 ratio and has been developed for treating MS. The US FDA has approved nabiximols and has also been approved in several European countries, Canada, and New Zealand for treating spasticity associated with MS.

Patients with multiple sclerosis suffer from spasticity and pain during their disease course. Baclofen, dantrolene, diazepam, and gabapentin have been used as first-line options to treat the conditions, with modest results. *Cannabis*-derived drugs have been tested and approved, and their use has been legalized as a therapeutic option. Pharmaceutical cannabinoids such as nabiximols, nabilone, dronabinol, and plant-based cannabinoids have been investigated for their therapeutic potential in treating multiple sclerosis symptoms [1]. The outcomes of disability and disability progression, pain, spasticity, bladder function, tremor/ataxia, quality of life (QOL), and adverse effects were examined in 11 systematic reviews with data from 32 studies, including ten moderate to high-quality randomized parallel or crossover trial (RCTs). Five reviews revealed sufficient evidence that cannabinoids are effective for symptoms of pain and spasticity in MS.

Multiple sclerosis causes multifocal and diffuse injuries to the central nervous system (CNS). Over time, most patients with MS (pwMS) develop significant symptoms with loss of function and a certain degree of disability. About 65% of pwMS report pain and approximately 26% have central neuropathic pain (NP), which significantly impacts their quality of life. About two-third of pwMS suffer

from spasticity (tightness/stiffness of the muscles), and 15% of pwMS report spasm-related pain. Spasticity and spasms often significantly impact sleep and everyday life and are limiting factors in rehabilitation.

The consequences of spinal cord injury (SCI) depend on the neurological level and the extent of the injury. Approximately 50% of patients with SCI (pwSCI) report significant chronic central or peripheral NP. In a recent survey in pwSCI, 71% reported spasticity and 46% received medical treatment for spasticity. No curative treatment exists for SCI. Hence, good rehabilitation offers optimal symptomatic treatment to prevent long-term complications.

Traditionally, spasticity has been treated with baclofen or tizanidine. However, the effects of the pharmaceuticals are often not sufficiently felt by many patients at the maximum dose, or they discontinue treatment due to side effects. Treating NP is challenging since the available analgesics often only have partial or no effect, and new treatment regimens, such as medical *Cannabis*, are being sought.

The Endocannabinoid System and MS

Spasticity is a complicating sign in multiple sclerosis that also develops in a model of chronic relapsing experimental autoimmune encephalomyelitis (CREAE) in mice. In areas associated with nerve damage, increased levels of the endocannabinoids, anandamide (arachidonylethanolamide [AEA]), and 2-arachidonoyl glycerol (2-AG), and of the AEA congener, palmitoylethanolamide (PEA), were detected, and comparable levels of the compounds were found in normal and non-spastic CREAE mice.

While exogenously administered endocannabinoids and PEA ameliorate spasticity, selective inhibitors of endocannabinoid re-uptake and hydrolysis – probably through the enhancement of endogenous levels of AEA and, possibly, 2-arachidonoyl glycerol – significantly ameliorated spasticity to an extent comparable with that observed previously with potent cannabinoid receptor agonists. The studies provide definitive evidence for the tonic control of spasticity by the endocannabinoid system and open new horizons to therapy of multiple sclerosis and other neuromuscular diseases based on agents modulating endocannabinoid levels and action, which exhibit little psychotropic activity [105].

Endocannabinoids play a role in the modulation of neuro-inflammation, and experimental findings suggest that they may be directly involved in the pathogenesis of multiple sclerosis. In a study conducted to measure eCB levels in the cerebrospinal fluid (CSF) of patients with MS, arachidonylethanolamine (AEA), palmitoylethanolamide, 2-arachidonoylglycerol, and oleoylethanolamide levels were measured in the CSF of 50 patients with MS and 20 control subjects by isotope dilution gas-chromatography/mass-spectrometry. The patients were 35 with MS in the relapsing-remitting (RR) form of the disease, 20 in a stable clinical phase and fifteen during a relapse, and 15 in the secondary progressive (SP) form.

Significantly reduced levels of all the tested eCBs were found in the CSF patients with MS compared to control subjects, with lower values detected in the SP MS group. Although below controls, higher levels of AEA and PEA were found in the CSF of RR MS patients during a relapse. Higher AEA, 2-AG, and OEA were found in patients with MRI gadolinium-enhancing (Gd+) lesions. The findings suggest the presence of an impaired eCB system in MS. Increased CSF levels of AEA during relapses or in RR patients with Gd + lesions suggest its potential role in limiting the ongoing inflammatory process with potential neuroprotective implications [106].

In Italy, *Cannabis* was authorized for treating several diseases, including chronic pain generally, spasticity in multiple sclerosis, cachexia and anorexia among AIDS and cancer patients, glaucoma, Tourette syndrome, and certain types of epilepsy. A retrospective case series analysis of all chronic pain patients treated with oral or vaporized *Cannabis* in six hubs during the initial year following the approval of the new Italian law.

Routes of administration, types of *Cannabis* products utilized, dosing, and effectiveness and safety of the treatment were analyzed. Only one of the six centers has extensively used cannabinoids for intractable chronic pain (614 out of 659). *Cannabis* tea was the primary mode of delivery, and in almost all cases, it was used in association with all the other pain treatments. Initial and follow-up cannabinoid concentrations were found to vary considerably. At the initial follow-up, 76.2% of the patients continued the treatment, and <15% stopped the treatment due to side effects, none of which were severe.

Of all the subjects, 181 were males, 422 were females, and data on gender were missing for 11 subjects. The average age of subjects was 61.33 (± 15.29) years. Of 614 patients whose data were used, 341 (55.5%) had at least one follow-up at 98.42 (± 144.66) days. At initial follow-up, 76.2% of patients continued the cannabinoid therapy (of whom 64.7% reported an improvement associated with the therapy, while 34.1% reported neither an improvement nor a worsening), and 23.8% discontinued treatment (3.7% due to a worsening of their pain, 61.7% due to side effects, 29.6% due to an unsatisfactory change of their clinical condition; for 4.9% of patients, the data were missing). In 89.2% of patients, *Cannabis* was prescribed without discontinuing their previous analgesic therapy. There were no complaints of severe side effects [107].

Cannabigerol in Multiple Sclerosis

Cannabigerol (CBG) is a precursor molecule for the most abundant cannabinoids. It exhibits affinity and activity characteristics between delta-9-THC and CBD at the cannabinoid receptors but appears to be unique in its interaction with α -2 adrenoceptors and 5-hydroxytryptamine (5-HT1A) [3]. Studies indicate that CBG has therapeutic potential in treating neurologic disorders such as Huntington's disease, Parkinson's disease, multiple sclerosis, and inflammatory bowel disease, as well as having antibacterial activity. Studies with animals have shown that chronic

exposure to *Cannabis* smoke, delta-9-tetrahydrocannabinol, or CB1 receptor agonists leads to the development of dependence. *Cannabis* withdrawal (CW) leads to somatic withdrawal signs (e.g., abdominal constriction, wet-dog shakes, head shakes, forepaw fluttering), anxiety-like behavior, and an increased release of the stress peptide corticotropin-releasing factor in the amygdala [44].

The negative affective state associated with *Cannabis* withdrawal provides powerful motivation for continuing drug use. There is evidence that *Cannabis* and delta-9-THC impair memory and cognition in rodents. Delta-9-THC inhibits the release of acetylcholine in the hippocampus, likely one mechanism by which it impairs memory. The observation supports that drugs that prevent the delta-9-THC-induced decrease in acetylcholine release in the hippocampus also prevent memory impairments [45].

It has also been indicated that repeated delta-9-THC administration leads to increased glutamate levels, which induces downregulation of glutamate receptors and a reduction in the density of dendritic spines on hippocampal neurons that may reduce synaptic plasticity and thereby cause memory impairments [47]. Memory impairments due to delta-9-THC exposure may gradually diminish over time. In one study, memory function returned to baseline after 4 weeks of abstinence [46]. Another study reported that memory function in mice was still impaired 3 weeks after administering one low dose of THC [48].

CBG is generated during the non-enzymatic decarboxylation of cannabigerolic acid, a key compound in the biosynthesis of phytocannabinoids (PCBs) and, consequently, the precursor to various phytocannabinoids. CBG exhibits a wide range of biological activities, among other things, anti-inflammatory, antibacterial, and antifungal activities, regulation of the redox balance, and neuromodulatory effects by interacting with G-protein-coupled receptors [4]. CBG is a promising compound for treating diseases requiring multidirectional pharmacotherapy due to its wide spectrum of biological activities. Also, due to the relatively rapid metabolism of cannabigerol, the phytocannabinoid concentration in blood or oral fluid can be used to determine *Cannabis* use.

In addition to CBG, this group includes cannabigerolic acid (CBGA), CBGA methyl ethers, and cannabigerovarin (CBGV) and its acid derivative, cannabigerovarinic acid (CBGVA). CBGV is an analog of CBG, containing a three-carbon side chain – in comparison, the side chain of a cannabigerol molecule contains five carbon atoms. The structure of CBG consists of 21 carbon atoms, 22 carbon atoms in the case of the acidic form, with the chemical formula of $C_{21}H_{32}O_2$ and a molar mass of 316.48 g/mol. According to the IUPAC nomenclature, the systematic name of cannabigerol is 2-[(2E)-3,7-dimethylocta-2,6-dienyl]-5-pentylbenzene-1,3-diol. It was noted that CBG melts at 52 °C compared to the melting point of cannabidiol at 68 °C. The thermal degradation of cannabigerol occurs at approximately 150 °C, and CBD is thermally degraded at 250 °C. For both CBG and CBD, the temperatures are close to their boiling points. Studies have shown that cannabigerol, like delta-9-THC, exhibits greater solubility in supercritical CO₂ as the temperature increases at constant pressure, while cannabidiol shows the opposite trend.

Cannabinoids present in plants are formed by biosynthesis from precursors in the form of the respective fatty acids or geranyl diphosphate (GPP) present within trichomes, with two possible mechanisms leading to the formation of CBG. In the first mechanism, biosynthesis of cannabigerol occurs by direct synthesis from GPP and olivetol (1,3-dihydroxy-5-pentylbenzene; OL), similar to the synthetic production of CBG. However, a more likely mechanism of CBG formation is the non-enzymatic decarboxylation of cannabigerolic acid formed from olivetolic acid (OLA) and geranyl diphosphate. That approach seems more realistic because olivetol is not detected in *Cannabis sativa* tissues despite the presence of olivetol synthase (OLS), an enzyme essential for its biosynthesis.

Another factor supporting the latter mechanism is that decarboxylation of CBGA and other phytocannabinoids synthesized in the acid form occurs by non-enzymatic means, including those occurring as a result of long-term storage, or under the influence of irradiation, from either sunlight and UV light or increased temperature. The lack of involvement of enzymes necessary for such a conversion explains the mode of action of orally ingested *Cannabis* in the form of heat-treated products as in smoking.

It was found that glucose produced through photosynthesis is converted to phosphoenolpyruvate, which, under the influence of pyruvate kinase and pyruvate dehydrogenase, is converted to acetyl-coenzyme A (acetyl-CoA). Acetyl-CoA is a key chemical that acts as a substrate in the formation of geranyl diphosphate and malonyl-CoA, which are necessary for the formation of olivetolic acid and olivetol, i.e., the precursors of cannabigerolic acid and cannabigerol, respectively [5]. Hexanoyl-CoA, on the other hand, is formed during the action of hexanoyl-CoA synthase on hexanoic acid formed in the process of biosynthesis of fatty acid.

The aldol condensation of hexanoyl-CoA and malonyl-CoA in the presence of olivetolic acid cyclase (OLAC) results in the formation of olivetolic acid, whereas in the presence of olivetol synthase (OLS), decarboxylation with simultaneous cyclization results in the formation of olivetol [6]. As a result of numerous transformations, acetyl-CoA is initially converted to mevalonic acid (MVA), which is then converted to geranyl diphosphate under the influence of kinases, decarboxylases, and isomerases [7].

Under the action of the respective enzymes tetrahydrocannabinolic acid synthase (THCAS) and cannabidiolic acid synthase (CBDAS), the resulting cannabigerolic acid is converted to delta-9-tetrahydrocannabinolic acid and cannabidiolic acid, which are then decarboxylated to form delta-9-THC and CBD, respectively. Due to the easy conversion to CBD, cannabigerol is treated as a structural analog of CBD with an open cyclohexylene ring [8].

The content of cannabigerol in *Cannabis sativa* is very low (up to 10% of the cannabinoid fraction), which stems from the fact that some of the cannabigerolic acids present in the plant are converted to acidic forms of other phytocannabinoids. Studies of the properties of cannabigerol extracted from *Cannabis sativa* are only possible owing to the last modification of the *Cannabis* genotype aimed at reducing the activity of CBGA-converting synthases to CBDA, delta-9-THCA, and CBCA, increasing the CBG content up to 90% of the cannabinoid fraction [9].

Cannabigerol is not one of the psychoactive compounds of *Cannabis*. It exhibits antibacterial, antifungal, and anti-inflammatory effects and prevents cell proliferation [10]. Like the other cannabinoids, cannabigerol's complex biological effects are believed to result from modifications of dependent redox and inflammatory processes, which modulate cellular metabolism.

Cannabigerol has been shown to regulate redox balance by reducing the activity of one of the main pro-oxidant factors, i.e., iNOS, activating the membrane receptor PARP- γ and modulating the expression of the superoxide dismutase SOD-1, whose activity is increased by pro-inflammatory factors. Consequently, CBG contributes to the inhibition of cell death by shifting the redox balance in the direction of the antioxidant [11]. The phytocannabinoid can also modify inflammatory processes by significantly reducing I κ B- α phosphorylation, thus reducing the transcriptional activity of the nuclear factor NF κ B, responsible for the transcription of pro-inflammatory cytokines, which results in reduced levels of cytokines, including TNF α and IL-1 β [12].

Based on the analyses of the biological activities of phytocannabinoids, some overlap exists with the activities of endocannabinoids, especially concerning their actions on G-protein-coupled membrane receptors and lipid mediators as phospholipid-metabolizing enzymes. Like endocannabinoids, phytocannabinoids belonging to the cannabigerol group also modify the activation of CB1 and CB2 cannabinoid receptors as agonists, with CBG interacting with receptors such as TRPV1 and PPAR [13].

In addition, cannabigerol decreases the activity of FAAH, an enzyme that metabolizes anandamide, thus affecting its levels and biological effects. However, compared to CBD, CBG is less effective as a FAAH activity inhibitor [14]. In contrast, phytocannabinoids from the cannabigerol group, e.g., CBG and CBGA, reduce the activity of DAGL, the enzyme responsible for the biosynthesis of 2-AG and the activities of COX-1 and COX-2, which metabolize PUFAs, mainly arachidonic acid, to lipid mediators; this causes altered levels of both endocannabinoids and other lipid mediators, which by acting on receptors both, directly and indirectly, modify both redox balance and inflammation [15].

Consequently, chronic exposure to the bioactive constituents of *Cannabis* leads to decreased CB1 receptor activation, resulting in decreased generation of ROS and TNF α [16]. CBG has a low affinity for CB1 and CB2 receptors (~fivefold and 27-fold lower than delta-9-THC), with CBG showing a higher affinity for CB1. The lower affinity of cannabigerol for CB1 compared to other phytocannabinoids explains this phytocannabinoid's lack of psychotropic effect [17]. Cannabigerol intermediates also show the ability to bind to cannabinoid receptors. CBGA, like CBG, is an agonist of CB1 and CB2 receptors, unlike olivetol (OL), which acts as an antagonist of CB1 and CB2 receptors.

Cannabigerol exhibits significant activity against several receptors from the, transient receptor potential (TRP) superfamily, including a strong TRPA1 agonist (TRP ankyrin type 1) and a weak TRPV1 agonist (TRP vanilloid type 1), and an even weaker agonist of TRPV2 and TRPV4. It is also a potent inhibitor of TRPM8 (TRP melastatin type 8) [18]. Also, CBG has been shown to act as a potent agonist

of $\alpha 2$ adrenergic receptors and to moderately block 5-HT_{1A} receptors, which may explain its biological activity considering its slight affinity for cannabinoid receptors.

By influencing the functioning of the endocannabinoid system, cannabigerol modulates many processes within the body, including immune responses, cancer formation, cardiovascular diseases, and pain perception. The neuromodulatory effect observed in terms of modifications in the activity of components of the endocannabinoid system is considered particularly important. CBG increases anandamide levels and its action in cells by inhibiting anandamide uptake. The ability of CBG to inhibit the proliferation of cancer cells (in breast, prostate, colorectal cancers, and gastric adenocarcinoma) through the activation of the TRPV1 receptor has been reported [19]. On the other hand, cannabigerol significantly reduces the apoptosis of transformed tumor cells by modulating the levels of Bax and Bcl2 proteins and may thus enhance the development of tumor processes. Moreover, CBG and its derivatives (both natural and synthetic) have been tested in terms of their potential use in alleviating the negative effects of chemotherapy, the treatment of mood disorders (including depression), neurodegenerative diseases, and diseases of the nervous system, and for their anesthetic effects.

To determine the potential use of cannabigerol in pharmacotherapy, in addition to the knowledge about its effect on the metabolic processes occurring in the human body under physiological and pathophysiological conditions, it is necessary to analyze the pharmacokinetics of the compound in question after its introduction into the body. In vivo studies have shown that cannabigerol suspended in mixtures of glycerol and ethylene oxide (cremophor EL) and ethanol and saline in a ratio of 1:1:18 administered orally or intraperitoneally to rats and mice in varying amounts concentrates in the blood plasma.

It has been found that the plasma concentration of CBG in rats was slightly higher after intraperitoneal administration compared to the concentration reached after oral administration. In contrast, the maximum plasma concentration of CBG in mice was over 60 times higher after oral administration than intraperitoneal administration. However, the level was reached as late as after 2 h; in the case of intraperitoneal administration, the maximum concentration was reached after 30 min. In addition, brain levels of CBG in both mice and rats were shown to be higher in the case of intraperitoneal administration, which favors significant increases in CBG levels. The studies also showed that intraperitoneal administration was accompanied by a longer elimination half-life of cannabigerol, with no common relationship between the mode of administration of CBG and the time required to reach its maximum concentration in both animal models tested [149].

***Cannabis* for Spasticity Symptoms and Neuropathic Pain**

Spasticity and chronic neuropathic pain are common and serious symptoms in people with multiple sclerosis. The symptoms increase with disease progression and lead to worsening disability, impaired activities of daily living, and quality of life.

Antispasticity medications and analgesics are of limited benefit or poorly tolerated. Cannabinoids may reduce spasticity and pain in people with MS, and the demand for symptomatic treatment with cannabinoids is high.

To assess the benefits and harms of cannabinoids for reducing symptoms for adults with MS, randomized parallel or crossover trials (RCTs) were evaluated for cannabinoids (including herbal *Cannabis*, *Cannabis* flowers, plant-based cannabinoids, or synthetic cannabinoids) irrespective of dose, route, frequency, or duration of use for adults with MS [61]. The Cochrane Risk of bias 2 tool for parallel RCTs and crossover trials was used to assess bias in the studies. The certainty of the evidence using the GRADE approach for the following outcomes was rated: reduction of 30% in the spasticity Numeric Rating Scale, pain relief of 50% or greater in the Numeric Rating Scale-Pain Intensity, much or very much improvement in the Patient Global Impression of Change (PGIC), Health-Related Quality of Life (HRQoL), withdrawals due to adverse events (AEs) (tolerability), serious adverse events (SAEs), nervous system disorders, psychiatric disorders, and physical dependence.

The study includes 25 RCTs with 3763 participants, of which 2290 received cannabinoids. The ages ranged from 18 to 60 years, and 50% and 88% of the participants were female [61]. The studies were 3–48 weeks long and compared nabiximols (13 studies); synthetic cannabinoids mimicking delta-9-THC (seven studies); an oral delta-9-THC extract of *Cannabis sativa* (two studies), and inhaled herbal *Cannabis* (one study) against placebo.

One study compared dronabinol, delta-9-THC extract of *Cannabis*, and placebo, and another compared inhaled herbal *Cannabis*, dronabinol, and placebo. Critical outcomes were spasticity: nabiximols probably increased the number of people who reported a reduction of perceived severity of spasticity compared with placebo (odds ratio (OR) 2.51, 95% confidence interval (CI) 1.56–4.04; 5 RCTs, 1143 participants; $I^2 = 67\%$; moderate-certainty evidence). The absolute effect was 216 more people (95% CI 99 more to 332 more) per 1000 reporting benefits with cannabinoids than with placebo.

Chronic neuropathic pain: one trial that measured the number of participants who reported substantial pain relief with a synthetic cannabinoid compared with placebo (OR 4.23, 95% CI 1.11–16.17; 1 study, 48 participants; very low-certainty evidence). It was uncertain whether cannabinoids reduce chronic neuropathic pain intensity. Treatment discontinuation due to AEs: Cannabinoids may increase slightly the number of participants who discontinue treatment compared with placebo (OR 2.41, 95% CI 1.51–3.84; 21 studies, 3110 participants; $I^2 = 17\%$; low-certainty evidence); the absolute effect is 39 more people (95% CI 15 more to 76 more) per 1000 people.

Cannabinoids probably increase the number of people who report “very much” or “much” improvement in health status compared with placebo (OR 1.80, 95% CI 1.37–2.36; eight studies, 1215 participants; $I^2 = 0\%$; moderate-certainty evidence). The absolute effect is 113 more people (95% CI 57 more to 175 more) per 1000 people reporting improvement. HRQoL: Cannabinoids may have little to no effect on HRQoL (SMD -0.08 , 95% CI -0.17 – 0.02 ; eight studies, 1942 participants;

$I^2 = 0\%$; low-certainty evidence). SAEs: Cannabinoids may result in little to no difference in the number of participants who have SAEs compared with placebo (OR 1.38, 95% CI 0.96–1.99; 20 studies, 3124 participants; $I^2 = 0\%$; low-certainty evidence). AEs of the nervous system: Cannabinoids may increase nervous system disorders compared with placebo (OR 2.61, 95% CI 1.53–4.44; seven studies, 1154 participants; $I^2 = 63\%$; low-certainty evidence). Psychiatric disorders: Cannabinoids may increase psychiatric disorders compared with placebo (OR 1.94, 95% CI 1.31–2.88; six studies, 1122 participants; $I^2 = 0\%$; low-certainty evidence). Drug tolerance: The evidence is very uncertain about the effect of cannabinoids on drug tolerance (OR 3.07, 95% CI 0.12–75.95; two studies, 458 participants; very low-certainty evidence).

Compared with a placebo, nabiximols reduced the severity of spasticity in the short term in people with MS. The effect on chronic neurological pain and health-related quality of life is uncertain. Cannabinoids may increase treatment discontinuation due to AEs, the nervous system, and psychiatric disorders compared with a placebo. The effect on drug tolerance is uncertain.

Research that supports *Cannabis* relief of MS-related pain and spasticity was evaluated in 1015 people with MS in the United States and Canada. They participated in an informational webinar on *Cannabis* to control MS symptoms [62]. The attendees were presented with three questions about their use of *Cannabis* for MS symptoms and their knowledge of the medical legality of *Cannabis*.

The associations between individual characteristics (demographics, disease severity, and legal status), *Cannabis* use, and knowledge of legality were analyzed. Of the 1015 webinar registrants, 54% ($n = 548$) answered the question. Statistically significant associations were seen between *Cannabis* use and local legal status of *Cannabis* (recreational vs. not legal: OR 4.55, 95% CI: [1.70–12.14], $p = 0.002$), years since medical legalization of *Cannabis* (for each year since legalization: OR 1.06, 95% CI: [1.02–1.10], $p = 0.004$), disease severity (severe vs. mild disability: OR 3.41, 95% CI: [1.23–9.46] $p = 0.018$), and gender (male vs. female: OR 2.33, 95% CI: [1.10–4.94], $p = 0.027$).

Accuracy of knowledge of local *Cannabis* legality was significantly associated with *Cannabis* use in the past year (users vs. non-users: OR 2.52, $p = 0.014$), the local legal status of *Cannabis* (medical only vs. not legal: OR 0.30, $p = 0.001$; recreational vs. not legal: OR 4.98, $p = 0.039$), years since the legalization of *Cannabis* (for each increased year since legalization: OR 1.15, $p < 0.001$), and country of residence (Canada vs. the United States: OR 0.42, $p = 0.021$). The data support that *Cannabis* uses for MS symptoms are more common where *Cannabis* laws are more permissive and where *Cannabis* has been legal for longer, in those with more severe MS and men. Accurate knowledge of local *Cannabis* legality is more common in those who report using *Cannabis* for their MS in the past year. Those living in states where *Cannabis* is legal for medical use only are less likely to be aware of its legal status than those in either recreationally legal or non-legal states. However, awareness of legal status has increased with time since medical legalization.

Cannabis is used recreationally by older adults and patients with neurological and psychiatric disorders to alleviate symptoms associated with their condition. A

large study with participants from 31 countries showed that 24.1% of *Cannabis* users are between the ages of 50 and 60, 5.8% between 61 and 70, and 0.6% are older than 70. *Cannabis* use has quadrupled among 55–59-year-olds (1.6–7.4%) and doubled among 60–64-year-olds (2.4–4.4%) between 2002 and 2012. A review of the perceived benefits and adverse mental health effects of *Cannabis* use in people with ALS, MS, AD, PD, bipolar disorder, and schizophrenia indicates that *Cannabis* use diminishes some associated symptoms.

Cannabis use decreases pain and spasticity in people with MS, reduces tremor, rigidity, and pain in people with PD, and improves the quality of life of ALS patients by improving appetite and decreasing pain and spasticity [43]. *Cannabis* use is more common among people with schizophrenia than among healthy controls. *Cannabis* use is a risk factor for schizophrenia which increases positive symptoms in schizophrenia patients and diminishes negative symptoms. *Cannabis* use worsens bipolar disorder, and there is no evidence that bipolar patients derive any benefit from *Cannabis*. In late-stage Alzheimer's patients, *Cannabis* products may improve food intake, sleep quality, and diminish agitation. However, chronic *Cannabis* use may lead to cognitive impairments and dependence.

The increased use of *Cannabis* for the treatment of neurological disorders is a combination of the dramatic increase in delta-9-THC levels in *Cannabis* which could lead to a rise in the number of people who experience psychological effects. In addition to *Cannabis*, *Cannabis*-based treatments such as nabiximols (trade name Sativex, *Cannabis* plant extract, 1:1 ratio of CBD and delta-9-THC), dronabinol (trade name Marinol, synthetic delta-9-THC), and nabilone (trade name Cesamet, synthetic cannabinoid with chemical structure similar to delta-9-THC) have also been used by people with brain disorders. Nabiximols are already being used in several countries to treat spasticity associated with multiple sclerosis. Nabilone and dronabinol have been approved by the US Food and Drug Administration to treat nausea and vomiting associated with chemotherapy for cancer and stimulate appetite in AIDS patients with wasting syndrome.

The American Academy of Neurology recommendations, an evidence-based guideline for complementary and alternative medicine (CAM) use in multiple sclerosis, stated that clinicians might offer oral *Cannabis* extract for spasticity symptoms and pain (excluding central neuropathic pain) (Level A). Clinicians might offer delta-9-tetrahydrocannabinol for spasticity symptoms and pain (excluding central neuropathic pain) (Level B). Clinicians should counsel patients that the agents are likely ineffective for objective spasticity (short term)/tremor (Level B) and likely effective for spasticity and pain (long term) (Level C).

Clinicians might offer Sativex oromucosal cannabinoid spray (nabiximols) for spasticity symptoms, pain and urinary frequency (Level B). Clinicians should counsel patients that the agents are probably ineffective for objective spasticity/urinary incontinence (Level B). Clinicians might choose not to offer the agents for tremors (Level C). Clinicians might counsel patients that magnetic therapy is probably effective for fatigue and probably ineffective for depression (Level B); fish oil is probably ineffective for relapses, disability, fatigue, MRI lesions, and quality of life (QOL) (Level B); *Ginkgo biloba* is ineffective for cognition (Level A) and possibly

effective for fatigue (Level C); reflexology is possibly effective for paresthesia (Level C); Cari Loder regimen is possibly ineffective for disability, symptoms, depression, and fatigue (Level C); and bee sting therapy is possibly ineffective for relapses, disability, fatigue, lesion burden/volume, and health-related QOL (Level C). Cannabinoids may cause adverse effects.

Complementary and alternative medicine (CAM) therapies are nonconventional therapies used in addition to or instead of physician-recommended therapies [116]. CAM use is prevalent in 33–80% of patients with MS, particularly among female patients with higher education levels. The safety/efficacy of other CAM/CAM interactions with MS disease-modifying therapies is unknown. Clinicians should exercise caution regarding standardized vs. non-standardized *Cannabis* extracts and overall CAM quality control/nonregulation.

Sativex is one of the first *Cannabis*-based medicines to undergo conventional clinical development and to be approved as prescription medicine. It is an oromucosal spray that allows flexible, individualized dosing. Patients self-titrate their overall dose and pattern of dosing according to their response to and tolerance of the medicine. That procedure usually results in administering approximately 8–12 sprays/per day [148]. Each spray delivers tetrahydrocannabinol 2.7 mg and cannabidiol 2.5 mg, giving an approximate average dose of tetrahydrocannabinol 22–32 mg/day and cannabidiol 20–30 mg/day.

Development has concentrated on treating symptoms of multiple sclerosis, notably spasticity and neuropathic pain, as well as treating neuropathic pain of other etiologies. Sativex has been approved for use in neuropathic pain due to multiple sclerosis. Positive results in placebo-controlled trials of using Sativex as an add-on therapy demonstrate that Sativex is efficacious and well-tolerated in treating the symptoms. Other studies replicate the previously observed results, further approvals for treating spasticity in multiple sclerosis and neuropathic pain.

The use of *Cannabis* as medical therapy to treat chronic pain and spasticity in patients with multiple sclerosis is increasing. In a prospective observational safety study, 28 patients with MS were treated with medical *Cannabis* oils (delta-9-THC-rich, CBD-rich, and delta-9-THC + CBD combined products). They were followed during a titration period of 4 weeks [59]. The patients were evaluated at treatment and after 4 weeks of treatment. At each visit, neurological examination (Expanded Disability Status Scale [EDSS]), ambulation (Timed 25-Foot Walk Test [T25FWT]), routine blood tests, plasma cannabinoids, dexterity (9-Hole Peg Test [9-HPT]), and processing speed (Symbol Digit Modalities Test [SDMT]) were tested. Adverse events and tolerability were reported during the 4-week visit. Second, the efficacy of medical *Cannabis* on pain, spasticity, and sleep disturbances was measured by a numeric rating scale (NRS-11) each day during the 4-week treatment period.

During treatment with *Cannabis* preparations containing 10–25 mg/mL delta-9-THC, the most common AEs were dry mouth, drowsiness, dizziness, and mild to moderate nausea. Two patients experienced pronounced symptoms of excessive dreaming and drowsiness, which stopped treatment during titration. Three serious adverse events were reported but were not associated with the treatment. The mean doses of delta-9-THC and CBD were 4.0 mg and 7.0 mg, respectively, primarily

administered as a once-daily evening dose. Pain decreased from a median NRS score of 7–4 ($p = 0.01$), spasticity decreased from a median NRS score of 6–2.5 ($p = 0.01$), and sleep disturbances decreased from a median NRS score of 7–3 ($p < 0.001$). The patients observed no impairment in disability, ambulation, dexterity, or processing speed. The treatment was safe and well tolerated and reduced pain intensity, spasticity, and sleep disturbances in MS patients.

Evidence supports that cannabinoids reduce self-reported spasticity and neuropathic pain in people with MS (PwMS). A survey on treatments used by PwMS, focusing on complementary and alternative medicine, was sent to PwMS living in Oregon and Southwest Washington [90]. The survey included questions about current and past *Cannabis* use, route of administration, perceived benefits, and personal demographics. Of 1188 returned surveys, 1000 had at least 75% complete responses and completed the current and past *Cannabis* use questions. Thirty percent ($n = 303$) of the respondents reported currently using *Cannabis*, 21% ($n = 210$) used in the past but not currently, and 49% ($n = 487$) had never used *Cannabis*. Among current users, rates of use by smoking, vaping, topicals, tinctures, oils, or edibles were similar (35–46%), and most (59%) reported using multiple routes of administration. Most (64–78%, varying by route) current and past users reported *Cannabis* being very or somewhat beneficial for their MS.

The odds of current *Cannabis* use were higher in PwMS who: were younger (OR 2.24 [95% CI 1.39–3.61] for those aged 18–40 compared with those greater than age 60); had lower household income (OR 3.94 [95% CI 2.55–6.09] with annual income <\$25 k compared with those with >\$100 k); had secondary progressive MS (OR 1.77 [95% CI 1.07–2.92]); had more than minimal MS disability (OR 2.05 [95% CI 1.03–4.10] for those using a walker compared to those with none/minimal disability). The odds of perceiving *Cannabis* as beneficial for MS were higher in (1) younger individuals (OR 5.61 [95% CI 2.62–11.98] for those aged 18–40 compared with age >60); (2) those with lower household income (OR 3.35 [95% CI 1.65–6.80] with annual income <\$25 k compared with those with >\$100 k), (3) those not currently using disease-modifying therapies (OR 2.32 [95% CI 1.30–4.13]), and (4) those with the greatest disability (OR 17.96; [95% CI 2.00–161.22]).

In the survey, 30% of PwMS reported currently using *Cannabis* for their MS, mostly by multiple routes of administration. Most of the people reported *Cannabis* being helpful for their MS. People who were younger, had lower household income, had progressive disease, and had more than minimal disability, were more likely to use *Cannabis* and report it was beneficial for their MS. People who were not using disease-modifying therapies were also more likely to report benefit from *Cannabis* use.

Canada has the highest incidence of MS worldwide. Anecdotal evidence reveals that people with MS smoke, ingest, or vaporize *Cannabis* for some reason. A consecutive sample of people with MS ($n = 246$) attending a neurology ($n = 118$) and a neuropsychiatry clinic ($n = 107$) was enrolled and asked to complete a questionnaire containing the demographic, disease, and *Cannabis*-related variables [74]. Of the 246 people who approached, 225 (91.8%) agreed to participate.

The attitude towards *Cannabis* revealed that 122 (54.3%) participants approved of the drug, while 75 (33.2%) were neutral. Legalization was endorsed by 98 (43.7%) participants, while 98 (43.7%) were in favor of legalization for medical use only. Forty-four (19.5%) people endorsed current use, with 125 (56.1%) reporting lifetime use. If *Cannabis* were legal, 113 (50.2%) participants would use it.

One in five persons currently uses *Cannabis* for reasons that differ between neuropsychiatry and neurology clinics. The participants attending the neuropsychiatry clinic were more likely to use *Cannabis* for managing depression ($\chi^2 = 4.99$; $p = 0.03$) and pain ($\chi^2 = 3.85$; $p = 0.05$). The most common symptoms for which *Cannabis* was being used were sleep (86%), pain (75%), anxiety (73%), and spasticity (68%).

A placebo-controlled, crossover trial involving adult patients with multiple sclerosis and spasticity was conducted with participants from a regional clinic or by referral from specialists [103]. The participants were randomly assigned to either the intervention (smoked *Cannabis* once daily for 3 days) or control (identical placebo cigarettes once daily for 3 days). Each participant was assessed daily before and after treatment. After a washout interval of 11 days, the participants crossed over to the opposite group.

The primary outcome was a change in spasticity as measured by patient score on the modified Ashworth scale. The secondary outcomes included patients' perception of pain (as measured using a visual analog scale), a timed walk, and changes in cognitive function (as measured by patient performance on the Paced Auditory Serial Addition Test) to ratings of fatigue.

Thirty-seven participants were randomized at the start of the study, and 30 completed the trial. Treatment with smoked *Cannabis* reduced patient scores on the modified Ashworth scale by an average of 2.74 points more than placebo ($p < 0.0001$). In addition, the treatment reduced pain scores on a visual analog scale by an average of 5.28 points more than the placebo ($p = 0.008$). Scores for the timed walk did not differ significantly between the treatment and placebo ($p = 0.2$). Scores on the Paced Auditory Serial Addition Test decreased by 8.67 points more with treatment than with placebo ($p = 0.003$). No serious adverse events occurred during the trial.

Smoked *Cannabis* was superior to placebo in symptom and pain reduction in participants with treatment-resistant spasticity. Future studies should examine whether different doses can produce similar beneficial effects with less cognitive impact. *Spasticity* is a common and disabling symptom that remains a substantial problem for many patients with multiple sclerosis. Some patients have adverse effects from conventional antispasticity medications; for others, spasticity persists despite treatment.

A report from the Institute of Medicine in the United States concluded that the active compounds of *Cannabis* are potentially effective in treating neurologic conditions. There is evidence that the cannabinoid receptors CB1 and CB2 may be involved in the control of spasticity in multiple sclerosis and that the endogenous ligand of CB1, anandamide, is an effective antispasticity agent [104]. CB1 receptors are primarily presynaptic; their activation inhibits calcium influx and glutamate

release and reduces neuronal excitability by activating somatic and dendritic potassium channels. Although many patients with multiple sclerosis endorse smoking *Cannabis* as therapy, evidence that it relieves spasticity is largely anecdotal, as most trials focus on orally administered cannabinoids. We sought to assess the safety and efficacy of smoked *Cannabis* versus placebo in patients with multiple sclerosis who have treatment-resistant spasticity.

Sativex, an oromucosal spray containing *Cannabis*-based products, effectively reduces spasticity symptoms in MS patients. A study examined the cost-effectiveness of Sativex plus oral antispasticity medicines compared with the current standard treatment for moderate or severe spasticity in MS patients. A Markov model was used to assess the costs of the treatments based on their effects on patients' quality of life. The main outcome was the incremental cost-effectiveness ratio (ICER) in terms of costs per additional QALY gained over 5 years of treatment.

One-way, multi-way, and probabilistic sensitivity analyses were conducted to explore the impact of uncertainties on the findings. In the base case, Sativex plus oral antispasticity medicines resulted in incremental costs of £7600 and a QALY gain of 0.15 per person over 5 years (ICER = £49,300 per QALY) [the year 2009 data for costs]. The findings were sensitive to the costs of Sativex (price and dose) and differences in utilities between responders and non-responders [118].

Using a willingness-to-pay threshold of £30,000 per QALY, Sativex appears unlikely to be considered cost-effective by UK healthcare funders for spasticity in MS. This is unfortunate since Sativex use is likely to benefit some patients in the management of this common consequence of MS.

The effectiveness and long-term safety of cannabinoids in Multiple sclerosis were evaluated in a follow-up to the main Cannabinoids in the multiple sclerosis study. Six hundred and thirty patients with stable MS with muscle spasticity from 33 UK centers were randomized to receive oral delta-9-tetrahydrocannabinol or placebo in the main 15-week CAMS study. The primary outcome was a change in the Ashworth spasticity scale. Secondary outcomes were the Rivermead Mobility Index (RMI), timed 10-meter walk, UK Neurological Disability Score, postal Barthel Index, General Health Questionnaire-30, and nine category rating scales (CRSs).

Following the main study, double-blinded patients were invited to continue medication for up to 12 months in the follow-up study. Analysis of data from the 80% of patients followed up for 12 months provide evidence of a small treatment effect on muscle spasticity as measured by the change in Ashworth score from baseline to 12 months (delta-9-THC mean reduction 1.82 ($n = 154$, 95% confidence interval (CI) 0.53–3.12), *Cannabis* extract 0.10 ($n = 172$, 95% CI –0.99 to 1.19), placebo –0.23 ($n = 176$, 95% CI –1.41 to 0.94); $p = 0.04$ unadjusted for ambulatory status and center, $p = 0.01$ adjusted). There was evidence for the treatment effects of delta-9-THC on some aspects of disability. There were no major safety concerns. Overall, patients felt that the drugs helped treat their disease [121].

A randomized, placebo-controlled trial was conducted to test the notion that cannabinoids are effective on spasticity and other symptoms in 630 multiple sclerosis patients.135 The patients were treated at 33 UK centers with oral *Cannabis* extract ($n = 211$), delta-9-tetrahydrocannabinol ($n = 206$), or placebo ($n = 213$). The trial

duration was 15 weeks, and the Ashworth Scale Analysis was used. Six hundred eleven of the 630 patients were followed up for the primary endpoint. No treatment effect of cannabinoids was observed on the primary outcome ($p = 0.40$). The estimated difference in a mean reduction in total Ashworth score for participants taking *Cannabis* extract compared with placebo was 0.32 (95% CI -1.04 to 1.67). For those taking delta-9-THC versus placebo, it was 0.94 (-0.44 to 2.31).

There was evidence of a treatment effect on patient-reported spasticity and pain ($p = 0.003$), with improvement in spasticity reported in 61% ($n = 121$, 95% CI 54.6 – 68.2), 60% ($n = 108$, 52.5 – 66.8), and 46% ($n = 91$, 39.0 – 52.9) of participants on *Cannabis* extract, delta-9-THC, and placebo, respectively. The treatment with cannabinoids did not have a beneficial effect on spasticity when assessed with the Ashworth scale. However, though there was a degree of unmasking among the active treatment groups, objective improvement in mobility and patients' opinion of an improvement in pain suggest cannabinoids might be clinically useful.

The efficacy, safety, and tolerability of a standardized oromucosal whole plant *Cannabis*-based medicine (CBM) containing delta-9 tetrahydrocannabinol and cannabidiol were investigated on spasticity in MS patients.¹³⁶ A total of 189 subjects with definite MS and spasticity were randomized to receive daily doses of active preparation ($n = 124$) or placebo ($n = 65$) in a double-blind study over 6 weeks. The primary endpoint was the change in a daily subject-recorded Numerical Rating Scale of spasticity. Secondary endpoints included a measure of spasticity (Ashworth Score) and a subjective measure of spasm.

CBM is a useful new agent for treating the symptomatic relief of spasticity in MS. The primary efficacy analysis on the intention to treat (ITT) population ($n = 184$) showed the active preparation to be significantly superior ($p = 0.048$). Secondary efficacy measures were all in favor of active preparation but did not achieve statistical significance. The responder analysis favored active preparation, 40% of subjects achieved $>30\%$ benefit ($p = 0.014$). Eight withdrawals were attributed to adverse events; six were on active preparation, and two were on placebo.

Maintenance of the blind-to-treatment allocation is one of the most important means of avoiding bias in randomized controlled clinical trials. This is of particular concern in studies where outcomes are patient-reported and products with a characteristic adverse event profile. The results of an evidence-based statistical approach to exploring the possible impact of unblinding to a *Cannabis*-based medicine, Sativex, have been reported in people with muscle spasticity due to multiple sclerosis. The relationship between factors that might permit patients to identify their treatment allocation and the effect of treatment on the self-reported primary outcome measure was investigated using a general linear model where the dependent variable was the change from baseline in patient self-reported spasticity severity and the various possible explanatory factors were regarded as fixed factors in the model [124].

The analysis included 666 patients in three phase III placebo-controlled studies. There was no significant relationship between the effect of Sativex on spasticity and the prior use of *Cannabis* or the incidence of the specific adverse events. Nor was there any significant relationship between the prior use of *Cannabis* and the

incidence of the specific adverse events, nor between prior use of *Cannabis* and dose of Sativex. Overall the proportion of patients with prior experience with *Cannabis* was fairly similar between the two treatment groups: 121 of 363 (33%) in the Sativex group and 110 of 303 (36%) in the Placebo group. After consolidating the AEs, there were 135 different preferred terms: 34 in gastrointestinal (GI) disorders, 23 in general disorders and administration site conditions, 45 in nervous system disorders, and 33 in psychiatric disorders. Twenty-five of them occurred in ten or more patients.

Five randomized controlled trials (RCTs) compared the benefits and harms of delta-9-THC/CBD spray with a placebo. Three hundred and sixty-eight patients with various neurological conditions, including MS, were recruited. The most common adverse events reported in trials were dizziness, sleepiness, fatigue, intoxication, and a bad taste. Delta-9-THC/CBD spray significantly reduced some trials' neuropathic pain, spasticity, muscle spasms, and sleep disturbances. Long-term safety and the potential for dependence, abuse, misuse, and diversion are unknown [125].

There is increasing evidence to suggest that *Cannabis* can ameliorate muscle spasticity in multiple sclerosis, as shown in experimental autoimmune encephalomyelitis (EAE) models. A study investigated the involvement of CB1 and CB2 cannabinoid receptors in the control of experimental spasticity. Spasticity was induced in wild-type (WT) and CB1-deficient mice after developing relapsing, experimental autoimmune encephalomyelitis. The resistance to flexion against a strain gauge following the administration of CB1 and CB2 agonists measured spastic-hindlimb stiffness [132].

The CB1 receptor controls spasticity, and cross-reactivity to this receptor accounts for the therapeutic action of some CB2 agonists. As previously suggested, some CB2-selective agonists (RWJ400065) could inhibit spasticity. However, the antispastic activity of RWJ400065 and the therapeutic effect of non-selective CB1/CB2 agonists (R(+)-WIN55212-2 and CP55, 940) were lost in spastic, CB1-deficit mice.

As the CB1 receptor also mediates cannabinoid-induced psychoactivity, it will be difficult to truly dissociate the therapeutic effects from the well-known adverse effects of cannabinoids when using *Cannabis* as a medicine. The lack of knowledge on the true diversity of the cannabinoid system and the lack of total specificity of current cannabinoid reagents makes interpreting in vivo results difficult if using a purely pharmacological approach. Gene knockout technology provides an important tool in target validation and indicates that the CB1 receptor is the main cannabinoid target for an antispastic effect [133].

Conventional drugs have a limited impact on spasticity associated with multiple sclerosis and are rarely satisfactory. A solution for oral transmucosal delivery (spray) containing a mixture of *Cannabis* extracts (2.7 mg of delta-9-tetrahydrocannabinol +2.5 mg of cannabidiol per spray) has been granted marketing authorization in France for patients who are inadequately relieved by standard treatments.

Three double-blind, placebo-controlled trials in about 300 patients tested this combination and ongoing treatment for 6–14 weeks [134]. Individually, none of the trials showed any tangible antispastic efficacy, but two combined analyses showed response rates of about 35% with the mixture versus about 25% with placebo. In the trial with 572 patients, 241 who responded after 4 weeks of treatment were randomized to continue using the *Cannabis* extract or receive a placebo. Twelve weeks later, 75% of patients using the extract were still responders, versus 51% of patients who switched to placebo.

The principal adverse effects of the *Cannabis* extracts consist of neuropsychiatric disorders that resolve on treatment withdrawal. The potential for abuse increases with the dose and is tangible from 16 sprays per day. Pharmacokinetic interactions due to P-glycoprotein inhibition are likely. Treatment during pregnancy may lead to neonatal withdrawal symptoms. In practice, about 10% of patients in whom standard antispastic medications are unsatisfactory benefit from a specific effect of the *Cannabis* extracts contained in this oral spray. There is increasing evidence to suggest that *Cannabis* can ameliorate muscle spasticity in multiple sclerosis, as shown in the experimental autoimmune encephalomyelitis models.

The lack of knowledge on the true diversity of the cannabinoid system coupled with the lack of total specificity of current cannabinoid reagents interprets in vivo results as difficult if using a purely pharmacological approach. Gene knockout technology provides an important tool in target validation and indicates that the CB1 receptor is the main cannabinoid target for an antispastic effect. The CB1 receptor controls spasticity, and cross-reactivity to this receptor accounts for the therapeutic action of some CB2 agonists. A study investigated the involvement of CB1 and CB2 cannabinoid receptors in the control of experimental spasticity [134].

Spasticity was induced in wild-type and CB1-deficient mice after developing relapsing, experimental autoimmune encephalomyelitis. The resistance to flexion against a strain gauge following the administration of CB1 and CB2 agonists measured spastic-hindlimb stiffness. As the CB1 receptor also mediates cannabinoid-induced psychoactivity, it is difficult to dissociate the therapeutic effects from the known adverse effects of cannabinoids when using *Cannabis* as a medicine. Some CB2-selective agonists (RWJ400065) inhibit spasticity. However, the antispastic activity of RWJ400065 and the therapeutic effect of non-selective CB1/CB2 agonists (R(+)-WIN55212-2 and CP55, 940) were lost in spastic, CB1-deficit mice.

A parallel-group, double-blind, randomized, placebo-controlled study was undertaken in three centers to determine whether a *Cannabis*-based medicinal extract (CBME) benefits a range of symptoms due to Multiple sclerosis [135]. The study involved 160 outpatients with MS who were experiencing significant problems from at least one of the following: spasticity, spasms, bladder problems, tremor, or pain. The interventions were oromucosal sprays of a matched placebo or whole plant CBME containing equal amounts of delta-9-tetrahydrocannabinol and cannabidiol at 2.5–120 mg each daily, in divided doses.

The primary outcome measure was a Visual Analogue Scale (VAS) score for each patient's most troublesome symptom. Additional measures included VAS scores of other symptoms and measures of disability, cognition, mood, sleep, and

fatigue. Following CBME, the primary symptom score reduced from the mean (SE) of 74.36 (11.1) to 48.89 (22.0) following CBME and from 74.31 (12.5) to 54.79 (26.3) following placebo [ns]. Spasticity VAS scores were significantly reduced by CBME (Sativex) in comparison with placebo ($p = 0.001$). There were no significant adverse effects on cognition or mood, and intoxication was generally mild.

VSN16R was synthesized as an anandamide analog to be used as an anti-metabolite to identify drugs that target spasticity in MS. Several biochemical, pharmacological, and electrophysiological approaches, using isolated cells, tissue-based assays, and in vivo animal models were used to demonstrate the activity, efficacy, pharmacokinetics, and mechanism of action of VSN16R. VSN16R had nanomolar activity in tissue-based, functional assays and dose-dependently inhibited spasticity in a mouse experimental encephalomyelitis model of MS. The effect occurred over a 1000-fold therapeutic window without affecting normal muscle tone. Efficacy was achieved at plasma levels that are feasible and safe in humans.

VSN16R did not bind to known CB1/CB2/GPR55 cannabinoid-related receptors in receptor-based assays but acted on a vascular cannabinoid target. The mechanism was identified as the major neuronal form of the big conductance, the calcium-activated potassium channel (BKCa). Drug-induced opening of neuronal BKCa channels induced membrane hyperpolarization, limiting excessive neural excitability and controlling spasticity [139]. The neuronal form of the BKCa channel, as the target for VSN16R, was identified and demonstrated that its activation alleviates neuronal excitability and spasticity in an experimental model of MS, revealing a novel mechanism to control spasticity.

Sativex, a synthetic combination of cannabidiol and delta-9-tetrahydrocannabinol, has been approved to treat symptoms of spasticity. In animals, CBD effectively reduces the amounts of T-cell infiltrates in the spinal cord, indicating that CBD has anti-inflammatory properties. CBD has been shown to delay symptom onset in animal models of multiple sclerosis and slow disease progression [2]. Importantly, combinations of CBD and delta-9-THC appear more effective in treating animal models of multiple sclerosis. While CBD reduces the amount of cell infiltrates in the spinal cord, delta-9-THC reduces spasticity scores. In human studies, the results are less encouraging and conflict with animal findings. Drugs that deliver a combination of delta-9-THC and CBD in a 1:1 ratio appear to be only moderately effective in reducing spasticity scores but appear to be almost as effective as current front-line treatments and cause less severe side effects than other treatments, such as baclofen, a GABA-B receptor agonist, and tizanidine, an $\alpha 2$ adrenergic receptor agonist [146].

Spasticity is a common neurologic condition in patients with multiple sclerosis, stroke, cerebral palsy, or an injured spinal cord. Animal studies suggest that delta-9-THC has an inhibitory effect on polysynaptic reflexes. Some spastic patients claim improvement after inhaling *Cannabis*. Muscle tone, reflexes, strength, and performance of EMGs before and after double-blinded oral administration of either 10 or 5 mg delta-9-THC or placebo were tested. The blinded examiner correctly identified the trials in which the patients received delta-9-THC in seven cases. For the group, 10 mg delta-9-THC significantly reduced spasticity by clinical measurement

($p = 0.01$). Quadriceps EMG interference pattern was reduced in the four patients with primarily extensor spasticity. Delta-9-THC was administered to eight other patients with spasticity and other CNS lesions [148]. Responses varied, but the benefit was seen in three patients with tonic spasms. No benefit was noted in patients with cerebellar disease.

Cannabis-Based Treatments for Tremors

Animal studies indicated that cannabinoid receptor agonists diminish tremors and spasticity in mouse models for MS. Preclinical studies revealed that spasticity, associated with MS, is diminished by CB1, but not CB2, receptor agonists [20]. Cannabinoids have neuroprotective effects due to their action on microglial cells [21]. *Cannabis* has decreased spasticity and pain in MS patients but negatively affects posture and balance [22]. Furthermore, both nabiximols and delta-9-THC decrease spasticity in MS patients. Oral *Cannabis* extract has proven to be very effective in treating central pain. In addition, people with MS often suffer from severe bladder dysfunction due to the disruption of neuronal transmission between the brain and the bladder. Evidence revealed that nabiximols, but not dronabinol or oral *Cannabis* extract improve bladder function in people with MS [23]. *Cannabis-based* treatments did not reduce tremors in patients with MS.

Chronic relapsing experimental allergic encephalomyelitis is an autoimmune model of multiple sclerosis. Although both diseases are typified by relapsing-remitting paralytic episodes, after CREA induction by sensitization to myelin antigens, Biozzi ABH mice also develop spasticity and tremor. The symptoms also occur during multiple sclerosis and are difficult to control, thus prompting some patients to find alternative medicines and perceive benefits from *Cannabis* use.

The value of *Cannabis* use in multiple sclerosis remains anecdotal, and cannabinoid receptor agonism using R(+)-WIN 55212, delta-9-tetrahydrocannabinol, methanandamide, and JWH-133 quantitatively ameliorated both tremor and spasticity in diseased mice. The exacerbation of the signs after antagonism of the CB1 and CB2 receptors, notably the CB1 receptor, using SR141716A and SR144528 indicates that the endogenous cannabinoid system may be tonically active in the control of tremor and spasticity. This evidence provides a rationale for patients' indications of the therapeutic potential of *Cannabis* in the control of the symptoms of multiple sclerosis. It provides a means of evaluating more selective cannabinoids [113].

Data from animal model experiments and subjective and small objective studies involving patients suggest that *Cannabis* may be an effective treatment for tremors associated with MS. A randomized, double-blind, placebo-controlled crossover trial was conducted to examine the effect of oral cannador (*Cannabis* extract) in 14 patients with MS with upper limb tremors. There were eight women and six men, with a mean age of 45 years and a mean Expanded Disability Status Scale score of 6.25. The patients were randomly assigned to receive each treatment, and the doses escalated over 2 weeks before each assessment. The primary outcome was changed

on a tremor index, measured using a validated tremor rating scale. Secondary outcomes included accelerometry, an ataxia scale, spiral drawing, finger tapping, and nine-hole pegboard test performance. The study was powered to detect a functionally significant 50% improvement in the tremor index.

The data analysis showed no significant improvement in any objective measures of upper limb tremor with *Cannabis* extract compared to placebo. Finger tapping was faster on placebo than on *Cannabis* extract ($p < 0.02$). However, there was no significant trend for patients to experience more subjective relief from their tremors while on *Cannabis* extract compared to placebo [119].

***Cannabis* and Cognitive Impairment**

Multiple sclerosis has been associated with cognitive impairments, depression, and anxiety. It has been estimated that about 50% of MS patients have cognitive deficits and suffer from depression. Multiple sclerosis patients who smoke *Cannabis* have more severe cognitive impairments than non-users. Patients with MS who smoke *Cannabis* perform poorly on tests for information processing speed, working memory, executive functioning, and visuospatial perception compared to ALS patients who do not smoke *Cannabis* [24]. Multiple sclerosis patients who used *Cannabis* were also twice as likely to be considered cognitively impaired.

Furthermore, in MS patients who use *Cannabis*, there is a correlation between cognitive impairments and reductions in gray and white matter volume in medial and lateral temporal regions, thalamus, basal ganglia, and prefrontal regions [25]. So far, there is no evidence that *Cannabis* use affects anxiety and depression in MS patients. Overall, studies indicate that *Cannabis* use may diminish spasticity and pain associated with MS, but chronic *Cannabis* use has a detrimental effect on cognition in MS patients.

Evidence suggests that smoking *Cannabis* further impairs cognition in people with MS and is linked to impaired functional brain changes. However, no such association has been reported between *Cannabis* use and structural brain changes. Twenty patients with MS who smoke *Cannabis* for symptom relief and 19 matched non-*Cannabis*-smoking MS patients were given the Brief Repeatable Neuropsychological Battery and structural MRI scans. Images were segmented into gray matter (GM) and white matter and subsequently analyzed with Partial Least Squares, a data-driven multivariate technique that explores brain-behavior associations.

In both groups, the Partial Least Squares analysis yielded significant correlations between cognitive scores and both gray matter (33% variance, $p < .0001$) and white matter (17% variance, $p < .05$) volume. Gray matter volume in the thalamus, basal ganglia, medial temporal, and medial prefrontal regions, and white matter volume in the fornix correlated with cognitive deficits. The analysis indicated that brain volume reductions were associated with more extensive cognitive impairment in the *Cannabis* versus the non-*Cannabis* MS group. The results indicate that *Cannabis*

use in MS results in more widespread cognitive deficits, which correlate with tissue volume in subcortical, medial temporal, and prefrontal regions.

Cognitive impairment occurs in approximately 40–60% of patients with multiple sclerosis and is associated with structural and functional brain changes [26]. Approximately 14–18% of MS patients use *Cannabis* for symptomatic relief from pain, spasticity, and insomnia. Evidence for the enduring effects of *Cannabis* on cognition and cerebral integrity in non-MS samples is equivocal, with negative studies offset by others revealing deficits in episodic memory, attention, and executive functioning, gray matter atrophy in the hippocampus and basal ganglia, and decreased white matter integrity in hippocampal efferent connections [27]. Given the effects of MS on the brain, patients who smoke *Cannabis* may be particularly vulnerable to similar cerebral changes and associated cognitive deficits.

Some patients with multiple sclerosis smoke *Cannabis* to relieve symptoms, including spasticity and pain. Recent evidence suggests that smoking *Cannabis* further impairs cognition in people with MS and is linked to impaired functional brain changes [25]. However, no such association has been reported between *Cannabis* use and structural brain changes.

Twenty patients with MS who smoke *Cannabis* for symptom relief and 19 matched non-*Cannabis*-smoking MS patients were given the Brief Repeatable Neuropsychological Battery and structural MRI scans. Images were segmented into gray matter and white matter and subsequently analyzed with Partial Least Squares, a data-driven multivariate technique that explores brain–behavior associations.

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Cognitive dysfunction affects 40–80% of patients with multiple sclerosis. Smoking *Cannabis* may add to the deficits, and it is unclear whether coming off *Cannabis* results in cognitive improvement. To address this dilemma, 40 patients with multiple sclerosis who started using *Cannabis* after the onset of multiple sclerosis and used it for at least 4 days a week over many years were divided by odd-even number selection into two groups: *Cannabis* continuation (CC) and *Cannabis* withdrawal.

Assessments took place at baseline and after 28 days and included serial versions of the Brief Repeatable Neuropsychological Battery for Multiple Sclerosis, containing tests of verbal and visual memory, processing speed, and executive function; structural and functional MRI, the latter entailing a compatible version of the Symbol Digit Modalities Test and urine for cannabinoid metabolites to detect compliance with abstinence.

Only the participants deemed globally impaired at baseline (failure on at least two cognitive domains) were enrolled. The results revealed that the two groups were well-matched demographically and neurologically. One subject was removed from

the withdrawal group because of failed abstinence. Urine analysis revealed that the cannabinoid consumed was predominantly delta-9-tetrahydrocannabinol. There were no baseline between-group cognitive differences, but by day 28, the withdrawal group performed significantly better on every cognitive index ($p < 0.0001$). Significant differences were present for every test, but only in the abstinent group ($p < 0.0001$). There were no between-group baselines or, on day 28, differences in structural MRI indices (global atrophy, total T1 and T2 lesion volume).

At index assessment, the two groups had a similar performance on the functional MRI-compatible Symbol Digit Modalities Test, and there were no group differences in brain activation. However, by day 28, the withdrawal group completed more trials correctly ($p < 0.012$) and had a faster reaction time ($p < 0.002$), associated with significantly increased activation in brain regions known to be associated with the performance of the test (bilateral inferior frontal gyri, caudate, and declive/cerebellum, $p < 0.001$).

The results reveal that patients with multiple sclerosis who are frequent, long-term *Cannabis* users can show significant improvements in memory, processing speed, and executive function after 28 days of drug abstinence. The absence of similar improvements in a matched multiple sclerosis group that remained on *Cannabis* shows that beneficial cognitive change after stopping *Cannabis* is not solely attributable to the effects of the practice [65].

Cannabis use has been negatively associated with cognitive function in PwMS. The morphological effects on cognitive function in *Cannabis* users and non-users with MS were investigated utilizing MRI [25]. It was found that regional volumes correlated with cognitive performance, although the regions and the strength of the correlations differed between the users and non-users. For example, white matter volume correlated only with tests involving processing speed in non-*Cannabis* using PwMS, while this metric correlated to all cognitive measures in *Cannabis* users, an indication of a maladaptive process in the *Cannabis* users, requiring additional brain circuit usage to perform cognitive tasks. No association between the resting activity of any region and PASAT scores was found in one study, and correlations performed for each group did not indicate an association between the variables [64]. The findings suggest that *Cannabis* use may enhance deleterious cognitive performance, as evidenced by the lower PASAT scores. However, the mechanism of the possible effect is not thoroughly understood.

To study the possible psychopathological symptoms and cognitive deficits, abuse induction, and general tolerability and effects on quality of life, fatigue, and motor function in *Cannabis*-naïve patients with multiple sclerosis, the patients were treated with a free-dose *Cannabis* plant extract (Sativex). In an 8-week, randomized, double-blind, placebo-controlled, parallel-group crossover trial, 17 *Cannabis*-naïve patients with MS were assessed at baseline and at the end of the *Cannabis* and placebo phases of the trial (each of 3 weeks) utilizing Symptom Checklist-90 Revised, Self-rating Anxiety Scale, Multiple sclerosis Functional Composite (of which one dimension is the Paced Auditory Serial Additional Test that was used to evaluate cognition), Visual Analogue Scale on health-related quality of life, Multiple sclerosis Impact Scale-29, and Fatigue Severity Scale.

Post-placebo versus post-cannabinoid scores showed no significant differences in all the variables under study. A significant positive correlation was found between delta-9-tetrahydrocannabinol blood levels and scores at the General Symptomatic Index and at the “interpersonal sensitivity,” “aggressive behavior,” and “paranoid tendencies” subscales of the Symptom Checklist-90 Revised. No serious adverse events, abuse tendencies, or direct withdrawal symptoms were reported. Increased desire for Sativex with secondary depression was reported in one subject. Cannabinoid treatment did not induce psychopathology and did not impair cognition in *Cannabis*-naïve patients with MS. However, the positive correlation between blood levels of delta-9-tetrahydrocannabinol and psychopathological scores suggests that at dosages higher than those used in therapeutic settings, interpersonal sensitivity, aggressiveness, and paranoid features might arise, although greater statistical power would be necessary to confirm the finding [108].

To determine the neuropsychological effects of *Cannabis* use in MS patients with cognitive deterioration, two groups of 25 patients with MS (*Cannabis* users and non-users) were administered the Minimal Assessment of Cognitive Function in MS battery of neuropsychological tests, the Hospital Anxiety and Depression Scale (HADS), and the Structured Clinical Interview for the DSM-IV Axis I Disorders (SCID-I). Group-matching and regression analysis were used to control the effects of age, sex, education, premorbid intelligence, disability, and disease course and duration on cognitive function.

The patients were 25 *Cannabis* users (11 women) and 25 non-users (12 women); the mean age was 43.60 (SD 10.7), with 33 patients (66.0%) married or co-habiting and 21 (42.0%) employed. The patients had received an average of 14.0 years (SD 2.8) of education. The average disease duration was 12.1 years (SD 9.4). The breakdown of disease course was as follows: relapsing-remitting 72.0%; secondary progressive 18.0%; primary progressive 10.0%. The median EDSS score was 3.0 (mean 3.32, SD 2.39, range 0–8.5). Twenty patients (40.0%) were taking disease-modifying drugs. Patients consumed a median of 2.0 alcoholic beverages per week (mean 3.0, SD 3.3, range 0–12). Four patients had taken steroids within the last 3 months.

The patients were administered the Minimal Assessment of Cognitive Function in MS battery of neuropsychological tests, the Hospital Anxiety and Depression Scale, and the Structured Clinical Interview for the DSM-IV Axis I Disorders to determine the neuropsychological effects of *Cannabis* in patients with cognitive deterioration. Group-matching and regression analysis were used to control the effects of age, sex, education, premorbid intelligence, disability, and disease course and duration on cognitive function. *Cannabis* users performed significantly more poorly than non-users on information processing speed, working memory, executive functions, and visuospatial perception. They were also twice as likely as non-users to be cognitively impaired globally.

Comparisons between *Cannabis* users and non-users on demographic and disease-related variables indicated no statistically significant group differences for age, sex, years of education, marital status, EDSS, disease course, duration of MS, and use of disease-modifying drugs. *Cannabis* users reported slightly higher alcohol consumption than non-users, although the difference did not reach

statistical significance. *Cannabis* users were significantly more likely to be unemployed [24].

To determine functional and structural neuroimaging correlates of cognitive dysfunction associated with *Cannabis* use in multiple sclerosis, a cross-sectional study of 20 patients with MS who smoked *Cannabis* and 19 non-*Cannabis* users with MS, matched on demographic and neurologic variables underwent fMRI while completing a test of working memory, the N-Back. Neuropsychological data on verbal (Selective Reminding Test-Revised) and visual (10/36 Spatial Recall Test) memory, information processing speed (Paced Auditory Serial Addition Test [2- and 3-second versions] and Symbol Digit Modalities Test), and attention (Word List Generation) were obtained. Resting-state fMRI and structural MRI data (lesion and normal-appearing brain tissue volumes, diffusion tensor imaging metrics) were also collected.

The *Cannabis* group performed more poorly on the more demanding Paced Auditory Serial Addition Test tasks (i.e., 2-second version) ($p < 0.02$) and the 10/36 Spatial Recall Test ($p < 0.03$). *Cannabis* users had more diffuse cerebral activation across all N-Back trials and made more errors on the 2-Back task ($p < 0.006$), during which they displayed increased activation relative to non-users in parietal ($p < 0.007$) and anterior cingulate ($p < 0.001$) regions implicated in working memory. No group differences in resting-state networks or structural MRI variables were found.

Patients with MS who smoke *Cannabis* are more cognitively impaired than non-users. *Cannabis* further compromises cerebral compensatory mechanisms, which are already faulty in MS. The imaging data boost the construct validity of the neuropsychological findings and act as a cautionary note to *Cannabis* users and prescribers [29].

***Cannabis* on Processing Speed, Memory, and Executive Functioning**

MS patients who smoke *Cannabis* show further deterioration in processing speed, memory, and executive functioning [28]. Moreover, *Cannabis* use is linked to less-efficient recruitment of brain regions during a working memory task. No MRI data show a specific association between structural brain changes and cognitive deficits in MS patients who smoked *Cannabis* [29]. Whether this reflects the absence of structural change or the limitations of the MRI analyses is unclear. Given the diffuse nature of MS-related cerebral pathology and the distribution of *Cannabis* receptors in the brain, multivariate analyses may uncover underlying effects not observable using standard univariate approaches.

Thirty-nine patients aged 18–60 years with a diagnosis of MS were recruited from MS clinics. A diagnosis of MS was confirmed according to the modified McDonald criteria. Of the 39 patient, 20 smoked *Cannabis* regularly, i.e., (daily use, $n = 17$; four to five times/week, $n = 2$; two to three times/week, $n = 1$). Concerning the reason for use, 14 of 20 patients smoked *Cannabis* to relieve physical

symptoms, two of 20 for recreational purposes, and four of 20 smoked *Cannabis* for medicinal and recreational purposes. In the *Cannabis*-smoking group, 16 of 20 patients had relapsing and remitting, and two had primary progressive. Two of 20 patients had secondary progressive MS. In the non-*Cannabis* group, 17 of 19 patients had relapsing and remitting, one of 19 patients had primary progressive, and one of 19 had secondary progressive MS.

Cannabis use was confirmed with a positive urine test for metabolites, a composite of THCCOOH glucuronide and delta-9-THC-COOH. Twelve hours before testing, the patients were asked to refrain from using *Cannabis* to prevent any behavioral effects of acute intoxication. To ensure compliance, saliva samples were collected before testing for delta-9-tetrahydrocannabinol within the last 4–6 h. The 19 MS patients who had never used *Cannabis* were matched to the *Cannabis* group on all demographic and disease-related variables. All subjects in the non-*Cannabis* group had negative urine and saliva tests.

Age, sex, handedness, years of education, employment status, disease-related variables, and Expanded Disability Status Scale scores were obtained at the testing time. Visual acuity was measured via the Snellen chart. Estimates of premorbid IQ were obtained via the Wechsler Test of Adult Reading. All patients were given the Brief Repeatable Battery of Neuropsychological Tests (BRNB) in MS, consisting of tests measuring verbal memory (Selective Reminding Test; SRT), visual memory (10/36 Spatial Recall Test), processing speed (Paced Auditory Serial Addition Test; PASAT, 2 and 3 s; Symbol Digit Modalities Test; SDMT), and verbal fluency (Word List Generation; WLG). Mood and anxiety symptoms were measured using the Hospital Anxiety and Depression Scale, using a cut-off score of eight or greater to denote clinically significant anxiety or depression. The modified fatigue impact scale (mFIS) was given to record subjective fatigue.

MRIs were collected on a 3 T MRI scanner using an 8-channel head coil. T1-weighted anatomical scans (repetition time [TR] = 8.1 ms, echo time [TE] = 3.2, flip angle = 58°, field of view [FOV] = 22 cm, 190 slices, slice thickness = 1 mm), PD/T2 scans (TR = 2500 ms, TE = 11.1/90, flip angle = 90°, FOV = 22 cm, 48 slices, slice thickness = 3 mm), and FLAIR images (TR = 9700 ms, TE = 140, FOV = 22 cm, 48 slices, slice thickness = 3 mm) were acquired for each subject. In addition, functional MRI scans were also acquired during a working memory task (n-back) and diffusion tensor imaging scans.

Images were preprocessed using SPM8, VBM8, and the Lesion Segmentation Toolbox (LST), using standard settings. This process first quantifies and fills WM lesions to limit segmentation errors caused by the presence of WM lesions [30]. After lesion filling, brain tissue was segmented and normalized to a common template space for voxel-wise testing. LST uses a lesion growth algorithm that simultaneously considers information from T1 and FLAIR images to identify white matter lesions. For the LST settings, a threshold value of $\kappa = 0.2$ was chosen and confirmed by visual inspection by a board-certified neurologist with expertise in MS as a setting that captured the majority of white matter lesions without including non-lesion tissue in the calculation of overall lesion volume.

After identification, the resultant lesion tissue was quantified and filled using each subject's average white matter intensity; this ensured an accurate estimate of gray matter (GM) volume and that our analysis included only healthy tissue. Filled T1 images were then segmented via VBM8 using default parameters: images were co-registered using linear and non-linear transformations, and tissue was segmented without tissue priors using partial volume estimation. Segmented GM and WM images were normalized using high-resolution affine registration and the non-linear DARTEL algorithm to the DARTEL template provided with VBM8 in MNI space. Images were resampled to a resolution of 1.5 mm^3 voxels and smoothed with an 8 mm full-width half-maximum Gaussian kernel.

Behavioral PLS was employed, which searches for latent variables that maximize the covariance between brain data (GM and WM voxels) and neuropsychological test scores. The analysis determines whether regional brain volume change patterns correlate with neuropsychological test scores and how the correlations may be similar or different across patient groups. Such an analysis would determine: Regional GM and WM volume are associated with cognitive performance within each patient group. Which brain regions and cognitive tests are correlated, and whether the patterns of those correlations differ between *Cannabis* and non-*Cannabis* groups.

For the analysis, raw total scores from all five neuropsychological tests (SRT, total learning score across trials; ten of thirty-six, total learning score across trials; WLG, the total number of words generated across trials; SDMT, total correct; PASAT-2, total correct) were entered simultaneously, each as individual variables of interest. Raw scores were used as both groups were well-matched in terms of age, level of education, and estimated IQ.

The statistical significance of extracted variables was evaluated by 1000 permutation tests using sampling without replacement. The robustness of each brain region's contribution to the latent variable is determined through bootstrap resampling that resampled the data 500 times with replacement to estimate the standard error of the weight of each voxel on the latent variable. Brain regions were considered reliable if they had a ratio of weight to standard error (i.e., bootstrap ratio, equivalent to a z -score) greater than 4.5, corresponding to a probability of $p < 0.0001$. Because the mathematical procedure was completed in a single step, there is no need for correcting for multiple comparisons.

Furthermore, segmented T1-weighted images using Freesurfer were used to obtain subcortical GM volumes and conduct ancillary regions of interest analyses. Specifically, the interest was in the thalamus and hippocampus, based on recent evidence demonstrating atrophy in the regions due to MS [31]. There were no significant differences between the *Cannabis* and non-*Cannabis* groups in terms of age (*Cannabis* $M = 41.30$, non-*Cannabis* $M = 43.89$, $t = 0.78$, $p = 0.44$), years of education (*Cannabis* $M = 14.30$, non-*Cannabis* $M = 15.20$, $t = -1.5$, $p = 0.14$), or IQ (*Cannabis* $M = 110.85$, non-*Cannabis* $M = 110.57$, $t = 0.97$, $p = 0.92$). There were no differences in disease duration (*Cannabis* $M = 9.50$, non-*Cannabis* $M = 9.90$, $t = -0.79$, $p = 0.44$), disability severity (EDSS; *Cannabis* $M = 2.83$, non-*Cannabis* $M = 2.47$, $t = -0.62$, $p = 0.54$), or mood symptoms (HADS-anxiety, *Cannabis*

= 13, non-*Cannabis* # = 11, $\chi^2 = 0.21$, $p = 0.65$; HADS-depression, *Cannabis* # = 11, non-*Cannabis* # = 8, $\chi^2 = 0.85$, $p = 0.42$). The *Cannabis* group showed significantly poorer performance on tests of spatial memory (10/36 recall; *Cannabis* $M = 16.40$, non-*Cannabis* $M = 20.79$ $t = -2.29$, $p = 0.03$) and information processing speed (PASAT 2 s; *Cannabis* $M = 28.35$, non-*Cannabis* $M = 39.47$, $t = -2.41$, $p = 0.03$). There were no differences between groups regarding total GM, WM, or lesion volume.

The behavioral PLS analysis extracted two latent variables that showed a significant association between tissue volume and cognition, accounting for 50% of the overall covariance. The first latent variable ($p < 0.0001$) represented the association between GM volume and cognitive performance (33% of the variance). Brain regions where GM was significantly correlated with cognitive performance included the thalamus and basal ganglia, medial temporal regions (hippocampus, amygdala, and fusiform gyrus), lateral temporal cortex, posterior parietal lobes, and both the lateral and medial prefrontal cortex.

The non-*Cannabis* and *Cannabis* groups showed differential patterns of brain-behavior correlations: In the non-*Cannabis* group, GM volume in the regions mentioned earlier was associated only with tests of processing speed (i.e., SDMT, PASAT-2), and WLG, a test of executive control that is timed and thus has a speeded component. However, in the *Cannabis* group, GM volume was associated with performance on measures of processing speed (SDMT, PASAT-2) and verbal memory (SRT).

As the PLS analysis revealed a significant correlation between subcortical GM and memory in the *Cannabis* group but not the non-*Cannabis* group, we ran additional region of interest analyses to test whether the correlations were significantly higher in the *Cannabis* group versus the non-*Cannabis* group. Specifically, as the analysis identified left hippocampal involvement in cognitive performance, correlations between verbal memory scores and left hippocampal volumes were computed via Freesurfer.

There was a significant correlation between left hippocampal volume and verbal memory in the *Cannabis* group ($r = .52$, $p = .02$) but not in the non-*Cannabis* group ($r = -.03$, $p = .90$). The magnitude of the correlation was significantly larger in *Cannabis* group compared to the non-*Cannabis* group (Fisher Z-difference = 1.73, $p = .04$, 1-tailed), indicating that *Cannabis* use moderated the association between cognition and hippocampal volume.

As a comparison, correlations between left thalamic volumes and processing speed performance were calculated (SDMT) in both groups, given the evidence suggesting the importance of the thalamus in cognitive deficits in MS, and given the SDMT is the most sensitive measure of cognitive performance in the patients [32]. There was a significant correlation between thalamic volume and processing speed in the *Cannabis* ($r = .58$, $p = .01$) and non-*Cannabis* groups ($r = .48$, $p = .04$). However, the correlations were not significantly higher in the *Cannabis* group (Fisher Z-difference = .39, $p = .35$, 1-tailed).

A link between structural brain changes and cognitive deficits was demonstrated in MS patients due to smoking *Cannabis*. GM and WM volume in medial and lateral temporal regions, thalamus, basal ganglia, and prefrontal cortex was associated with more widespread cognitive deficits in *Cannabis*-smoking MS patients compared to patients who were *Cannabis*-naïve. Decreased regional brain volume was associated with poorer performance on all neuropsychological tests in MS patients who smoked *Cannabis*, whereas only speeded tasks correlated with brain volumes in non-*Cannabis* patients. Verbal memory was significantly correlated with hippocampal volume in the *Cannabis* group but not in the non-*Cannabis* group. *Cannabis*-smoking MS patients also showed significantly lower performance on the ten of 36 spatial recall test and the PASAT.

The pattern of the results raises the notion that MS patients who smoke *Cannabis* may be less able to compensate for declines in memory due to regional volume reductions. Given that there were no group differences in overall brain volume or subcortical structures, non-*Cannabis* MS patients may utilize other strategies to compensate for their memory but not their processing speed deficits.

The results complement the fMRI data, which revealed an increased and more anatomically diffused cerebral activation pattern during a working memory task in the *Cannabis* group. Conversely, *Cannabis*-smoking MS patients, who show significantly lower processing speed and visual memory scores, appear unable to compensate for either. Thus, lower regional brain volumes in the patients were associated with poorer scores on all cognitive tests. Crucially, the functional and structural imaging findings cannot be explained by differences in demographic factors such as premorbid intelligence, disease severity, or global measures of the lesion, GM, or WM volumes, as they were not significantly different between the two MS groups.

A meta-analysis of structural brain changes in healthy, long-term users concluded that selective brain regions, such as the hippocampus, were particularly vulnerable to atrophy [33]. A systematic review of 43 studies involving chronic *Cannabis* users and matched control subjects who were non-users reported similar results, namely, selective atrophy of medial prefrontal cortices and the cerebellum in the *Cannabis* group [34]. A subsequent review confirmed the findings, highlighting the vulnerability of medial temporal and frontal regions to the deleterious effects of *Cannabis* [35].

The imaging data help explain some of the cognitive findings to emerge from studies looking at long-term, non-psychotic *Cannabis* smokers. As with the earlier structural neuroimaging data, initial findings were equivocal, with greater certainty regarding the acute adverse effects of delta-9-THC on verbal and working memory but less clarity on whether the deficits endure beyond the period of intoxication. A longitudinal study suggested that impairment may continue, particularly if *Cannabis* use started as an adolescent and continued for years.

Of particular concern is the finding that multifocal cognitive deficits can persist even after *Cannabis* cessation [36]. While the conclusion has been called into question because of a failure to control for socio-economic status; it is germane to note that some findings of cognitive impairment in non-clinical samples of *Cannabis* smokers, and a pattern of gender-specific cognitive deficits, greater memory

difficulties in females, more executive challenges in males, fit with the structural brain MRI findings of selective hippocampal and prefrontal cortex atrophy. A systematic review noted a link between hippocampal volume and memory performance in *Cannabis* users [34]. That region may be particularly sensitive to the effects of *Cannabis*, given its high concentration of endocannabinoid receptors [37].

The regions that showed structural changes with *Cannabis* use in the general population are the same as those associated with cognitive impairment. Specifically, correlations were found between GM volume in the hippocampus and poorer memory performance only in *Cannabis* users. Furthermore, it was revealed that WM volume in the fornix and connecting fimbria was associated with more widespread cognitive deficits in *Cannabis*-smoking MS patients, the same WM tracts affected by long-term *Cannabis* use in non-MS patients [27].

The results indicate that the hippocampus and its associated WM tracts are particularly vulnerable in MS patients who use *Cannabis*. The findings are also consistent with cerebral atrophy due to MS-related pathology. It is well-established that GM atrophy reliably occurs in the thalamus and putamen, with brain changes robustly linked to cognitive deficits and more extensive neurological disability [31, 32]. Hippocampal atrophy also occurs in MS and is associated with memory deficits [38]. Both patient groups showed significant correlations between thalamic volumes and tests of information processing speed.

Interestingly, it was found that GM volume reductions in the default-mode network (DMN, i.e., lateral temporal regions, precuneus, lateral parietal cortices, and medial prefrontal cortex) were also correlated with poorer cognitive performance [39]. Also, cognitively-impaired MS patients showed decreased connectivity between DMN structures compared to cognitively intact MS patients [40]. Regional volume reductions in the DMN are associated with more widespread cognitive deficits in *Cannabis*-smoking MS patients; one may assume that DMN integrity is necessary to mount any functional compensation for cognitive deficits. Thus, the region's volume loss would be associated with more global cognitive decline. The point is emphasized by a study showing that in a sample of MS patients who received cognitive rehabilitation, the extent of functional connectivity within the DMN was predictive of lasting treatment gains [41].

The lack of a matched healthy control group precluded the ability to determine whether patients' brains showed cerebral atrophy relative to healthy brains. Nevertheless, the evidence demonstrates the adverse effects of *Cannabis* use on both GM and WM in MS: for patients who smoke *Cannabis*, decreased GM, and WM volume is associated with a wider array of cognitive deficits beyond that explained by MS alone. Consequently, the potential clinical benefits of *Cannabis* use in terms of symptom relief must be weighed against the negative impact on brain health and cognition, the full extent of which is only beginning to emerge. The findings also raise the question of what amount of *Cannabis* use is required before such effects manifest or whether cognitive deficits are reversible with cessation of *Cannabis* use [42].

Studies with animals have shown that chronic exposure to *Cannabis* smoke, delta-9-tetrahydrocannabinol, or CB1 receptor agonists leads to the development of

dependence. *Cannabis* withdrawal leads to somatic withdrawal signs (e.g., abdominal constriction, wet-dog shakes, head shakes, forepaw fluttering), anxiety-like behavior, and an increased release of the stress peptide corticotropin-releasing factor in the amygdala [44]. The negative affective state associated with *Cannabis* withdrawal provides powerful motivation for continuing drug use. There is evidence that *Cannabis* and delta-9-THC impair memory and cognition in rodents.

Delta-9-THC inhibits the release of acetylcholine in the hippocampus, likely one mechanism by which it impairs memory. The observation supports that drugs that prevent the delta-9-THC-induced decrease in acetylcholine release in the hippocampus also prevent memory impairments [45]. It has also been indicated that repeated delta-9-THC administration leads to increased glutamate levels, which induces downregulation of glutamate receptors and a reduction in the density of dendritic spines on hippocampal neurons that may reduce synaptic plasticity and thereby cause memory impairments [47]. Memory impairments due to delta-9-THC exposure may gradually diminish over time. In one study, memory function returned to baseline after 4 weeks of abstinence [46]. Another study reported that memory function in mice was still impaired 3 weeks after administering one low dose of THC [48].

The added value of new psychometric methods (Rasch measurement and Item Response Theory) over traditional psychometric approaches was measured by comparing and contrasting their psychometric evaluations of existing sets of rating scale data. The Rasch measurement was focused on rather than Item Response Theory because it is the more advantageous method for health measurement from a conceptual, theoretical, and practical perspective. The intention is to provide an authoritative document that describes the principles of Rasch measurement and the practice of Rasch analysis in a clear, detailed, non-technical form that is accurate and accessible to clinicians and researchers in health measurement.

The comparison was undertaken of traditional and new psychometric methods in five large sets of rating scale data: Evaluation of the Rivermead Mobility Index (RMI) in data from 666 participants in the *Cannabis* in Multiple sclerosis studies, evaluation of the Multiple sclerosis Impact Scale (MSIS-29) in data from 1725 people with Multiple sclerosis, evaluation of test–retest reliability of MSIS-29 in data from 150 people with Multiple sclerosis, examination of the use of Rasch analysis to equate scales purporting to measure the same health construct in 585 people with Multiple sclerosis and comparison of relative responsiveness of the Barthel Index and Functional Independence Measure in data from 1400 people undergoing neurorehabilitation.

Findings from the five studies show that Rasch analysis is empirically superior to traditional psychometric methods for evaluating rating scales, developing rating scales, analyzing rating scale data, understanding and measuring stability and change, and understanding the health constructs we seek to quantify. There is considerable added value in using Rasch analysis rather than traditional psychometric methods in health measurement [112].

Acute Effects of *Cannabis* Use

Cannabis has a wide range of subjective effects. The effects may vary between light and heavy users and can include feelings of intoxication, euphoria, altered sensory perception, cognitive and perceptual distortions, anxiety, dizziness, and increased appetite. The most reliable markers of acute *Cannabis* exposure are intoxication and tachycardia. In terms of cognitive processes, there is extensive evidence that acute *Cannabis* exposure impairs attentional tasks, consolidation and retrieval of memory, working memory, verbal memory, learning, and executive functions. Impaired performance has been consistently found in multiple aspects of attention, including sustained, divided, selective, and focused.

Additionally, studies have found executive dysfunction related to *Cannabis* exposure, including disinhibition and impaired decision-making. Acute *Cannabis* intoxication in healthy young people causes slower reaction times, impaired accuracy, and impaired response inhibition. Other frontal dysfunctions observed include decreased information processing speed, poor planning, lack of self-monitoring, and inability to alter behaviors to suit changing tasks. There can also be alterations in mathematical abilities and time perception, along with changes in gross and fine motor skills.

Chronic Effects of *Cannabis* Use

One of the main adverse effects of *Cannabis* use is the development of dependence. Almost 10% of adults use *Cannabis* and one-third of users meet the criteria for *Cannabis* use disorder. *Cannabis* use disorder is characterized by a strong desire to use *Cannabis*, using larger amounts than intended and continued usage despite negative social and physical consequences, craving, tolerance, and withdrawal. It has been estimated that 30% of regular *Cannabis* users and 50–95% of heavy users experience a *Cannabis* withdrawal syndrome. *Cannabis* withdrawal is characterized by anxiety, depression, irritability, decreased sleep quality and quantity, and stomach pain.

The negative affective state plays a critical role in the maintenance of drug addiction. Some longitudinal studies have shown persistent adverse effects of *Cannabis* use on neurocognitive performance. The effects depend on the length of abstinence, age at the onset, or cumulative lifetime exposure. In one study in which participants were followed from birth to age 38, persistent *Cannabis* users had a 6-point reduction in IQ compared to non-users. Significant psychomotor dysfunction has also been reported in chronic *Cannabis* users. Some recovery of cognitive function might occur after cessation of *Cannabis* use [49].

Adolescent *Cannabis* users with 3 weeks of sobriety demonstrated resolution of learning and verbal memory deficits but continued to have difficulty with attentional tasks. In one longitudinal study in young adults, episodic memory improved over an

8-year abstinence period; this indicates that chronic *Cannabis* use can lead to loss of control over *Cannabis* use and cognitive impairments, which may diminish gradually after a prolonged abstinence period.

Chronic *Cannabis* use can also lead to what is called an amotivational syndrome. The amotivational syndrome is characterized by apathy, lack of motivation, and poor educational performance. Animal studies suggest that the amotivational syndrome is due to a delta-9-THC-induced dysregulation of dopaminergic systems [50]. A study of *Cannabis* users showed that *Cannabis* users with the highest level of apathy had the lowest dopamine synthesis capacity in the striatum [51].

A study was conducted to monitor the safety and efficacy of the long-term use of an oromucosal *Cannabis*-based medicine in patients with multiple sclerosis. A total of 137 MS patients with symptoms not controlled satisfactorily using standard drugs entered this open-label trial following a 10-week, placebo-controlled study. Patients were assessed every 8 weeks using visual analog scales and diary scores of main symptoms and were followed for an average of 434 days (range: 21–814). A total of 58 patients (42.3%) withdrew due to lack of efficacy (24), adverse events (seventeen), withdrew consent (six), loss of follow-up (three), and others (eight). The patients reported 292 unwanted effects, of which 251 (86%) were mild to moderate, including oral pain (28), dizziness (twenty), diarrhea (seventeen), nausea (fifteen), and oromucosal disorder (12).

Three patients had five serious adverse events: two seizures, one fall, one aspiration pneumonia, and one with gastroenteritis. Four patients had first-ever seizures. The improvements recorded and dosage taken in the acute study remained stable. The sudden interruption of CBM for 2 weeks in 25 patients (of 62 approached) did not cause a consistent withdrawal syndrome, although eleven (46%) patients reported at least one of tiredness, interrupted sleep, hot and cold flushes, mood alteration, reduced appetite, emotional lability, intoxication or vivid dreams. Twenty-two (88%) patients re-started CBM treatment [111].

Structural and Functional Abnormalities in Young *Cannabis* Users

There is evidence of structural and functional abnormalities in young *Cannabis* users. Chronic *Cannabis* use changes gray (cell bodies, dendrites, and synapses) and white matter (myelinated neuronal tracts) architecture [52]. There is evidence that *Cannabis* use may increase the volume of subregions of the cerebellum and amygdala in adolescents [53]. The changes in the volume of the brain sites were associated with poor executive functioning (cerebellum) and internalizing problems (amygdala). An increase in the volume of a brain site might be due to disrupted pruning of gray matter during a critical period in adolescence or possibly abnormal connectivity patterns that develop to compensate for cognitive deficits [54].

Although some studies reported that *Cannabis* use could increase the size of brain regions, most studies found that *Cannabis* use decreases the volume of brain regions. *Cannabis* use-induced decreases in brain volume have been reported for the orbitofrontal cortex, hippocampus, striatum, and amygdala [55]. One of the most consistent findings has been that *Cannabis* use decreases the volume of the hippocampus, which correlates with the amount of *Cannabis* used and the level of dependence.

Cannabis use is most common among adolescents and young adults, and this period is critical for brain myelination. Several studies investigating neuronal tracts in the brain using diffusor tensor imaging have found reductions in white matter integrity throughout the frontal and temporal lobes in adolescent *Cannabis* users [57]. Cannabinoid receptors on myelinating glial cells play a role in white matter integrity and connectivity [56]. The abnormalities associated with psychological symptoms and cognitive impairments also lead to impairments in cerebrovascular functioning, which has been associated with an increased risk for stroke [58].

The Effects of *Cannabis* on Serum Cytokines

A study to evaluate the prevalence of *Cannabis* abuse in multiple sclerosis cases and to evaluate the effect of *Cannabis* on serum cytokines was conducted [60]. One hundred fifty multiple sclerosis cases and 150 healthy controls were included during the study. The cases were subjected to history taking, neurological examination, and routine investigations. They were asked about *Cannabis* intake, which was confirmed by a urine test. Serum cytokines, including IL-1, IL-2, IL-4, IL-10, IL-12, IL-17, IL-22, IFN- γ , IFN- β 1, and TNF- α , were evaluated for all cases and control groups. Twenty-eight cases were *Cannabis* abusers (MS/*Cannabis* group, 18.67%). The remaining 122 cases represented the MS group.

Fifteen cases (53.6%) reported that *Cannabis* improved their symptoms. There was no significant difference between the three groups regarding age, disease duration, or MS type. The male gender was more predominant in the MS/*Cannabis* group, and the number of relapses was significantly lower in the same group. Proinflammatory cytokines were significantly elevated in the MS group compared to the MS/*Cannabis* and control groups.

Most clinical symptoms were improved considerably in the MS/*Cannabis* group compared to the MS group, apart from sexual dysfunction, bladder symptoms, and visual disturbances. Additionally, anti-inflammatory cytokines had significantly lower values in the MS group compared to the MS/*Cannabis* and control groups. Mild side effects of *Cannabis* were also reported.

***Cannabis* Consumption and Urinary Functions**

Most multiple sclerosis patients develop troublesome lower urinary tract symptoms (LUTS). Anecdotal reports suggest that *Cannabis* may alleviate LUTS, and cannabinoid receptors in the bladder and nervous system are potential pharmacological targets. In an open trial, the safety, tolerability, dose range, and efficacy of two whole-plant extracts of *Cannabis* were evaluated in patients with advanced MS and refractory LUTS. The patients took extracts containing delta-9-tetrahydrocannabinol and cannabidiol, 2.5 mg of each per spray, for 8 weeks, followed by delta-9-THC-only (2.5 mg delta-9-THC per spray) for a further 8 weeks, and then into a long-term extension [123].

Twenty-one patients were recruited, and data from fifteen were evaluated. Assessments included urinary frequency and volume charts, incontinence pad weights, cystometry, and visual analog scales for troublesome secondary symptoms. Urinary urgency, the number and volume of incontinence episodes, frequency, and nocturia decreased significantly following treatment ($p < 0.05$, Wilcoxon's signed rank test). Daily total voided, catheterized, and urinary incontinence pad weights decreased significantly on both extracts. Patient self-assessment of pain, spasticity, and sleep quality improved significantly ($p < 0.05$, Wilcoxon's signed rank test), with pain improvement continuing to a median of 35 weeks. There were few troublesome side effects, suggesting that *Cannabis*-based medicinal extracts are a safe and effective treatment for urinary and other problems in patients with advanced MS.

For the sub-study, the patients completed incontinence diaries. The three groups showed a significant reduction, $p < 0.01$, in adjusted episode rate (i.e., correcting for baseline imbalance) from baseline to the end of treatment, *Cannabis* extract, 38%; delta-9-THC, 33%; and placebo, 18%. Both active treatments showed significant effects over placebo (*Cannabis* extract, $p = 0.005$; delta-9-THC, $p = 0.039$). The findings indicated a clinical effect of *Cannabis* on incontinence episodes in patients with MS. This is in contrast to the negative finding of the CAMS study, where no difference was seen in the primary outcome of spasticity.

An exploratory study was conducted to determine the trends of *Cannabis* consumption among people with MS and the association with perceived benefits on MS symptoms. A cross-sectional survey study of PwMS, recruited from an MS Clinic, was undertaken [63]. Logistic regression analyses were performed to assess the associations between *Cannabis* consumption and improvement in bladder function symptoms. Out of 2899 PwMS, 775 were contacted by email. Among the respondents, 734 reported *Cannabis* use in the previous 3 months. 275 (37.5%) respondents reported *Cannabis* use in the prior 3 months, and 73.8% reported at least weekly use of *Cannabis*. Among the users, 78.1% reported a primary medical or therapeutic indication for consumption. The most common modes of *Cannabis* consumption were oral-edible (69.0%) and smoked (57.1%), 59.3% used more than one mode of consumption, and 2.6% used five different modes. The most common reasons for *Cannabis* use were for sleep (58.3%), pain (51.5%), relaxation (44.4%), muscle spasms (40.2%), anxiety (33.8%), and depression (22.9%).

Among the 19 participants who reported bladder symptoms as the main reason for *Cannabis* use, 89.5% reported improvement in bladder symptoms when using *Cannabis*. *Cannabis* consumption in the previous 3 months was associated with a two-fold increase in the odds of reporting improvement in urinary frequency, urinary urgency, bladder leakage, wetness, pad use, and bladder emptying.

Most patients diagnosed with multiple sclerosis will develop lower urinary tract symptoms during the disease. Antimuscarinic (anticholinergic) treatment is currently the mainstay of conservative treatment of neurogenic detrusor overactivity (NDO), including MS-induced NDO. When antimuscarinic medications fail to prove efficacious, a further option is intradetrusor injections of onabotulinumtoxin A. In several studies, more than half, 76% of the patients treated with onabotulinumtoxin A experienced significant improvement in symptoms or achieved complete continence.

Cannabis extracts have shown promise as an effective treatment. Many disease-modifying drugs have been approved and introduced to treat MS [143]. The drugs may not affect the MS disease process but the disease symptoms, including LUTS. However, MS is not primarily a bladder disease, and treatment of the underlying pathophysiology is the main goal of treatment. Since most urology drugs target LUTS, the drugs should be regarded as a supplemental treatment to the underlying disorder.

Glucose Uptake and Cognitive Functions in Multiple Sclerosis

Estimates show that 20–66% of people with multiple sclerosis (PwMS) are currently using *Cannabis* even though there is little empirical evidence showing its efficacy. Several short-term interventional studies have generally shown some beneficial effects on spasticity and pain and have led to loose recommendations for *Cannabis* use. While some benefits have been observed, several studies reported that PwMS using *Cannabis* performed worse on cognitive tasks.

In healthy *Cannabis* users, several studies have shown lower global and regional glucose uptake (GU), suggesting a neuro-adaptive process to regular *Cannabis* use, possibly related to the downregulation of the CB1 receptors due to regular delta-9-THC exposure [66]. The possible scenario is supported by normalizing brain activity with prolonged abstinence [67]. Reduced brain glucose uptake in *Cannabis* users could account for the impairments in motor performance reported in the subjects [68]. However, reduced brain GU was typically associated with minimal or no change in behavioral performance during task performance in imaging studies [69].

Several studies have shown lower brain glucose uptake in PwMS. Reduced glucose uptake in gray and white matter may relate to direct or indirect effects of MS lesions [70, 71]. It indicates a different neuro-adaptive process in the MS brain compared with non-MS brains. An integral factor in nervous system glucose uptake is circulating levels of lactate. PwMS have been shown to have an increased lactate level, which is thought to stem from increased inflammation and neurodegeneration

within the central nervous system [72]. Infusing lactate peripherally lowered nervous system glucose uptake in healthy individuals [73]. *Cannabis* use in PwMS may lead to lowered inflammation and neuro-degeneration and thereby have an offsetting effect between speculated CB1 downregulation and reduced nervous system inflammation, leading to a zero-net change in glucose uptake.

Positron emission tomography (PET), with the glucose analog [F]-Fluorodeoxyglucose (FDG), has been used to investigate brain function in healthy and clinical populations. FDG-PET studies have shown regular heavy alcohol use with *Cannabis* and other illegal drugs could lead to lower resting nervous system glucose uptake [66]. It has been suggested that lower brain glucose uptake may be responsible for some cognitive and physical impairments in drug abuse populations. In PwMS, lower brain glucose uptake has been associated with clinical symptoms such as fatigue, cognitive ability, and walking speed. Brain glucose uptake may be an effective biomarker for tracking disease progression and disability in PwMS.

Physical and Psychological Impact *Cannabis*

Patients with stable MS at 22 UK centers were randomized to oral *Cannabis* extract (CE) ($n = 144$) or placebo ($n = 135$), stratified by center, walking ability, and use of antispastic medication. A double-blind, placebo-controlled, phase III study with a screening period, a 2-week dose titration phase from 5 mg to a maximum of 25 mg of tetrahydrocannabinol daily, and a 10-week maintenance phase [75]. The primary outcome measure was a category rating scale (CRS) measuring the patient-reported change in muscle stiffness from baseline. Category rating scales also assessed body pain, spasms, and sleep quality. Three validated MS-specific patient-reported outcome measures assessed spasticity, physical and psychological impact, and walking ability.

The rate of relief from muscle stiffness after 12 weeks was almost twice as high with *Cannabis* extract as with placebo (29.4% vs. 15.7%; OR 2.26; 95% CI 1.24–4.13; $p = 0.004$, one-sided). Similar results were found after 4 and 8 weeks and for all other category rating scales. Results from the MS scales supported the findings to demonstrate the superiority of *Cannabis* extract over placebo in the treatment of muscle stiffness in MS. This was supported by results for secondary efficacy variables. Adverse events in the participants treated with *Cannabis* extract were consistent with the known side effects of cannabinoids. No new safety concerns were observed.

One hundred and fifteen MS patients completed a 36-question survey online or on paper which queried aspects of their use of *Cannabis*, including frequency of use, effect on symptoms, and changes in their use of prescription medications, as well as some key demographic variables such as age, gender, disease duration, and clinical course. All the patients were treated at a multiple sclerosis clinic in

Connecticut and enrolled in the Connecticut Medical Marijuana Program (CTMMP) [76].

Self-reported benefit from *Cannabis* use for two or more symptoms of MS was associated with relapsing-remitting MS (RRMS) vs. progressive (PMS) (OR 3.043, 95% CI 1.026–9.028, $p = 0.038$) and less benefit for two or more symptoms for those who required a wheelchair vs. those who ambulated without assistance (OR .246, 95% CI .195–.797, $p = 0.016$). General benefit from *Cannabis* use was reported for mood disorders ($p < 0.001$), insomnia ($p < 0.001$), sensory symptoms, including pain ($p < 0.001$), and muscle cramps and spasms ($p < 0.001$). The benefit was also significantly associated with symptom severity in the case of insomnia (OR 9.735, 95% CI 2.751–34.445, $p < 0.001$) and cramps and spasms (OR 5.234, 95% CI 1.261–21.729, $p = 0.014$). A significant proportion of respondents had stopped or reduced prescription medications (86% vs. 55%, $p < 0.001$) to find *Cannabis* more effective than prescription medications. Those included opioids, benzodiazepines, muscle relaxers, and other pain medications.

Medical *Cannabis* use among people with MS can lead to the reduction or discontinuation of several categories of prescription medications for symptoms of MS. Persons reporting the most benefit from Medical *Cannabis* use tended to have a milder form of MS with less disability, in contrast to previous studies. This study confirms the benefit of *Cannabis* in several common MS symptoms, extending the findings to show that benefit can be related to the baseline severity of some symptoms.

Some experiments performed with mice with chronic relapsing experimental allergic encephalomyelitis (CREATE) have shown that cannabinoid-induced reductions in tremor and spasticity are mediated by cannabinoid receptors CB1 and CB2. Endocannabinoid concentrations are elevated in the brains and spinal cords of CREATE mice with spasticity. In line with this observation, spasticity exhibited by CREAE mice can be ameliorated by inhibitors of endocannabinoid membrane transport or enzymic hydrolysis [79].

A sample of 140 patients with MS was interviewed with the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) Axis I disorders (SCID-IV), from which details of *Cannabis* use were recorded. Cognition was assessed using the Neuropsychological Battery for MS supplemented with the Symbol Digit Modalities Test (SDMT), an index of information processing speed, working memory, and sustained attention.

Ten participants (7.7%) were defined as current *Cannabis* users based on use within the last month. Compared to non-*Cannabis* users ($n = 130$), they were younger ($p = 0.001$). Each of the ten current *Cannabis* users was matched on demographic and disease variables to four subjects with MS who did not use *Cannabis* (total control sample $n = 40$). Group comparisons revealed that the proportion of patients meeting DSM-IV criteria for a psychiatric diagnosis was higher in *Cannabis* users ($p = 0.04$). In addition, on the SDMT, *Cannabis* users had a slower mean performance time ($p = 0.006$) and a different response pattern than matched controls (group $\times$ time interaction; $p = .001$) [28]. Inhaled *Cannabis* is associated with

impaired mentation in patients with multiple sclerosis, particularly concerning cognition.

An anonymous questionnaire was sent to all 3606 patients at the Danish multiple sclerosis Center, Rigshospitalet University of Copenhagen. The questionnaire included sociodemographic factors, clinical characteristics, and medical or recreational *Cannabis* use [78]. The questionnaires were completed by 2244 of 3606 (62%), of which 2009 questionnaires from patients with MS or clinically isolated syndrome (CIS) were valid for analysis. Forty-nine percent (980 of 2009) had used *Cannabis* at least once. *Cannabis* was used within the past year (the current user) by 21%, and only 21% of those received prescribed *Cannabis*-based medicine. Recreational use was reported by 17%.

The primary reasons for use were to alleviate pain (61%), spasticity (52%), and sleep disturbances (46%). The most common adverse effects were drowsiness (30%), feeling quiet/subdued (23%), and dizziness (13%). Almost half (44%) of the non-*Cannabis* users would consider using *Cannabis* to alleviate MS symptoms if the drug was legalized, an indication that illegal *Cannabis* use is common among Danes with MS as only 21% of the current *Cannabis* users received prescribed *Cannabis*-based medicine. Current *Cannabis* users reported high efficacy in relieving pain, spasticity, and sleep disturbances. Only mild to moderate severities of adverse effects were reported.

Clinical evidence from trials has shown that delta-9-tetrahydrocannabinol and nabilone can produce objective and subjective relief from spasticity, pain, tremor, and nocturia in patients with Multiple sclerosis (eight trials) or spinal cord injury (one trial). Results from experiments with animal models of multiple sclerosis support the clinical evidence.

Forty people with MS who smoked *Cannabis* almost daily were assigned by odd-even case number selection to either a *Cannabis* continuation (CC) or *Cannabis* withdrawal (CW) group [80]. Both groups were followed for 28 days. All participants completed, at baseline and day 28, the Brief Repeatable Battery of Neuropsychological Tests (BRNB) in MS for measures of processing speed, memory, and executive function; modified fatigue impact scale (mFIS) for self-report indices of cognitive functioning.

No significant baseline differences between the groups on the BRNB and mFIS were observed. On day 28, significant improvement within the group was seen on all measures of the BRNB, but only in the CW group ($p = .0001$). A repeated measure ANOVA did not find any significant group (CC vs. CW) $\times$ time (baseline and day 28) interactions for the self-report cognitive measures on the mFIS.

Cannabis abstainers reported less ability to function away from home. All 19 participants in the CW group reverted to using *Cannabis* on study completion despite being informed individually of their cognitive improvement. The inability of pwMS to accurately appraise their memory and executive function may help to explain, in part, why they continue to smoke *Cannabis* despite objective evidence of the deleterious cognitive side effects of this behavior.

People with MS completed a questionnaire assessing personal opinions about *Cannabis* use, characteristics of *Cannabis* use, MS history, and sociodemographic

details, as well as the self-reported disability – Patient Determined Disease Steps (PDDS), overall quality of life-the, Patient Reported Outcome Measure Information System (PROMIS-10), and cognition-the Neuro-QoL ACGC vs. 1.0 measures.

Thirty-eight percent ($n = 96$) of PwMS were current *Cannabis* users (CUs). However, there were no sociodemographic or clinical differences ($p \leq 0.05$) between CUs and Non-*Cannabis* users (NUs), CUs had significantly higher median disability compared to NUs (PDDS = 2 vs. 1; $p = 0.02$). Among CUs, 57% categorized their use as strictly medicinal. CUs reported using *Cannabis* most often for pain and insomnia/poor sleep and experienced greater than 60% benefit/relief from *Cannabis* use. Over 90% of respondents desire more research on *Cannabis* for MS, and 74% indicated they would consider using *Cannabis* for their MS [81].

Patients with MS from the North American Research Committee on multiple sclerosis (NARCOMS) Registry (aged 18 years or older) were invited to complete a supplemental survey on *Cannabis* use. The participants reported *Cannabis* use, treated symptoms, patterns, preferences, methods of use, and the factors limiting use. Of the 6934 participants invited, 3249 responded. Of the respondents, 31% reported having used *Cannabis* to treat MS symptoms, and 20% currently use *Cannabis*. The remaining 69% had never used *Cannabis* for MS symptoms for reasons including not enough data about efficacy (40%) and safety (27%) and concerns about legality (25%) and cost (18%).

The most common symptoms current users were attempting to treat were spasticity (80%), pain (69%), and sleep problems (61%). Ever users (vs. never users) were more likely to be younger, have lower education, be unemployed, be younger at symptom onset, be currently smoking, and have higher levels of disability and MS-related symptoms (all $p < .001$) [82]. Despite concerns about insufficient safety and efficacy data, legality, and cost, almost one-third of NARCOMS Registry respondents, report having tried non-prescription *Cannabis* products to alleviate their symptoms.

During an inpatient rehabilitation program, 57 patients were enrolled in a prospective, randomized, double-blind, placebo-controlled crossover study of *Cannabis*-extract capsules standardized to 2.5 mg tetrahydrocannabinol and 0.9 mg cannabidiol each. The patients in group A started with a drug escalation phase from 15 to 30 mg delta-9-THC by 5 mg per day if well tolerated, being on active medication for fourteen days before starting the placebo. Patients in group B started with a placebo for seven days, crossed to the active period (fourteen days), and closed with a three-day placebo period (active drug dose escalation and placebo sham escalation as in group A). Measures used included daily self-report of spasm frequency and symptoms, Ashworth Scale, Rivermead Mobility Index, 10-m timed walk, nine-hole peg test, paced auditory serial addition test (PASAT), and the digit span test.

In the 50 patients included in the intention-to-treat analysis set, no statistically significant differences were associated with active treatment compared to placebo. However, trends favoring active treatment were seen for spasm frequency, mobility, and sleeping. In the 37 patients (per-protocol set) who received at least 90% of their prescribed dose, improvements in spasm frequency ($p = 0.013$) and mobility after excluding a patient who fell and stopped walking were seen ($p = 0.01$). Minor

adverse events were more frequent and severe during active treatment, and mild toxicity symptoms were generally more pronounced in the active phase [109].

Pain, spasticity, tremor, spasms, poor sleep quality, and bladder and bowel dysfunction are some of the symptoms contributing to the disability and impaired quality of life of patients with multiple sclerosis. Motor symptoms referable to the basal ganglia, especially paroxysmal dystonia, occur rarely and contribute to the experience of distress. A substantial percentage of patients with MS report subjective benefits from an extract of the *Cannabis sativa* plant; the main cannabinoids include delta-9-tetrahydrocannabinol and cannabidiol.

Clinical trials of *Cannabis* plant extracts and synthetic delta-9-THC provide support for therapeutic benefit on at least some patient self-report measures. A case of a 52-year-old woman with MS, paroxysmal dystonia, complex vocal tics, and marijuana dependence was presented. The patient started on an empirical trial of dronabinol, an encapsulated form of synthetic delta-9-THC usually prescribed as an adjunctive medication for cancer chemotherapy patients. The patient reported a dramatic craving reduction; she did not experience the “high” on the prescribed medication. The patient reported improved sleep quality with diminished awakenings during the night, decreased vocalizations and the tension associated with their emission, decreased anxiety, and a decreased frequency of paroxysmal dystonia [110].

The prevalence of MS in Alberta has been described as among the highest reported worldwide, estimated at 217 per 100,000. Numerous anecdotal reports and a few small empirical investigations have suggested that *Cannabis* use may relieve the symptom experienced by those with MS. A study was conducted to examine *Cannabis* use by the patient group. Information on peoples’ beliefs, practices, and experiences related to use was investigated.

A questionnaire was mailed to a sample of 780 adults with MS in southern Alberta, Canada. The completed questionnaires were returned by 420 of 673 eligible subjects (response rate of 62%). The mean sample age was 48 years, and 75% were women. The respondents ranged from mildly to severely impaired. Most respondents (96%) knew that *Cannabis* was potentially therapeutically useful for MS, and most (72%) supported legalization for medicinal purposes. Forty-three percent had tried *Cannabis* at some point in their lives and 16% for medicinal purposes. The ameliorated symptoms included anxiety/depression, spasticity, and chronic pain. Reasons for not trying *Cannabis* were that it is an illegal substance, concern about side effects, and lack of knowledge on how to obtain it. Subjective improvements in symptom experience were reported by the majority of people with MS who currently use *Cannabis* [114].

Anecdotal reports indicate that many PwMS use *Cannabis* as a supplement to improve their mobility. A web-based survey hosted by the National multiple sclerosis Society stated that 66% of PwMS use *Cannabis* for symptom treatment. A study from Canada states that approximately 50% of PwMS would use *Cannabis* if the legal status is clear and scientific evidence is available. Based on the considerations, CBD supplementation may be advisable for PwMS to reduce fatigue, pain, and spasticity and ultimately improve mobility. Despite the common use of and interest in *Cannabis* by people with MS, there is limited empirical data on its impact on

physical mobility. However, data indicate that *Cannabis*, with a 1:1 or greater CBD to delta-9-THC ratio, reduces muscle spasticity and pain in PwMS. The American Academy of Neurology has highlighted *Cannabis*'s safety profile and benefits. Studies have suggested that cannabinoids may positively affect health by decreasing inflammation and pain [120].

Furthermore, inflammation plays an important role in the generation of MS-related fatigue. Specifically, chronic peripheral inflammation and a resulting overactivity of the vagus nerve are related to fatigue in PwMS. There is indirect evidence that reductions in spasticity, pain, and fatigue may result in improvements in the mobility of PwMS. Furthermore, it is suggested that CBD produced a dose-dependent antidepressant-like effect in the animal model. Depression is an important contributory factor to the observed impaired mobility in PwMS. Based on extant evidence, the impact of cannabidiol on mobility has been investigated.

Medications continue to be widely prescribed for most PwMS suffering from pain, spasticity, anxiety, and panic disorders. Common side effects of opioid administration include physical dependence, dizziness, sedation, nausea, vomiting, tolerance, constipation, and respiratory depression. Physical dependence and addiction are clinical concerns that may prevent accurate prescribing and, in turn, poor pain management.

Traditional benzodiazepines are associated with sleep disturbances and anterograde amnesia. Another concern with long-duration benzodiazepines, such as diazepam or flurazepam, is drowsiness and the "hangover effect." Antidepressants can cause many unpleasant side effects, including nausea, fatigue and drowsiness, blurred vision, dizziness, and anxiety. Those drugs delay or even prevent successful physical rehabilitation.

An epidemiological study showed that among people that frequently used opioids, over three-quarters (77%) indicated that they had reduced their use since they started taking *Cannabis*. Approximately two-thirds of patients decreased their use of antianxiety (72%), migraine (67%), and sleep (65%) drugs following medical *Cannabis*, which significantly exceeded the reduction in antidepressants or alcohol use. Complete or partial replacement of the drugs with specific *Cannabis* products should be the long-term goal.

Objections to the notion that cannabinoids should be used to improve mobility in PwMS include the following: limited scientific evidence for the effectiveness of *Cannabis* on mobility in PwMS; the uncertainty of legal status; social stigmatization from friends, family, and authorities such as employers, landlords, and law enforcement; incidence of dependency and negative psychoactive effects of *Cannabis*. The objections have some merit and should be taken into consideration. It is important to note that the psychoactive effects of *Cannabis*, such as cognitive impairments, psychosis, and anxiety, are due to delta-9-tetrahydrocannabinol.

However, CBD has antipsychotic properties and can counter several negative side effects of delta-9-THC. Most PwMS prefer to avoid feeling *high*. Therefore, individuals should seek out strains of *Cannabis* containing equal or higher levels of CBD than delta-9-THC. Another concern is the risk of *addiction*. It is estimated that ~9% of individuals utilizing *Cannabis* will become dependent on it. Although a

significant risk, this incidence of dependency is significantly lower than approved chronic pain management pharmaceuticals.

Five randomized controlled trials (RCTs) compared the benefits and harms of delta-9-THC/CBD spray with a placebo. Three hundred and sixty-eight patients with various neurological conditions, including MS, were recruited. The most common adverse events reported in the trials were dizziness, sleepiness, fatigue, intoxication, and a bad taste. Delta-9-THC/CBD spray significantly reduced some trials' neuropathic pain, spasticity, muscle spasms, and sleep disturbances. Long-term safety and the potential for dependence, abuse, misuse, and diversion are unknown [127].

Generally, individuals who subsequently develop MS are more likely to be smokers, have suffered glandular fever, and have a low blood vitamin D level. A questionnaire was completed by 94 patients with clinically definite MS. Their responses were compared to a control group of 59 patients with benign headaches [128]. The questions explored the pre-symptomatic experience of alcohol, smoking, substance abuse, glandular fever, blood transfusion, hazardous sport, gambling, sexual history, gynecologic infection, number of pregnancies, and pregnancy terminations. Data were adjusted for age of first symptoms, gender, and smoking.

Compared to the headache group, MS subjects showed significant differences prior to symptom onset, adjusted for age of first symptoms, gender and smoking, with odds ratios for reporting or means and 95% confidence intervals as follows: consuming alcoholic drinks, OR 6.91 (1.74–27.45; $p = 0.006$) and at an earlier age, mean 16.9 years (16.4–17.5; $p = 0.046$); cigarette smoking, OR 2.24 (1.09–4.59; $p = 0.028$) and to have smoked more per day, mean 9.45 (5.55–13.35; $p = 0.001$); history of glandular fever/infectious mononucleosis, OR 3.07 (1.11–8.49; $p = 0.031$); consumed recreational drugs, OR 3.90 (1.32–11.50; $p = 0.014$), notably *Cannabis*, OR 4.10 (1.40–12.09; $p = 0.011$); used a car seat belt, OR 4.45 (1.43–13.83; $p = 0.010$); attended all-night parties, OR 2.45 (1.12–5.36; $p = 0.025$); sunbathed, OR 2.770 (1.02–7.52; $p = 0.046$); had more sexual partners, mean 3.97 (2.00–5.95; $p < 0.001$); more pregnancies, mean 1.43 (0.99–1.86; $p = 0.015$), and one or more terminations of pregnancy, OR 5.05 (1.003–25.386; $p = 0.049$).

All but one of the statistically significant variables, car seat belts, supports the hypothesis that MS subjects lead a riskier premorbid lifestyle. Strong associations were found between smoking, alcohol, and glandular fever. Associations were also found for recreational drug use, attending all-night parties, gambling on the lottery, more sexual partners, more pregnancies, and one or more pregnancy terminations.

***Cannabis* Use in Depression**

To assess whether symptoms of depression change when people with multiple sclerosis (pwMS) discontinue *Cannabis* use, 40 cognitively impaired people with multiple sclerosis who smoked *Cannabis* almost daily were randomly assigned to either a *Cannabis* continuation (CC) or *Cannabis* withdrawal (CW) group [77].

Both groups were followed for 28 days. All participants completed the Hospital Anxiety and Depression Scale. At day 20, the 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH)/creatinine ratio in the CW group declined to zero ($p = 0.0001$) but remained unchanged in the CC group ($p = 0.709$).

Depression scores in those people with multiple sclerosis who were using *Cannabis* to manage their depression remained statistically unchanged in the CC group but declined in the CW group ($p = 0.006$). Despite people with multiple sclerosis using *Cannabis* to help their mood, depression improved significantly off the drug. The finding provides a cautionary note concerning *Cannabis* use in people with multiple sclerosis, at least for depression.

Pain Management in MS

Chronic pain is prevalent in multiple sclerosis, and pain heterogeneity may contribute to poor treatment outcomes. The International Association for the Study of Pain (IASP) has defined three mechanistically based pain types categories [83]. Given the axonal injury associated with MS, pain is often assumed to be neuropathic [84].

Neuropathic pain results from a lesion or disease of the somatosensory nervous system. Nociceptive pain, by contrast, arises from the activation of nociceptors in the periphery due to actual or threatened tissue damage (including inflammation) as opposed to dysfunction in the somatosensory nervous system; nociceptive pain is not well characterized in MS. Nociplastic pain arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain. This type of pain, often termed “centralized pain” or “central sensitization,” is believed to be due to CNS alterations in pain processing, as opposed to ongoing inflammation (nociceptive) or damaged neural pathways (neuropathic).

Most work on pain in persons with MS has focused on pain associated with focal demyelinating lesions, with little attention given to concomitant nociceptive/inflammatory or nociplastic mechanisms or differences in perceived treatment effects related to pain mechanisms. One study of the natural history of pain in MS indicated that those with chronic pain developed more widespread pain over time (a characteristic of central sensitization). However, an examination of nociplastic pain in MS has not been undertaken. Work to identify subgroups with different underlying pain mechanisms will likely improve pain outcomes in MS by providing the opportunity to tailor therapies to an individual’s specific type(s) of pain [85].

A study was conducted to characterize pain phenotypes distributions in persons with MS and compare pain phenotypes in terms of pain intensity, frequency of chronic overlapping pain conditions, and use and analgesic effects of different pain medications [86]. Data were collected through a national web-based survey with measures of neuropathic (painDETECT) and nociplastic pain (Fibromyalgia Survey

Criteria), chronic overlapping pain conditions, and pain medication use and pain relief.

In a sample of 842 adults with chronic pain and MS, the largest proportion (41%) showed evidence of nociceptive pain, 27% had mixed neuropathic/nociplastic pain, 23% had nociplastic pain, and 9% had neuropathic pain. Nociplastic pain was associated with significantly higher pain intensity and frequency of chronic overlapping pain conditions. Across all pain types, a high frequency of pain medication use and poor-modest pain relief was reported.

Cannabis use for pain was more common, and pain relief ratings were higher among those with nociplastic pain relative to nociceptive pain. Although NSAID use was highest among those with nociplastic pain (80%), pain relief ratings for NSAIDs were highest among those with nociceptive pain. The findings underscore the need for a multidimensional assessment of pain in MS with greater emphasis on identifying pain phenotype.

Immunomodulatory Effects of Cannabinoids

Cannabinoids affect immune responses in ways that may be beneficial for autoimmune diseases. To determine whether chronic *Cannabis* use differentially modulates a select number of immune parameters in healthy controls and individuals with Multiple sclerosis (MS cases), patients were enrolled. They consented to a single blood draw, matched for age and BMI. The cases met the definition of MS by international diagnostic criteria. Monocyte migration was isolated from each subject, and plasma levels of endocannabinoids and cytokines were measured [87]. A set ratio of phytocannabinoids similarly inhibited monocyte cell migration measured in control subjects and individuals with MS. The plasma levels of CCL2 and IL17 were reduced in non-naïve *Cannabis* users irrespective of the cohorts.

A significant increase was detected in the endocannabinoid arachidonylethanolamine (AEA) serum from individuals with MS compared to control subjects. There was no significant difference in levels of other endocannabinoids, and signaling lipids irrespective of *Cannabis* use [88]. Chronic *Cannabis* use may affect the immune response to a similar extent in individuals with MS and control subjects through the ability of phytocannabinoids to reduce both monocyte migration and cytokine levels in serum. From a panel of signaling lipids, only the levels of AEA were increased in individuals with MS, irrespective of *Cannabis* use. The results indicated that MS cases and controls respond similarly to chronic *Cannabis* use for the immune parameters measured in the study.

It is known that regimented use of phytocannabinoids (PCBs) may induce prolonged immunosuppressive and anti-inflammatory effects. Clinical studies show that heavy use of smoked *Cannabis* decreased lymphocyte proliferation and suppressed cell-mediated immunity [89]. Peripheral blood mononuclear cells from chronic smokers have altered basal levels of the ratio of CB1:CB2 cannabinoid receptors compared to controls [90]. Delta-9-THC acting on T-cells induces a shift

toward a TH2 phenotype in animal infection models [91]. Accordingly, long-term *Cannabis* use decreases T-cell proliferation and IL-2 levels (a TH1 cytokine) and increases IL-10 levels (a TH2 cytokine) in human blood [92]. Thus, chronic use of *Cannabis* might bias immune effector mechanisms, thereby affecting autoimmune reactions such as in MS.

Evidence shows that PCBs are likely to modulate the phenotype of circulating monocytes. The phenotype of circulating monocytes is relatively “plastic,” evidenced by cell-surface protein expression profiles, migratory potentials, and differential cytokine secretion in response to specific environmental cues [93]. Accordingly, phenotypic variability in circulating monocytes will influence the phenotype of the ensuing tissue macrophages.

A single low dose of delta-9-THC in mice induced the recruitment of bone marrow monocytes (myeloid-derived suppressor cells, MDSC) into the blood and altered cytokine release (also using cannabidiol), the second most abundant PCB. The direct modulation of the phenotype of MDSCs induced a fourfold increase in CD11bGR1+ cells [94]. MDSC are known to inhibit T-cell proliferation in humans after a viral illness. Together, the data provide a mechanistic framework indicating that regimented use of PCB might affect the phenotype of circulating monocytes. The prevalence of medicinal *Cannabis* use by individuals with MS is estimated at 14% in Nova Scotia, 17% in Spain, and 22% in the UK [95].

Given the critical role of circulating immune cells in MS and the immunomodulatory actions of PCB on the cells, a study was conducted to determine if *Cannabis* use differentially modulates three parameters: ex-vivo migration of circulating monocytes, the quantity of cytokines, and eCB released in serum [96].

The participant was recruited from 2009 to 2011 from the MS Center at the University of Washington (UW), the MS Center at Swedish Hospital in Seattle, and the MS Society of Greater Seattle and from medical, nursing, and research staff at UW with approval from the UW Human Subjects Committee. Before enrollment, the participants were screened by telephone for inclusion/exclusion criteria. Written informed consent was obtained before fasting and ante-cubital venipuncture using an approved protocol. Individuals with stable MS either were or were not currently using disease-modifying therapy (DMT). As part of the protocol, the participants were asked not to use ibuprofen the day before their blood draw. Subjects were stratified based on current *Cannabis* exposure.

All of the subjects were ≥ 21 and ≤ 50 . MS diagnostic criteria: Positive diagnosis in the last ten years of relapsing-remitting MS (made by a neurologist, according to international guidelines); an EDSS score between three and five; not currently in exacerbation and no exacerbation less than thirty prior to the blood draw. Exclusion criteria: BMI ≤ 19 and ≥ 27 ; diagnosis of diabetes; diagnosis of other chronic inflammatory or autoimmune diseases; current pregnancy; previous DSM IV diagnosis; tobacco use; or currently a performance athlete. The criteria were to minimize variables influencing the eCS or immune function. The participants were stratified and matched by *Cannabis* exposure: non-naïve is currently *Cannabis* use at least two to three times per week, and naïve is “never used” nor has no history of use in the last 3 years.

To validate the method used in this study, freshly isolated monocytes from a healthy subject were used and confirmed by a correlation between the fluorescence signal (near-infrared) emitted by cells that had migrated through the filter and an actual number of cells by counting them. The result extends what was found in a previous study in which a correlation between the fluorescent signal and actual cell number in mouse microglial cells in culture [97].

Both CCL2 (monocyte chemo-attractant protein 1) and serum freshly isolated from this subject were tested to stimulate the migration of human monocytes. Both stimuli enhanced cell migration ($419 \pm 38\%$ and $861 \pm 253\%$, respectively; $n = 17$). To determine whether the stimulatory response induced by freshly isolated serum is due to CCL2, serum was incubated with a CCL2 neutralizing antibody ($10 \mu\text{g/mL}$). It revealed that the antibody blocked the stimulatory response induced by serum. The result indicates that most of the migratory stimulatory response induced by freshly isolated serum on monocyte migration requires CCL2, confirming and extending the previous reports [98]. The results validate the approach to studying *human monocytes'* ex vivo migratory response.

Monocytes from ten healthy human subjects were isolated. Five subjects were naïve to *Cannabis* (i.e., either never used this plant or had been naïve for more than 3 years), and five subjects were non-naïve (currently using *Cannabis* more than twice a week). Tests on how CCL2, serum, and PCB regulate their cell migration show that monocytes isolated from *Cannabis* non-naïve subjects exhibit a reduced response to both CCL2 and serum compared to monocytes isolated from naïve subjects. It was also found that both basal and stimulated (CCL2 and serum) migration of monocytes isolated from *Cannabis* non-naïve subjects were inhibited by PCB. In contrast, the responses measured in monocytes isolated from *Cannabis* naïve subjects were unaffected.

The results indicate that monocytes isolated from *Cannabis* naïve and non-naïve subjects exhibit different migratory responses and that only monocytes isolated from *Cannabis* non-naïve subjects are sensitive to PCB. This selective sensitivity to PCB is specific because both synthetic cannabinoids and eCB do not affect the migratory response of monocytes isolated from both groups of subjects. Synthetic compounds and eCBs (other than PEA) did not affect the migration of monocytes isolated from both *Cannabis* naïve and non-naïve subjects.

Because of the small number of monocytes isolated from each subject and the absence of antibodies that specifically label cannabinoid receptors, changes in CB receptor mRNA expression by qPCR using TBP mRNA as the housekeeping gene was measured. It was revealed that monocytes isolated from *Cannabis* non-naïve subjects express fourfold higher amounts of CB1 receptor mRNA than cells isolated from *Cannabis* naïve subjects, whereas CB2 receptor mRNA remained unchanged between the two cohorts. The results show that the sensitivity of monocytes isolated from non-naïve subjects to PCB correlates with enhanced expression of CB1 receptors.

Circulating monocytes play a fundamental role in initiating inflammatory and immune responses [99]. The molecular components controlling the ability to circulate monocytes to infiltrate diseased tissue, including crossing the blood–brain

barrier (BBB), represent promising targets for drugs designed to treat diseases involving chronic inflammatory and immune responses. Monocytes isolated from human subjects naïve and non-naïve to *Cannabis* respond differentially to specific modulators of cell migration. CCL2, a potent chemo-attractant, is produced by various cell types (including endothelial cells, fibroblasts, astrocytes, and microglia) in response to growth factors, cytokines, and oxidative stress [100].

Thus, CCL-2 gradients direct circulating monocytes toward injured tissue, such as the CNS in the case of Multiple sclerosis, cerebral ischemia, and brain tumors. Indeed, CCL2 production increases in diseased tissue, and many immune cells express CCL2 receptors. It was shown that the CCL2-stimulated migration of monocytes is attenuated in non-naïve subjects. While the molecular details of the reduced response are not thoroughly understood (e.g., whether it is due to decreases in CCL2 receptor expression and coupling), the result suggests that chronic use of *Cannabis* affects the ability to circulate monocytes to respond to a potent chemo-attractant known to recruit monocytes toward damaged and inflamed tissues. The result is remarkable, considering numerous patients regularly use *Cannabis* as medicine (an estimated 20% of patients with MS).

Most subtypes of immune cells express cannabinoid receptors [101]. Monocytes isolated from patients who are non-naïve to *Cannabis* express more CB1 receptors than monocytes isolated from subjects who are naïve to *Cannabis*; a result corroborated a study reporting a similar response in PBMCs [90]. A study tested whether cannabinoids affected the migration of freshly isolated human monocytes and noted that the cannabinoid compound tested was JWH-015 at 20 μ M, a concentration that leads to both CB1, CB2, and GPR55 cross-activation and receptor-independent effects [102]. A test of JWH-015 at 3 μ M, a concentration that still cross-activates CB1, CB2, and GPR55 receptors, while minimizing receptor-independent effects, revealed that the aminoalkylindole compound does not regulate the migration of freshly isolated monocytes. None of the synthetic cannabinoids that were tested affected monocyte migration. Considering this lack of response to synthetic cannabinoids, the inhibitory response induced by phyto-CB that was measured only in monocytes isolated from non-naïve subjects suggests a mechanism involving the selective sensitization to this subfamily of compounds acting on CB receptors. This is the first example of *Cannabis* use leading to the selective sensitizing of immune cells to PCBs.

The study indicates that chronic *Cannabis* use affects key aspects of monocyte migration, basal migration, and response to potent chemo-attractant stimulants. Accordingly, the ability to circulate monocytes to invade tissues under healthy and diseased conditions and how therapeutic compounds control this step may differ when considering subjects naïve or not to *Cannabis*, as in patients with MS.

A double-blind, randomized trial was conducted to assess the potential therapeutic effects of *Cannabis* seed, evening primrose oils, and a Hot-nature dietary intervention on RRMS patients. One hundred MS patients with EDSS <6 were allocated into three groups: Group A received co-supplemented *Cannabis* seed and evening primrose oils with an advised Hot-nature diet, Group B received olive oil, and Group C received the co-supplemented oils. Mizadj, clinical EDSS, relapse rate,

and immunological factors IL-4, IFN- γ , and IL-17 were assessed at baseline and after 6 months.

The mean follow-up was 180 ± 2.9 SD days ($n = 65$, 23 Males and 42 Females aged 34.25 ± 8.07 years with a disease duration of 6.80 ± 4.33 years). There was no significant difference in study parameters at baseline. After 6 months, significant improvements in Mizadj, EDSS, and relapse rate were found in groups A and C, while group B showed a significant border decrease in relapse rate. Immunological parameters showed improvement in groups A and C, whereas there was a worsening condition for group B after the intervention [122]. The co-supplemented *Cannabis* seed and evening primrose oils with a hot-nature diet have beneficial effects in improving the clinical score in RRMS patients, confirmed by immunological findings.

Liver enzyme serum levels (GGT, AST, and ALT) were serially monitored. Clinically, EDSS and serum levels of liver enzymes GGT, AST, and ALT were assessed at baseline and after 6 months [145]. There was no significant difference in the study parameters at baseline. The intervention was associated with liver function alteration in three groups. Significance decreased in EDSS score, and the levels of liver enzymes were found in groups A and C. In contrast, elevated serum liver enzymes and EDSS scores were observed in group B after the intervention. Selecting foods according to their total antioxidant capacities, such as co-supplemented hemp seed and evening primrose oils with the hot-nature diet, affects antioxidant intake and can have beneficial effects on improving EDSS score and activity of liver enzymes in RRMS patients.

The risk of developing multiple sclerosis is associated with increased dietary intake of saturated fatty acids. For many years, it has been suspected that this disease might be associated with an imbalance between unsaturated and saturated fatty acids. Erythrocyte membrane fatty acids levels and their correlation with the Expanded Disability Status Scale (EDSS) at baseline were studied after 6 months of intervention in MS patients. After 6 months, EDSS, immunological parameters, and the erythrocyte cell membrane-specific fatty acids showed improvement in groups A and C. In contrast, there was a worsening condition for group B after the intervention. The conclusion is that the hot-nature dietary intervention with co-supplemented hemp seed and evening primrose oils increased PUFAs in MS patients and improved erythrocyte membrane fatty acids composition, indicating restored plasma stores and a reflection of disease severity reduction [115].

The correlations between the Expanded Disability Status scale and cytokines were examined. Clinical EDSS and immunological factors (plasma cytokines of IL-4, IFN- γ , and IL-17) were assessed at baseline and after 6 months. After 6 months, significant improvements in extended disability status scores (EDSS) were found in the patients with decreased cytokines of IFN- γ and IL-17 and increased cytokines of IL-4. Clinical and immunological parameters showed improvement in the patients after the intervention [117].

Cannabis extracts and several cannabinoids have been shown to exert broad anti-inflammatory activities in experimental models of inflammatory CNS degenerative

diseases. Their psychotropic effects limit the clinical use of many cannabinoids. However, phytocannabinoids like cannabidiol are potentially safe and effective alternatives for alleviating neuroinflammation and neurodegeneration. An experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) was used in C57BL/6 mice as a model of Multiple sclerosis [126]. Immunocytochemistry and cell proliferation assays of the effects of CBD on microglial activation in MOG-immunized animals and on MOG-specific T-cell proliferation were examined.

Treatment with CBD during disease onset ameliorated the severity of the clinical signs of EAE. The effect of CBD was accompanied by diminished axonal damage and inflammation, as well as microglial activation and T-cell recruitment in the spinal cord of MOG-injected mice. The effect of CBD inhibition on MOG-induced T-cell proliferation in vitro at low and high myelin antigen concentrations was not mediated via cannabinoid CB1 and CB2 receptors; the suppression appears to contribute to the beneficial effects.

A study was conducted to investigate the efficacy of crude extracts of *Cannabis* on motor symptoms in the chronic relapsing experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis. It has been shown that the endocannabinoid system is altered in MS and that the exogenous cannabinoids may play a possible role in its therapeutic management [129]. The effect of the combined treatment with delta-9-tetrahydrocannabinol and cannabidiol extracts has also been investigated. CREAE-induced mice were injected with crude ethanolic extracts from *Cannabis* containing delta-9-tetrahydrocannabinol, cannabidiol, or cannabinoid-free. All of the extracts were administered in acute and chronic experimental protocols.

The chronic administration of delta-9-tetrahydrocannabinol-rich extract significantly reduced neurological deficits until the end of the observations. The acute and chronic treatments with the cannabidiol-rich extract could not induce changes in neurological signs. However, a significant decrease in neurological scores was observed during the relapse phase. The combined treatment with delta-9-tetrahydrocannabinol and cannabidiol extracts was ineffective, whereas the acute administration of the cannabinoid-free extract significantly improved.

Endocannabinoids and some phytocannabinoids bind to CB1 and CB2 cannabinoid receptors, transient receptor potential vanilloid 1 (TRPV1) receptor, and the orphan G protein receptor fifty-five (GPR55). Studies using C57BL/10 and C57BL/6 (Cnr2 (tm1Zim)) CB2 cannabinoid receptor knockout mice have demonstrated an immune-augmenting effect in experimental autoimmune encephalomyelitis (EAE) models of multiple sclerosis. However, other EAE studies in Biozzi ABH mice often failed to produce any treatment effect of either CB2 receptor agonism or antagonism on inhibition of T-cell autoimmunity.

The influence of genetic background on the induction of EAE in endocannabinoid system-related gene knockout mice was examined, and it was found that C57BL/6. CB2 (Cnr2 (Dgen)) receptor knockout mice developed augmented severity of disease consistent with the genetically and pharmacologically distinct, Cnr2 (tm1Zim) mice [130]. GPR55 knockout mice developed the less severe disease,

notably in female mice, following active induction with myelin oligodendrocyte glycoprotein 35–55 peptide, in contrast to C57BL/6. Likewise, the CB1 receptor and transient receptor potential vanilloid one knockout mice on the ABH background demonstrated no alteration in immune-susceptibility, in terms of disease incidence and severity of EAE, in contrast to that reported in some C57BL/6 mouse studies.

However, when the knockout gene was bred into the ABH mouse background and EAE induced with spinal cord autoantigens, the immune-enhancing effect of CB2 receptor deletion was lost. The immune-modulating influence of GPR55 was marginal on the ABH mouse background. While sedative doses of tetrahydrocannabinol could induce immunosuppression, this was associated with a CB1 receptor rather than a CB2 receptor-mediated effect. The data support that non-psychoactive doses of medical *Cannabis* significantly influence the immune response in MS.

CBD has been approved to treat inflammation, pain, and spasticity associated with multiple sclerosis, of which demyelination and oligodendrocyte loss are hallmarks. The protective effects of CBD against damage to oligodendrocyte progenitor cells (OPCs) mediated by the immune system were investigated. Doses of 1 μ M CBD protect OPCs from oxidative stress by decreasing the production of reactive oxygen species. CBD also protects OPCs from apoptosis induced by LPS/IFN γ by decreasing caspase-3 induction via mechanisms that do not involve CB1, CB2, TRPV1, or PPAR γ receptors.

Tunicamycin-induced OPC death was attenuated by CBD, indicating a role of endoplasmic reticulum (ER) stress in the mode of action of CBD. The protection against ER stress-induced apoptosis was associated with reduced phosphorylation of eIF2 α , one of the initiators of the ER stress pathway. CBD diminished the phosphorylation of PKR and eIF2 α induced by LPS/IFN γ . The pro-survival effects of CBD in OPCs were accompanied by decreased ER apoptotic effectors expression (CHOP, Bax, and caspase-12) and increased anti-apoptotic Bcl-2. The findings suggest that attenuation of the ER stress pathway is involved in CBD's oligoprotective effects during inflammation [147].

Multiple sclerosis has an inflammatory/autoimmune origin; nevertheless, neuronal cell death mechanisms should not be underestimated. A study was conducted to test the effects of intraperitoneal administration of cannabidiol in an experimental model of MS to evaluate the capability of CBD to thwart the cascade of mediators involved in MS-induced apoptosis. Experimental autoimmune encephalomyelitis was induced by immunization with myelin oligodendroglial glycoprotein (MOG)35-55 peptide in mice. After immunization, the mice were observed daily for signs of EAE and weight loss [132]. Disease signs were evaluated using a standardized scoring system. Immunohistochemical and Western blot assessments of key apoptotic markers reveal that CBD treatment can avoid Fas pathway activation, phospho-ERK p42/44, cleaved caspase-3 triggering, and alterations in mitochondrial permeability due to Bax/Bcl-2 unbalance.

Most of the therapeutic properties of *Cannabis* are ascribed to the psychotropic effects of the phenylterpenoid delta-9 tetrahydrocannabinol. Moreover, CBD

interferes with p53-p21 axis activation. The absence of tissue apobody formation in spinal cord tissues of EAE mice treated with CBD was established.

The mouse model of multiple sclerosis was used for the inflammatory demyelinating disease. Rapamycin (RAPA) may reduce inflammatory responses to experimental autoimmune encephalomyelitis (EAE). The immunological factors include genes expression of the regulatory-associated protein of the mammalian target of rapamycin (RAPTOR), regulatory-associated companion of mammalian target of rapamycin (RICTOR), interferon (IFN)- γ , interleukin (IL)-10, signal transducer and activator of transcription factors (STAT3), forkhead box P3 (FOXP3), and IL-17 of splenocytes were evaluated by real time-polymerase chain reaction (RT-PCR) [157]. The data showed that EP/HS oil reduced the severity of EAE and inhibited the development of the disease. EP/HS oil treatment significantly inhibited the expression of RAPTOR, IFN- γ , IL-17, and STAT3 genes and promoted the expression of RICTOR, IL-10, and FOXP3 genes. The EP/HS oil is likely to be involved in the transcription of factors in favor of EAE improvement and participating in remyelination in the EAE spinal cord. It suggests effective therapeutic approaches for MS.

Cannabis is increasingly prescribed as an analgesic for patients with multiple sclerosis. A study was conducted to investigate the immune-cell modulatory properties of *Cannabis* [136]. Healthy volunteers were asked to ingest medical *Cannabis*, and kinome profiling was used to generate comprehensive descriptions of the *Cannabis* challenge on inflammatory signal transduction in the peripheral blood of the volunteers. The results were related to short-term and long-term effects in patients experimentally treated with a *Cannabis* preparation for abdominal pain due to chronic pancreatitis or other causes.

The results reveal an immunosuppressive effect of cannabinoid preparations via the deactivation of signaling through the pro-inflammatory p38 MAP kinase and mTOR pathways and a concomitant deactivation of the pro-mitogenic ERK pathway. Long-term *Cannabis* exposure in two patients resulted in a reversal of the effect. While the data provide a powerful mechanistic rationale for the clinical use of medical *Cannabis* in inflammatory and oncological diseases, caution should be advised with sustained use of such preparations.

Cannabidiol has potent anti-inflammatory and immunosuppressive properties. Inflammation in the central nervous system is a complex process that involves several molecules and effectors. It requires the transmigration of blood leukocytes across the blood-brain barrier (BBB) and activating resident immune cells. Using a viral model of multiple sclerosis demonstrates that CBD decreases the transmigration of blood leukocytes by downregulating the expression of vascular cell adhesion molecule-1 (VCAM-1), chemokines (CCL2 and CCL5), and the pro-inflammatory cytokine IL-1 β , as well as by attenuating the activation of microglia.

Cannabidiol administration at the time of viral infection exerts long-lasting effects, ameliorating motor deficits in the chronic phase of the disease in conjunction with reduced microglial activation and pro-inflammatory cytokine production. Adenosine A2A receptors participate in some of the anti-inflammatory effects of CBD, as the A2A antagonist ZM241385 partially blocks the protective effects of CBD in the initial stages of inflammation [141]. The findings highlight the

anti-inflammatory effects of CBD in the viral model of MS and demonstrate the significant therapeutic potential of CBD for treating pathologies with an inflammatory component.

Activation of the peripheral immune system and the infiltration of immune cells into the central nervous system are key features of the experimental autoimmune encephalomyelitis model. By exploring how the endocannabinoid system modulates this response, we can understand how exogenous cannabinoids could be used to modulate the immune responses of multiple sclerosis patients. A study examined the role of the CB1 receptor in IFN- γ and IL-17A production in the EAE model and in vitro stimulations of naive splenocytes using *Cnr1*^{-/-} mice and wild-type (WT) littermates [137]. Scoring spinal cord histological sections to show the differences in disease severity between *Cnr1*^{-/-} and WT mice with EAE were employed.

Clinical scores of *Cnr1*^{-/-}/EAE and WT/EAE mice showed more severe disease progression in *Cnr1*^{-/-} mice, which was confirmed using our new histological scoring method. In the peripheral immune system, the production of IFN- γ by restimulated splenocytes from *Cnr1*^{-/-}/EAE mice was increased compared with WT/EAE mice, and the primary source of IFN- γ was a CD3⁺-cell population. However, IFN- γ production by *Cnr1*^{-/-} splenocytes was decreased compared with WT splenocytes when the primary source of IFN- γ was CD3⁺ T cells in cultures from naive mice stimulated by either anti-CD3/anti-CD28 antibodies or Staphylococcal superantigens. The findings suggest a duality to the CB1 receptor's effects on the peripheral immune response, which varies based on the specific cell types stimulated.

***Cannabis* and Cytochrome P450**

Serious drug interactions have not been seen with CBD or other drugs. CBD and other cannabinoids can potentially interact with many pharmaceuticals. For example, the activity of liver enzymes such as cytochrome P450 is impacted. This group of enzymes metabolizes more than 60% of marketed pharmaceuticals. CBD will temporarily deactivate the liver enzymes at high enough dosages, altering how a wide range of compounds is metabolized.

A major concern is the often labeling accuracy of CBD extracts. A range of CBD concentrations among CBD products contains 26% less CBD than labeled, which could negate any potential clinical benefit. The over-labeling of CBD products and that delta-9-THC was detected (up to 6.43 mg/mL) in 18 of the 84 samples tested indicating a need for federal and state regulatory agencies to take steps to ensure label accuracy of CBD products sold online and in dispensaries.

In CBD products without delta-9-THC, a urine test would not yield a positive result for delta-9-THC metabolites. However, most CBD products contain minimal amounts of delta-9-THC in CBD. An important aspect of cannabinoid compounds is the entourage effect. The entourage effect means that the compounds in *Cannabis* work more efficiently together than if the compounds are isolated. Therefore, CBD products may contain more *Cannabis* compounds, including delta-9-THC, to

increase the product's effectiveness. Studies showed that CBD could be converted into delta-9-THC after prolonged exposure to simulated gastric acid. However, there is no scientific evidence that this reaction occurs *in vivo* in humans.

Anti-inflammatory Effects of Cannabidiol

To better understand how CBD limits excessive neuroinflammation, CBD was administered by gavage, at a dose of 20 mg/kg, in a murine model of multiple sclerosis known as experimental autoimmune encephalomyelitis. Single-cell RNA sequencing (scRNA Seq) and array-based transcriptomics were used to delineate how CBD limits excessive inflammation within the central nervous system (CNS) and the intestinal lining in EAE. In-depth scRNA Seq analysis of CNS tissue demonstrated that CBD treatment resulted in a significant reduction in CXCL9, CXCL10, and IL-1 β expression within the CNS, leading to inhibited infiltration of inflammatory macrophages. CBD inhibited IL-1 β production independent of the classical cannabinoid receptors, CB1 and CB2. CBD treatment also led to the induction of Myeloid-derived Suppressor Cells (MDSCs) both in the CNS and periphery [138].

Interestingly, CBD treatment of EAE mice revealed significant suppression of inflammation in the gastrointestinal (GI) tract. The intestinal epithelial cells (IECs) of CBD-treated mice demonstrated a transcriptional inhibition of a family of pyroptosis initiators that drive localized inflammation known as gasdermins (GSDMs). Further investigation into the GI tract via 16 s sequencing of cecal and fecal contents demonstrated that oral administration of CBD resulted in no significant changes in the intestinal microbiota composition.

Neuroinflammation provoked by the activated microglia is emerging as an important therapeutic target for neurological dysfunction in the central nervous system. In pathology, the hyperactivation of microglia leads to neuro-inflammation by releasing neurotoxic molecules, such as reactive oxygen species, proteinases, pro-inflammatory cytokines, and chemokines. CBD has demonstrated promising therapeutic effects based on clinical and preclinical studies of neurological disorders such as multiple sclerosis. Several preclinical studies indicated that CBD exhibited potent inhibitory effects of neurotoxic molecules and inflammatory modulators, highlighting its remarkable therapeutic potential for treating neurological disorders [140].

In MS, microglia-mediated pro-inflammatory cytokines, IL-1 β , IFN- γ , and IL-12, break the myelin sheath in a demyelination process that ultimately damages the axon, cutting the connection between neurons [142]. *In vivo* studies explored the effectiveness of CBD in various MS mouse models. CBD, administered intraperitoneally at 5 mg/kg, ameliorated the severity of the symptoms of EAE assessed by the histology and immunocytochemistry assays on spinal cord sections. The diminished axonal damage was accompanied by reduced microglia activation and T-cell recruitment.

The study concluded that the action of CBD was not mediated via the known CB2 receptors. Another study suggested that the adenosine A2A receptors are involved in the anti-inflammatory action of CBD using a viral model of MS in mice. The potent anti-neuroinflammatory activity of CBD, at a dose of 5 mg/kg administered intraperitoneally, was shown to attenuate the activation of microglia and inhibit pro-inflammatory cytokines (IL-1 β), chemokines (CCL2 and CCL5), and the vascular cell adhesion molecule-1 (VCAM-1) [143]. It was also demonstrated that a CBD treatment, administered intraperitoneally at a dose of 5 mg/kg, ameliorated the cuprizone-induced demyelination in mice. The mechanisms were associated with suppressing microglia accumulation and reducing oxidative stress.

Two neurologically and demographically matched samples of MS patients were enrolled in a study to determine the functional brain correlates of information processing speed in MS patients who smoke *Cannabis* and those who are drug naïve. The participants who smoked *Cannabis* daily ($n = 20$) and those who were *Cannabis* naïve ($n = 19$) completed the Brief Repeatable Battery of Neuropsychological Tests and underwent fMRI testing, during which they were administered a modified version of the Symbol Digit Modalities Test (mSDMT).

The *Cannabis* group responded slower in nine of 11 blocks of the mSDMT ($p < 0.001$), showing a trend toward a slower response time ($p < 0.08$) but did not differ in the accuracy of response ($p < 0.18$). Both groups displayed activation in a prefrontal cortex-parietal network associated with information processing speed. When compared to the *Cannabis*-naïve group, *Cannabis* users showed less activation in the right ($p = 0.009$) and left ($p = 0.001$) thalami and increased activation in the anterior cingulate ($p = 0.006$) [142].

Risk Factors of Multiple Sclerosis

The effect of nutrition and dietary supplements are possible factors affecting both disease risk and progression in multiple sclerosis with complex genetic-risk profiles. A study was conducted to assess the regulation of surface-membrane enzymes such as delta-6-desaturase (FADS2), secretory Phospholipase A2(sPLA2) by *Cannabis* seed and evening primrose oils, and hot-natured dietary intervention in relapsing-remitting multiple sclerosis (RRMS) patients [136]. In a double-blind, randomized trial, 100 RRMS patients with extended disability status score (EDSS) < 6 were allocated into three groups: Group A received co-supplemented *Cannabis* seed and evening primrose oils along with an advised hot-natured diet, Group B received olive oil, and Group C received the co-supplemented oils. Clinically EDSS, functional score, and biochemical parameters [blood cells polyunsaturated fatty acid (PUFA), FADS2, sPLA2] were assessed at baseline and after 6 months.

The mean follow-up was 180 ± 2.9 SD days ($n = 65$, 23 males and 42 females aged 34.25 ± 8.07 years with a disease duration of 6.80 ± 4.33 years). There was no significant difference in the study parameters at baseline. After 6 months, significant improvements in EDSS and functional scores were found in groups A and C, while

EDSS and the pyramidal score showed a significant increase in group B. Alteration of biochemical parameters showed improvement in groups A and C. In contrast, the co-supplemented *Cannabis* seed and evening primrose oils with the hot-natured diet can have beneficial effects in improving clinical symptoms and signs in RRMS patients, which were confirmed by the regulation of surface-membrane enzymes.

A study was conducted to investigate the potential effect of drug or substance exposure on the onset of MS diagnosis. A population-based incident case-control study of cases ($n = 547$) with persons 15–50 years of age with MS was identified by the Iranian multiple sclerosis Society (IMSS). The population-based controls ($n = 1057$) were recruited by random digit telephone dialing. Inverse-probability-of-treatment weighting (IPTW) using two sets of propensity scores (PSs) was used to estimate marginal incidence odds ratios (ORs) for MS contrasting pre-specified substance use.

The estimated marginal OR was 6.03 (95% confidence interval: 3.54;10.3, using trimmed weights at the 95th percentile of the stabilized weight distribution) in both IPTW analyses comparing lifetime substance use (opioids, *Cannabis*, inhalants, hallucinogens, and stimulants) for at least one time monthly during a 6 months or longer period versus no history of drug use. Subject to the limitation of causal claims based on case–control studies, the study suggests that monthly drug or substance use for at least six consecutive months may increase the risk of MS by a factor of 3.5 or higher [131].

The combination of evening primrose/*Cannabis* seed oil (EPO/HSO) has essential fatty acids (EFAs) for optimal human health due to the favorable ratio of omega-6/omega-3 and antioxidant properties. The immune response is influenced by the administration of omega-3 polyunsaturated fatty acids (PUFA). Multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE), are affected by PUFA. A study was conducted to evaluate the effects of EPO/HSO on improving the membrane fatty acids composition of spleen and blood cells and immunologic factors compared to rapamycin (RAPA) in the EAE model [144].

Chronic EAE was induced by induction of MOG in C57BL/6 J mice (female, age 6–8 weeks, weight 18–21). Mice were assigned to five groups (6/group) to evaluate the therapeutic effects of EPO/HSO supplement compared with rapamycin: A group; EPO/HSO + RAPA, B group; RAPA, C group; EPO/HSO. The results were compared to two control groups (EAE and naive). The fatty acid profile of the spleen and blood cell membrane was evaluated. Real-time polymerase chain reaction was used to evaluate the gene expression levels of interleukin (IL)-4, IL-5, and IL-13 in lymphocytes. Also, IL-4 in serum was evaluated by enzyme-linked immunosorbent assay (ELISA). EPO/HSO therapy significantly increased the percentage of essential fatty acids in the spleen and blood cell membrane. The relative expression of IL-4, IL-5, and IL-13 genes in lymphocytes and serum level of IL-4 was significantly increased in the HSO/EPO treated group versus other groups [146].

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Chapter 10

Cannabis and Cancer



The International Agency for Research on Cancer report on the global burden of cancer estimated 18.1 million new cancer cases (17.0 million excluding nonmelanoma skin cancers) and 9.6 million cancer deaths (9.5 million excluding nonmelanoma skin cancers) in 2018. In both sexes combined, lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths), closely followed by female breast cancer (11.6%), prostate cancer (7.1%), and colorectal cancer (6.1%) for incidence and colorectal cancer (9.2%), stomach cancer (8.2%), and liver cancer (8.2%) for mortality [33].

Lung cancer is the most frequent cancer and the leading cause of cancer death among males, followed by prostate and colorectal cancer (for incidence) and liver and stomach cancer (for mortality). Among females, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death, followed by colorectal and lung cancer (for incidence) and vice versa (for mortality); cervical cancer ranks fourth for both incidence and mortality.

However, the most frequently diagnosed cancer and the leading cause of cancer death substantially vary across countries and within each country, depending on the degree of economic development and associated social and lifestyle factors. It is noteworthy that high-quality cancer registry data, the basis for planning and implementing evidence-based cancer control programs, are not available in most low- and middle-income countries.

Cancer is a multifactorial disease encompassing a combination of factors, such as genetic susceptibility, exposure to carcinogens, random gene mutations, and chronic inflammation. Phytocannabinoids trigger cancer cell death via various signal transduction pathways, including oxidative stress, cell-cycle arrest, endoplasmic reticulum stress, autophagy, and apoptosis. Studies have demonstrated that phytocannabinoids potentially possess anticancer properties, including the inhibition of cell migration, proliferation, and angiogenesis and the induction of apoptosis in skin, lung, breast, prostate, and glioma cancer cells.

Endocannabinoids and Cancer

Endocannabinoids are endogenous lipid-signaling molecules generated in the cell membrane from phospholipid precursors. The endocannabinoid system is implicated in various physiological and pathological conditions, such as inflammation, immunomodulation, analgesia, and cancer. The main active ingredient of *Cannabis*, delta-9-tetrahydrocannabinol, produces its effects through the activation of CB1 and CB2 receptors. CB1 receptors are expressed at high levels in the central nervous system, whereas CB2 receptors are concentrated predominantly, although not exclusively, in immune system cells.

The two best-characterized endocannabinoids are anandamide and 2-arachidonoylglycerol. The relationship between the endocannabinoid system and antitumor actions, such as inhibition of cell proliferation and migration, induction of apoptosis, and reduction of tumor growth of the cannabinoids in different types of cancer, was examined. The activation of the endocannabinoid system impacts breast, prostate, and bone cancers in both in vitro and in vivo systems. Identifying safe and effective treatments to manage and improve cancer therapy is critical to improving the quality of life and reducing unnecessary suffering in cancer patients. In this regard, *Cannabis*-like compounds offer therapeutic potential for treating patients' breast, prostate, and bone cancers.

In cancer cell lines, CB1 and CB2 agonists were first shown to modulate the activity of ERK, p38 MAPK, and JNK1/2. However, there was a clear difference in the activity produced, sustained stimulation vs. inhibition, depending upon the agonist used and the cancer cell line studied. Initially, the production of ceramide leading to sustained upregulation of ERK activity by treatment with CB1 and CB2 agonists was shown to be an essential component of receptor-mediated signal transduction leading to the inhibition of brain cancer cells growth, both in culture and in vivo, and in targeting primary tumor growth to include de novo synthesis of ceramide leading to the endoplasmic reticulum stress and induction of TRIB3 resulting in inhibition of pAkt/mTOR and the production of autophagy-mediated cell death [1].

N-arachidonylethanolamine was the first to be discovered for its euphoric effects. AEA binds to and activates the same receptor as delta-9-tetrahydrocannabinol, and its synthesis is catalyzed by an *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD). The formation of 2-AG occurs by hydrolysis of inositol phospholipids through a specific phospholipase C (PLC) to generate diacylglycerol (DAG), which is then converted into 2-AG by a sn-1-DAG lipase (DAGL).

Two specific enzymes control ECs degradation: the fatty acid amide hydrolase (FAAH), which preferentially degrades AEA and 2-AG to a less extent, and the monoacylglycerol-lipase (MAGL) that hydrolyzes 2-AG. In the brain, 2-AG is present at higher levels than AEA under most physiological conditions, indicating that 2-AG is the most efficacious endogenous natural ligand for the cannabinoid receptors. AEA, but not 2-AG, also behaves as an endovanilloid, binding to and activating the type-1 vanilloid receptor (transient receptor potential vanilloid 1, TRPV1) at an intracellular site.

Antitumorigenic Effects

Studies indicate that CB receptors are upregulated in humans with tumors, and their overexpression correlates with cancer [43]. The activation of CB receptors in human cells in vitro and mice models often has an antitumorigenic effect, activating cancer cell proliferation, migration, apoptosis, and tumor vascularization. The mechanisms are complex and can differ between cancer types. The inactivation might target a selected number of tumors instead of stimulating cannabinoid receptors. This highlights the complexity of the endocannabinoid system and the need for careful molecular characterization of the tumors and patients' stratification before agonists or antagonists of cannabinoid receptors are employed.

Although the activation of CB receptors has been associated with an anticancer effect in a variety of human cells and mice models, *Cannabis* use has also been evaluated as a risk factor for testicular cancer development by increasing the incidence of testicular tumors. The reported association between *Cannabis* exposure and increased incidence of testicular tumors concerns exclusively nonseminomas. The active compounds in phytocannabinoids could likely target the hypothalamic–testis axis by binding to cannabinoid receptors at a central level, thus disrupting normal hormone regulation of spermatogenesis and leading to carcinogenesis. Also, cannabinoids could bind to CB receptors expressed in germ cells or somatic cells at the periphery of the testis, altering germ cell development and firing the cancerous transformation.

Genome and Epigenome

The interest is increased following identifying epigenetic implications of *Cannabis* exposure in germ cells. The cells are unique in their ability to transfer genetic/epigenetic information from generation to generation. As such, the integrity of their genome and epigenome is paramount to the health of organisms of the next generation. On this basis, studies have highlighted that fathering cannabinoid exposure-induced alterations in the sperm epigenome and alterations were associated with increased defects in the offspring, including behavioral and neurobiological abnormalities, and reduced growth at birth and fetal life. Since testicular tumors are believed to arise from the failure of normal maturation of gonocytes, the risk of a potential predisposition in the offspring to TGCT or metabolic and chronic diseases in adulthood should be considered.

Endocannabinoids in Male Reproductive Functions

The biological actions of eCBs are mediated by the two main cannabinoid receptors. Endocannabinoids are released “on-demand” from membrane phospholipid precursors, and their tone is established by the balance between their biosynthesis

and degradation. Endocannabinoids and their receptors are present in the testis of invertebrates and vertebrates, including sea urchins, frogs, rats, mice, boars, and humans [39]. The conserved expression across species supports that endocannabinoid signaling plays an important role in male reproductive functions.

Strong evidence indicates that eCBs and signaling cascades via cannabinoid receptors regulate different biological activities on male reproduction by acting at central and gonadal levels; they modulate the pituitary–gonad (HPG) axis, Sertoli and Leydig cells functions, germ cell differentiation, and sperm functions [41]. Consequently, any changes in the tone of eCBs and the expression of cannabinoid receptors CB1 or CB2 may have consequences on reproductive health. Accordingly, in humans, low AEA levels and decreased cannabinoid receptor mRNA transcripts have been found in the seminal plasma of men with asthenozoospermia or oligoasthenoteratozoospermia.

In the mouse, ECS has been hypothesized to control at least two critical steps of the germ cell differentiation required for the success of spermatogenesis. From the mitotic to haploid stage, mammalian male germ cells have a complete and active ECS modulated during spermatogenesis [42]. At an early phase of spermatogenesis, testicular 2-AG, produced at a high level by spermatogonia, might act as an autocrine factor via CB2, promoting their meiotic entry. In the final steps of spermatogenesis, AEA, produced by Sertoli cells and spermatids, might act on spermatids as a paracrine/autocrine factor via CB1, regulating spermiogenesis. In this way, continuous sperm production throughout the reproductive lifetime is guaranteed by the action of ECS, which controls the balance between meiotic entry of spermatogonia and spermiation. A similar expression pattern of many components of the ECS has been recently described in human germ cells, suggesting that eCBs might also play a role in regulating human spermatogenesis.

Consequently, exogenous cannabinoid exposure, as in *Cannabis* users, could over-activate the system and negatively impact male fertility. Interestingly, in humans, *Cannabis* use is associated with impotence, decreased testosterone level, impairment of spermatogenesis, and reduction of sperm motility and viability; it has been associated with alterations in sperm epigenome in humans, rats, and mice. This observation is strongly relevant for the potential impact on the gamete function of transmitting information to the next generation.

Cannabinoids

Cannabinoids have been applied to treat and manage several diseases and conditions, including pain, cramps, migraine, limb muscle spasms, asthma, sleep disorders, depression, insomnia, and emesis. Intense research has been conducted to evaluate the clinical and pharmacological potential of cannabinoids, alone and in combination, for treating different pathological conditions. Cannabinoid-based medicines that are already approved for clinical use include nabiximols (Sativex), a 1:1 mixture of delta-9-THC and CBD used in Multiple sclerosis, dronabinol

(Marinol) and nabilone (Cesamet), two delta-9-THC synthetic analogs used to relieve chemotherapy-related side effects, such as vomiting and nausea, and CBD oil (Epidiolex) for the treatment of some pediatric epilepsy conditions.

Cannabinoids have been evaluated for other conditions and diseases, including cancer. It was postulated that the ECS might be involved in controlling cell proliferation and survival, considering the expression of endocannabinoids in living organisms and their ability to modulate the activity of proteins responsible for cell proliferation, differentiation, and apoptosis. However, there are conflicting data regarding the role in tumor development since some studies suggest it may be over-activated. In contrast, others demonstrate that the activation of CBs reduces tumor growth, suggesting that the ECS may display tumor-suppressive actions.

The biphasic effect seems to be dose-dependent, as low doses of cannabinoids promoted cell proliferation while higher doses induced antiproliferative actions. The antiproliferative effects were demonstrated when delta-9-THC and CBN inhibited lung adenocarcinoma cell growth [45]. Cannabinoids, including the endocannabinoids AEA and 2-AG and the synthetic cannabinoids WIN55212-2 and HU-210, have also been highlighted for their antitumor actions in vitro and in vivo [91].

The primary antitumor effects of cannabinoids rely on cell-cycle arrest through the inhibition of the expression of growth factors and induction of apoptosis. They can also avoid angiogenesis and block invasion and metastasis by impairing the activation of the vascular endothelial growth factor (VEGF) pathway. The actions of cannabinoids have been identified in different tumors, including gliomas, melanomas, lymphomas, breast cancer, skin cancer, lung carcinoma, liver cancer, pancreatic cancer, colon cancer, and prostate cancer, indicating that the antitumor actions are not tumor-type specific.

Several clinical trials have evaluated the safety and effectiveness of cannabinoids or cannabinoid-based preparations alone or combined with chemotherapy agents such as temozolomide, bortezomib, fluorouracil, oxaliplatin, leucovorin, bevacizumab, irinotecan, palonosetron, and dexamethasone. The results thus far indicate that, generally, cannabinoids are well-tolerated compounds without significant side effects and with a high potential for the modulation of pain and chemotherapy-related side effects [44].

Although the knowledge concerning the pharmacology of a majority of the cannabinoids is poor, it is accepted that delta-9-tetrahydrocannabinol is the most abundant cannabinoid in the *Cannabis* plant. Other cannabinoids include delta-8-tetrahydrocannabinol, which is almost as potent as delta-9-tetrahydrocannabinol but is found in smaller quantities. Tetrahydrocannabinol affects several biological functions by mimicking endogenous substances that activate cannabinoid receptors.

Cannabinoid receptor is a term to describe receptors that respond to cannabinoid drugs derived from *Cannabis sativa* and its biologically active synthetic analogs. Synthetic cannabinoids, developed in scientific laboratories, have structural similarities to natural and endogenous cannabinoids, such as WIN-55212-2, JWH-133, and (*R*)-methanandamide. Exogenous agonists include synthetic agonists, such as CP55940, and plant-derived natural products, such as tetrahydrocannabinol. In

particular, synthetic agonists that bind to cannabinoid receptors include delta-9-tetrahydrocannabinol-like analogs and aminoalkylindole compounds typified by R-WIN-55212.

Studies have shown that potent agonists and antagonists with marked selectivity for CB1 or CB2 receptors can be constructed. Likewise, CB1, CB2, and CB1/CB2 knockout mice have been developed. CB1 has been found in rat, mouse, and human tissues and is identical in 97–99% of its amino acid sequence across species. *CB1* mRNA and protein are detected predominately in the brain and neuronal tissue.

Cannabinoid receptor 1 is abundant in the brain and it is expressed in some peripheral presynaptic nerve terminals and many extra-neural locations. CB1 receptor consists of a seven-transmembrane domain receptor. Its function is associated with biological responses and information about the structure–activity relationships of ligands for interaction with this receptor. Stimulation of the CB1 receptors suppresses the release of neurotransmitters, such as gamma-aminobutyric acid, allowing the endocannabinoid system to reduce the activity of the gamma-aminobutyric acid terminal in a retrograde manner back across the synapse.

There is a 48% homology between CB1 and CB2. Mouse CB2 has been cloned, and its sequence is 82% identical to human CB2. The mRNA of CB2 was first detected in immune tissue. Thus, it was thought that CB2 only existed in the immune system. However, it has been described as expressed in many cell types, such as natural killer cells, macrophages, monocytes, β -lymphocytes, microglial cells, and inflammatory cells, in various human body organs, such as the brain and the gastrointestinal tract. Notably, CB2 mRNA is missing from normal nervous tissue. Delta-9-tetrahydrocannabinol-like, aminoalkylindole, and eicosanoid ligands are the basic ligands of expressed CB2 protein. CB2 triggers a sustained activation of ceramide biosynthesis [69].

Both CB1 and CB2 are coupled to toxin-sensitive Gi/o proteins. Gi proteins are heterotrimeric GTP-binding proteins that inhibit plasma membrane adenylyl cyclase. Binding to an agonist with CB1 or CB2 leads to a signaling cascade in which the activated receptor activates Gi, which inhibits adenylyl cyclase, reducing intracellular cyclic adenosine monophosphate (cAMP). Essentially, the classical theory of receptor occupancy and activation is a conformational induction theory that fits equivalently with Koshland's induced-fit model for enzymes. In particular, when agonist A binds to the resting-state receptor R, conformational changes are provoked, leading to the activation of receptor R.

CB1 is coupled through Gi/o proteins to various potassium and calcium channels. Additionally, in 2007, a new cannabinoid receptor called G protein-coupled receptor 55 (GPR55) was identified, which binds to and is activated by CP55940 with potential action on the brain. This action was confirmed in explaining how GPR55 mediates anxiolytic-like effects in the medial orbital cortex of mice. The effect, in conjunction with analgesia, appetite regulation, and antiemetic actions, is referred to as palliative methods for symptoms of malignancies, with potential use as a new treatment for pancreatic cancer.

The antiproliferative potential of cannabinoids was first reported when it was shown that oral administration of tetrahydrocannabinol in mice inhibits Lewis lung

adenocarcinoma tumor growth [45]. Later, studies reported that either endogenous or synthetic cannabinoids induce antitumor effects on a wide spectrum of tumor cells in culture. In particular, it was found that in several types of cancer cells, such as glioma, lymphoma, prostate, breast, pancreatic, and skin cancer cells, cannabinoids reduced tumor size and induced apoptosis.

As various biochemical and pharmacological approaches indicate, the antitumor effects require the efficiency of CB1 and CB2. Elective cannabinoid receptor agonists and antagonists, which ligate to CB1 and CB2, can be used to study their expression and mode of action (apoptotic or stimulatory) in malignant cells. However, at certain doses and in specific cellular contexts, some cannabinoids have been reported to induce the proliferation of cancer cells *in vitro*. Studies in animal tumor models, such as Wistar rats inoculated with C6 gliomas, and xenografts in athymic nude mice implanted with KiMol or MBA-MD-231 breast cancer cells, revealed that cannabinoid receptor agonists exerted antiproliferative actions.

Cannabinoid receptors, when activated by specific G-protein coupling with cannabinoids, induce several cellular pathways. The cAMP–protein kinase A pathway is inhibited, and the activity of Ca^{2+} and K^{+} channels is modulated. The changes lead to the inhibition of neurotransmitter release. CB1 participates in various cellular signaling pathways that control cell fate. In particular, CB1 receptor coupling urges the activation of mitogen-activated protein kinase (MAPK) cascades, such as the extracellular-signal-regulated kinase (ERK), the stress-activated kinases Jun amino-terminal kinase (JNK), and p38 MAPK.

The signaling cascades exert dominant roles in cell proliferation and differentiation regulation. Cannabinoid-induced MAPK stimulation has been reported in neural cell lines, primary neural cells, lymphoid cells, vascular endothelial cells, and Chinese hamster ovary cells transfected with cannabinoid receptor complementary DNAs. An *in vitro* study in a neuronal-like cell line showed that CB1 receptor activation attenuates ERK [70].

Cannabinoid receptors activate the phosphatidylinositol 3-kinase (PI3K)-AKT serine/threonine kinase 1 (AKT) survival pathway. Particularly, the phosphorylation of AKT induces the inhibition of nuclear translocation of forkhead transcription factors [71]. As a result, the expression of proapoptotic proteins is prevented. A study reported that cannabinoid receptors negatively regulated AKT activation. Some systems showed that PI3K is an upstream component of cannabinoid-induced ERK activation, while others reported contradictory results [72]. Data claim that nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and nitric oxide synthase (NOS) are other targets of CB1 activation, which are involved in the control of cell fate. Nevertheless, it is not clear if the components are activated or inhibited by cannabinoids.

Cannabinoid receptors are also involved in the modulation of sphingolipid-metabolizing pathways. CB1 activation induces sphingomyelin breakdown, increasing ceramide levels. Ceramide is a lipid second messenger that stimulates apoptosis and cell-cycle arrest. Interestingly, the actions are not based on the G-protein structure but are cannabinoid receptor-dependent. It is reported that an adaptor factor associated with neutral sphingomyelinase activation (FAN) is involved. Furthermore,

cannabinoid receptor activation has the potential to urge a sustained peak of ceramide expression through enhanced de novo synthesis [73].

Overall, cannabinoid receptors are implicated in antitumor effects by various mechanisms, as their activation stimulates transformed-cell death, inhibits transformed-cell proliferation, and limits tumor angiogenesis and metastasis. De novo ceramide accumulation sustained ERK activation, and AKT inhibition induced apoptosis. In glioma cells, ceramide levels are increased after cannabinoid receptor activation. The increase induces the activation of RAF1–mitogen-activated protein kinase (MEK)–ERK signaling cascade and the AKT inhibition. ERK activation stimulates cell proliferation. Many factors can affect ERK activation and cell fate, given that prolonged ERK stimulation mediates cell-cycle arrest and cell death. It has been reported that pharmacological inhibition of de novo ceramide synthesis in prostate tumor cells led to the prevention of cannabinoid-induced cell death [74].

A study on breast cancer cells found that CB1 receptor activation induced cell-cycle arrest at the G1-S transition by inhibiting adenylyl cyclase and the cAMP signaling cascade. Given that the phosphorylation of protein kinase A reduces RAF1 expression, cannabinoid receptors block the inhibition of RAF1. As a result, the RAF1–MEK–ERK pathway is stimulated. Corresponding research in thyroid epithelioma cells transformed with the Kirsten rat sarcoma (*KRAS*) oncogene showed that CB1 receptor activation also blocks the cell cycle at the G1-S transition. Similar results of cell-cycle arrest following cannabinoid receptor activation have been reported in skin and prostate cancer cells. In those studies, growth-factor-receptor signaling is inhibited. A general underlying mechanism of cannabinoid antitumor action likely exists.

Delta-9-THC

Delta-9-THC was shown to inhibit the growth of tumors, inhibits angiogenesis, and promotes apoptosis in various cancer cells in vitro and in vivo. Delta-9-THC and CBD exhibited synergistic inhibition of cell proliferation in GBM cell lines. Cannabidiol inhibits the invasiveness of breast cancer and GBM cells at sublethal concentrations by downregulating the matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). An MMP–TIMP imbalance results in proteolysis of the matrix that may be associated with different pathological processes, including tumor invasion. In vivo, delta-9-THC and CBD reduced GBM tumor growth. Studies have demonstrated CBG anticancer activity in mouse melanomas, human oral epithelioid carcinoma cells, human breast carcinomas, and colorectal cancer cells.

Delta-9-THC inhibits tubulin polymerization, which perturbs the formation and function of the microtubules of the mitotic spindle. The disruption of the mitotic machinery perturbs proper chromosomal segregation during anaphase. It causes micronucleus formation, the primary locus, and the cause of the chromosomal pulverization of chromothripsis and downstream genotoxic events, including oncogene induction and tumor suppressor silencing. Complementing multiple positive

Cannabis-cancer epidemiological studies and replicated dose–response relationships with established mechanisms fulfills causal criteria.

The data is also consistent with the acceleration of the aging process by addictive drugs, including alcohol, tobacco, *Cannabis*, stimulants, and opioids. Delta-9-THC shows a nonlinear sigmoidal dose–response relationship in multiple pertinent *in vitro* and preclinical genotoxicity assays and, in this respect, is similar to the serious major human mutagen thalidomide. Rising community exposure, tissue storage of cannabinoids, and increasingly potent phytocannabinoid sources suggest that the threshold mutagenic dose for cancerogenesis will increasingly be crossed beyond the developing world and raise transgenerational transmission of teratogenicity as an increasing concern.

Two fractions of a high-delta-9-THC *Cannabis* strain extract produced significant cytotoxic activity against Human GBM cell lines and GSCs derived from Human tumor specimens. The two fractions were composed of different phytocannabinoid combinations, with CBG or delta-9-THC as the most abundant compound. The active fractions induced apoptosis and the expression of ER-stress-associated genes. The fractions altered cell cytoskeletons, reduced cell invasion, and inhibited cell migration and colony formation. The study demonstrated the therapeutic potential of combinations of *Cannabis* compounds in exerting cytotoxic, antiproliferative, and antimigratory effects on human GBM cells. The activity of combinations was higher than the purified primary compound, each fraction, and the crude extract.

Cannabigerol

Cannabigerol (CBG) is a *Cannabis*-derived cannabinoid that interacts with specific targets involved in carcinogenesis. Specifically, cannabigerol potently blocks transient receptor potential (TRP) M8 (TRPM8), activates TRPA1, TRPV1, and TRPV2 channels, blocks 5-hydroxytryptamine receptor 1A (5-HT1A) receptors, and inhibits the reuptake of endocannabinoids [68].

Cannabigerol protection against colon tumorigenesis was investigated in colorectal cancer cells using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide and 3-amino-7-dimethylamino-2-methylphenazine hydrochloride assays. Apoptosis was examined by histology and by assessing caspase-3/7 activity; reactive oxygen species production by a fluorescent probe; CB receptors, TRP, and CCAAT/enhancer-binding protein homologous protein (CHOP) messenger RNA (mRNA) expression were quantified by reverse transcription-polymerase chain reaction; small hairpin RNA-vector silencing of TRPM8 was performed by electroporation. The *in vivo* antineoplastic effect of cannabigerol was assessed using mouse models of colon cancer. Colorectal cancer cells expressed TRPM8, CB1, CB2, 5-HT1A receptors, TRPA1, TRPV1, and TRPV2 mRNA.

Cannabigerol hampers colon cancer progression *in vivo* and selectively inhibits the growth of colorectal cancer cells, an effect shared by other TRPM8 antagonists. Cannabigerol promoted apoptosis, stimulated reactive oxygen species production,

upregulated CHOP mRNA, and reduced cell growth in colorectal cancer cells. Cannabigerol effect on cell growth was independent of TRPA1, TRPV1, and TRPV2 channels activation, was further increased by a CB2 receptor antagonist, and mimicked by other TRPM8 channel blockers but not by a 5-HT1A antagonist. The effect of cannabigerol on cell growth and CHOP mRNA expression was reduced in TRPM8-silenced cells. In vivo, cannabigerol inhibited the growth of xenograft tumors and chemically induced colon carcinogenesis.

Cannabigerol is one of the main active phytocannabinoids produced by *Cannabis* plants; however, it is considered a minor phytocannabinoid due to its lower abundance relative to delta-9-THC and CBD. *Cannabis* cultivars that tend to have higher cannabigerol content are referred to as Type IV *Cannabis* [92].

Cannabigerol is derived from its acidic precursor cannabigerolic acid (CBGA), which also serves as the precursor molecule for the production of delta-9-THC and CBD. Cannabigerol has attracted more attention for its use in therapeutics due to its lack of intoxicating effects, and more commercial varieties have been developed with CBG and CBGA as the main phytocannabinoids present. Using [³H]-CP-55940, CBG competed with low micromolar K_i values the binding to CB₁R and CB₂R. Homogeneous binding in living cells, which is only technically possible for the CB₂R, provided a 152 nM K_i value. Also interesting, CBG competed the binding of [³H]-WIN-55,212-2 to CB₂R but not to CB₁R (K_i : 2.7 versus >30 μ M). The phytocannabinoid modulated signaling mediated by receptors and receptor heteromers even at low concentrations of 0.1–1 μ M. cAMP, pERK, β -arrestin recruitment, and label-free assays in HEK-293T cells expressing the receptors and treated with endocannabinoids or selective agonists proved that CBG is a partial agonist of CB₂R. The action on cells expressing heteromers was similar to that obtained in cells expressing the CB₂R [93].

Two studies examined the potential therapeutic benefits of cannabigerol in mouse skin melanoma cells and oral epithelioid carcinoma (KB) cells. In mouse skin melanoma cells, CBG produced significant inhibitory effects on proliferation, with an IC₅₀ of 3 μ g/mL. In KB cell lines, CBG over a concentration range of 1–100 μ M was the most effective of the cannabinoids tested at reducing cell viability [94].

The antitumor activities of cannabidiol, cannabigerol, cannabichromene, cannabidiol acid, and THC acid were assessed for the advantage in using *Cannabis* extracts enriched in either cannabidiol or delta-9-THC over pure cannabinoids. The results obtained from a panel of tumor cell lines clearly indicate that, of the five natural compounds tested, cannabidiol is the most potent inhibitor of cancer cell growth (IC₅₀ between 6.0 and 10.6 μ M), with significantly lower potency in noncancer cells; whereas cannabigerol and cannabichromene followed in the rank of potency. Both cannabidiol and the cannabidiol-rich extract inhibited the growth of xenograft tumors obtained by subcutaneous injection into athymic mice of human MDA-MB-231 breast carcinoma or rat v-K-ras-transformed thyroid epithelial cells and reduced lung metastases deriving from intrapaw injection of MDA-MB-231 cells. Judging from several experiments on its possible cellular and molecular mechanisms of action, we propose that cannabidiol lacks a unique mode of action in the cell lines investigated. At least for MDA-MB-231 cells, however, our experiments indicate that cannabidiol effect is due to its capability of inducing apoptosis

via: direct or indirect activation of cannabinoid CB2 and vanilloid transient receptor potential vanilloid type-1 receptors and cannabinoid/vanilloid receptor-independent elevation of intracellular Ca^{2+} and reactive oxygen species [10].

CBG also stimulated apoptosis, ROS production, upregulated C/EBP homologous protein (CHOP) mRNA and inhibited cell proliferation in colorectal cancer (CRC) cells. In vivo, CBG was shown to decrease the growth of xenograft tumors in a murine model, and that this effect was largely due to its activity as an antagonist at TRPM8 receptors on CRC cells [68].

Tetrahydrocannabivarin

Tetrahydrocannabivarin (THCV) is a homologue of delta-9-THC, where different side chains contribute to a variety of effects that are distinct from delta-9-THC. Most *Cannabis* cultivars only contain trace amounts of THCV, but some cultivars from hybridized African genetics may have higher levels of THCV. As for most other minor cannabinoids, little has been demonstrated regarding the effects of THCV in cancer. Some cytotoxic effects were observed for tetrahydrocannabivarin in prostate cancer cell lines DU-145 and LNCaP, with IC_{50} values above $17.5\text{ }\mu\text{M}$ [95].

Cannabinol

Cannabinol (CBN) is present in the *Cannabis* plant and, particularly in aged *Cannabis*. It is the degraded product of tetrahydrocannabinolic acid. It was the first of the phytocannabinoids to be isolated but remains poorly studied. Cannabinol's psychoactive effects are estimated to be 10 times lower than that of delta-9-THC [96].

Some cytotoxic effects were observed for cannabinol in prostate cancer cell lines DU-145 and LNCaP. The observed IC_{50} was reported to be superior to the highest dose tested ($25\text{ }\mu\text{M}$) in most experiments. CBN has also been shown to have some antiproliferative effects in aggressive breast cancer cells. Multidrug transporters are an ongoing issue in the treatment of cancers due to their ability to confer resistance to multiple anticancer agents. In one study, CBN inhibited multidrug transporter ABCG2 and promoted the accumulation of mitoxantrone, a substrate for this transporter [97].

Cannabidivarin

Cannabidivarin's (CBDV) structure is similar to cannabidiol except with a shortened side chain. *Cannabis* cultivars with relatively high levels of CBDV have been identified in India and Nepal. In one study, CBDV was assessed for potential

cytotoxic effects on various human prostate carcinoma cell lines. Results showed an IC_{50} of around 20 μM [95]. In colon cancer cells, CBDV inhibited cell viability in a concentration-dependent manner, with an IC_{50} of 10 μM [68].

Cannabichromene

Cannabichromene (CBC) is considered another of the minor phytocannabinoids produced by *Cannabis* plants, due to its lower abundance than the major cannabinoids delta-9-THC and CBD. In the United States, cannabichromene has been found to be the second most abundant type of cannabinoid present in some strains of *Cannabis*, particularly abundant in dry-type *Cannabis* material. Though CBC is commonly found in many *Cannabis* strains, there is much to be discovered about its pharmacology. Like CBD, cannabichromene lacks intoxicating effects and is therefore appealing to researchers in terms of its potential therapeutic effects in human health and medicine [98].

The potential anticancer effects of cannabichromene have not been extensively studied. In prostate carcinoma (PCC) cells DU-145 and LNCaP, cannabichromene was found to be the second most potent inhibitor of cell viability behind CBD, and CBC at 10 μM and had very little effect on caspase-3/7 activity. In serum deprived PCC 22RV1 and PC-3 cells, treatment with CBC at 20 μM resulted in significant activation of caspase-3/7, and CBC caused elevated intracellular Ca^{2+} in all four PCC cell lines mentioned [95].

In colorectal cancer Caco-2 cells, CBC, at a concentration of 30 μM inhibited cell growth [68]. In MDA-MB-231 and MCF-7 breast cancer cell lines, CBC demonstrated high potency as an inhibitor of cell viability [10].

Cannabidiol

Data strongly support the therapeutic potential of cannabidiol as a direct inhibitor of tumor progression and for enhancing the activity of first-line therapies. Anecdotal reports by cancer patients using various formulations of *Cannabis* suggest significant efficacy, and pharmaceutical companies have initiated GMP-grade cannabinoids for targeting glioblastoma. In many cases, phytocannabinoid concentrations used in vitro do not coincide with those safely achievable in vivo.

The discovery that CB1 and CB2 agonists demonstrate antitumor activity led scientists to investigate the potential antitumor activity of additional plant-derived cannabinoids. The compounds either do not or are inefficient at activating CB1 receptors, producing little to no psychoactivity. Of the compounds, CBD has been the most extensively studied. In culture, the most unifying theme for CBD-dependent inhibition of cancer cell aggressiveness is the production of reactive oxygen species (ROS). The first evidence in vivo is that CBD-dependent generation of ROS is, in

part, responsible for the antitumor activity of the cannabinoid. CBD inhibited disease progression in tumors derived from glioma stem cells (GSCs). However, a portion of therapeutic resistance to the treatment in this subpopulation of tumor cells was the upregulation of antioxidant response genes [2].

Despite the lack of interaction with classical cannabinoid receptors, nonpsychoactive cannabinoids appear to share similar mechanisms for targeting tumor progression [4]. Paradoxically, CBD is neuroprotective, and multiple groups have shown selectivity for CBD inhibition of cancer cell growth compared to match non-transformed cells [3]. Additionally, CBD has antioxidant properties in neuronal cultures.

Cannabidiol inhibits human GBM viability in culture, and ROS scavenger α -tocopherol/vitamin E reversed the effect. A study was conducted to evaluate the *in vitro* antiproliferative ability of cannabidiol on the U87 and U373 human glioma cell lines [5]. Adding CBD to the culture medium led to a dramatic drop in mitochondrial oxidative metabolism [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide test] and viability in glioma cells in a concentration-dependent manner that was evident 24 h after CBD exposure, with an IC_{50} of 25 μ M. The antiproliferative effect of CBD was partially prevented by the CB2 receptor antagonist N-[(1S)-endo-1,3,3-trimethylbicyclo[2,2,1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528; SR2) and α -tocopherol.

By contrast, the CB1 cannabinoid receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride (SR141716; SR1), capsazepine (vanilloid receptor antagonist), the inhibitors of ceramide generation, or pertussis toxin did not counteract the effects of CBD. It was also shown that the antiproliferative effect of CBD was correlated to the induction of apoptosis, as determined by cytofluorimetric analysis and single-strand DNA staining, which cannabinoid antagonists did not revert. Cannabidiol administered subcutaneously to nude mice at the dose of 0.5 mg/mouse significantly inhibited the growth of subcutaneously implanted U87 human glioma cells. Cannabidiol produced a significant antitumor activity both *in vitro* and *in vivo*.

It was further demonstrated that CBD-dependent ROS production was accompanied by a reduction in glutathione (GSH) and GSH-related enzymes [6]. GSH is an important antioxidant that prevents damage to cellular components by ROS. The source of CBD-dependent stress partially originated in the mitochondria; it led to the activation of multiple caspases involved in intrinsic and extrinsic pathways of apoptosis. Cannabidiol produced a gradual, time-dependent activation of caspase-3, which preceded the appearance of apoptotic death.

The exposure to CBD caused, in glioma cells, early production of ROS, depletion of intracellular glutathione, and increased activity of glutathione reductase and glutathione peroxidase enzymes. Additionally, the release of Cytochrome C and -9 and caspase-8 activation were detected. Under the same experimental condition, CBD did not impair primary glia. A different sensitivity to the antiproliferative effect of CBD in human glioma cells and nontransformed cells that appears closely

related to a selective ability of CBD in inducing ROS production and caspase activation in tumor cells was detected.

Studies analyzing CBD-treated GBM tumor tissue revealed that inhibition of lipoxygenase (LOX) signaling played a role in CBD antitumor activity. In addition, the indirect modulation of the endocannabinoid system by CBD may be attributed to the observed antitumor activity. The *Cannabis* constituents, cannabidiol (1), delta-8-tetrahydrocannabinol (3), and cannabinol (5), were oxidized to their respective paraquinones 2, 4, and 6. The oxidized product 4 had been assigned a paraquinone structure, later modified to an orthoquinone.

A detailed NMR evaluation was conducted to distinguish between the two possible quinone structures, and the original paraquinone form was confirmed. X-ray crystallography elucidated the structures of crystallines 2 and 6. All three compounds displayed antiproliferative activity in several human cancer cell lines in vitro, and quinone 2 significantly reduced cancer growth of HT-29 cancer in nude mice [7]. CBD-hydroxyquinone (HU-311) inhibited prostate cancer cell viability and tumor progression in vivo.

It was later demonstrated that HU-311, a new anticancer quinone synthesized from cannabidiol, shows significantly high efficacy against human cancer cell lines in vitro and against in vivo tumor grafts in nude mice. Its mode of action and evidence of its mechanism was investigated. HU-331 does not cause cancer cell-cycle arrest, cell apoptosis, or caspase activation. HU-331-caused cell death of human cancer cell lines is not mediated by reactive oxygen intermediates/species, as exposure to HU-331 failed to elicit the generation of reactive oxygen species. HU-331 inhibits DNA topoisomerase II even at nanomolar concentrations but has a slight nonsignificant effect on DNA topoisomerase I action. The cannabinoid quinone HU-331 is a highly specific inhibitor of topoisomerase II compared with the most known anticancer quinones and represents a new potent anticancer drug [8].

The results revealed that cannabidiol, acting through CB2 and regulating Nox4 and p22(phox) expression, may be a highly selective treatment for leukemia [8]. Exposure of leukemia cells to cannabidiol led to cannabinoid receptor 2-mediated reduction in cell viability and induction in apoptosis. Furthermore, cannabidiol treatment led to a significant decrease in tumor burden and an increase in apoptotic tumors in vivo.

From a mechanistic standpoint, cannabidiol exposure resulted in the activation of caspase-8, caspase-9, and caspase-3, cleavage of poly(ADP-ribose) polymerase, and decreased the full-length BID, suggesting possible cross-talk between the intrinsic and extrinsic apoptotic pathways. The role of the mitochondria was further suggested as exposure to cannabidiol led to the loss of mitochondrial membrane potential and release of Cytochrome C.

Cannabidiol exposure decreased p-p38 mitogen-activated protein kinase levels, which a CB2-selective antagonist or reactive oxygen species scavenger treatment could block. Cannabidiol exposure also increased reactive oxygen species production and the expression of the NAD(P)H oxidases Nox4 and p22(phox). Cannabidiol-induced apoptosis and reactive oxygen species levels could be blocked by treatment with the reactive oxygen species scavengers or the NAD(P)H oxidase inhibitors.

The role of COX-2 and PPAR- γ in cannabidiol's proapoptotic and tumor-regressive action was investigated in lung cancer cell lines A549, H460, and primary cells from a patient with lung cancer. Apoptotic cell death by cannabidiol was suppressed by NS-398 (COX-2 inhibitor), GW9662 (PPAR- γ antagonist), and siRNA targeting COX-2 and PPAR- γ . Cannabidiol elicited decreased viability associated with apoptosis. Cannabidiol-induced apoptosis was paralleled by upregulation of COX-2 and PPAR- γ mRNA and protein expression with a maximum induction of COX-2 mRNA after 8 h and continuous increases of PPAR- γ mRNA when compared with vehicle [9].

In response to cannabidiol, tumor cell lines exhibited increased levels of COX-2-dependent prostaglandins (PG), among which PGD(2) and 15-deoxy- Δ (12,14)-PGJ(2) (15d-PGJ(2)) caused a translocation of PPAR- γ to the nucleus and induced PPAR- γ -dependent apoptotic cell death. Moreover, in A549-xenografted nude mice, cannabidiol produced upregulation of COX-2 and PPAR- γ in tumor tissue and tumor regression that was reversible by GW9662. Together, the data revealed a novel proapoptotic mechanism of cannabidiol involving initial upregulation of COX-2 and PPAR- γ and subsequent nuclear translocation of PPAR- γ by COX-2-dependent PGs.

Imaging studies of hippocampal cultures using fura-2 AM suggested that CBD-mediated $\text{Ca}(2^+)$ regulation is bidirectional, depending on the excitability of cells. Under physiological $\text{K}(^+)/\text{Ca}(2^+)$ levels, CBD produced a subtle rise in $[\text{Ca}(2^+)]$. In contrast, CBD reduced $[\text{Ca}(2^+)]$ and prevented $\text{Ca}(2^+)$ oscillations under high-excitability conditions (high $\text{K}(^+)$ or exposure to the $\text{K}(^+)$ channel antagonist 4AP). Regulation of $[\text{Ca}(2^+)]$ was not primarily mediated by interactions with ryanodine or IP(3) receptors of the endoplasmic reticulum [10].

Application of carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) and the mitochondrial $\text{Na}(^+)/\text{Ca}(2^+)$ exchange inhibitor, CGP 37157, but not the mitochondrial permeability transition pore inhibitor cyclosporin A, prevented subsequent CBD-induced $\text{Ca}(2^+)$ responses. Instead, dual-calcium imaging experiments with a cytosolic (fura-2 AM) and a mitochondrial (Rhod-FF, AM) fluorophore implied that mitochondria act as sinks and sources for CBD's $[\text{Ca}(2^+)]$ regulation. In established human neuroblastoma cell lines, SH-SY5Y treated with mitochondrial toxins, CBD (0.1 and 1 μM) was neuroprotective against the uncoupler FCCP (53% protection) and modestly protective against hydrogen peroxide-mediated cell death (16%) and oligomycin-mediated cell death (15%). A pattern confirmed in cultured hippocampal neurons that under pathological conditions involving mitochondrial dysfunction and $\text{Ca}(2^+)$ dysregulation, CBD proved beneficial in preventing apoptotic signaling via restoring $\text{Ca}(2^+)$ homeostasis.

The antitumor activities of cannabinoids, i.e., cannabidiol, cannabigerol, cannabichromene, cannabidiol acid, and delta-9-THC, were assessed to determine whether there is an advantage in using *Cannabis* extracts (enriched in either cannabidiol or delta-9-THC) over pure cannabinoid [10]. In oligodendrocytes, CBD produced a concentration-dependent increase in calcium leading to alterations in mitochondrial membrane potential, production of ROS, and ultimately cytotoxicity.

In hippocampal cultures, the activity of CBD was not related to the activation of CB1, CB2, or TRPV1.

The results obtained in a panel of tumor cell lines indicate that of the five natural compounds tested, cannabidiol is the most potent inhibitor of cancer cell growth (IC_{50} between 6.0 and 10.6 μM), with significantly lower potency in noncancer cells. The cannabidiol-rich extract was equipotent to cannabidiol, whereas cannabigerol and cannabichromene followed in potency. Both cannabidiol and the cannabidiol-rich extract inhibited the growth of xenograft tumors obtained by subcutaneous injection into athymic mice of human MDA-MB-231 breast carcinoma or rat v-K-ras-transformed thyroid epithelial cells and reduced lung metastases deriving from intrapaw injection of MDA-MB-231 cells.

The data on cellular and molecular mechanisms of action determined that cannabidiol lacks a unique mode of action in the cell lines investigated, at least for MDA-MB-231 cells. However, the experiments indicate that the cannabidiol effect is due to its capability of inducing apoptosis via direct and indirect activation of cannabinoid CB2, vanilloid transient receptor potential, vanilloid type-1 receptors, and cannabinoid/vanilloid receptor-independent elevation of intracellular $Ca(2^+)$, and reactive oxygen species.

Cannabidiol inhibits cell proliferation, induces the death of cancer cells, and activates immune cells. It is not an agonist of the classical CB1/CB2 cannabinoid receptors, and the mechanism by which it functions is not thoroughly clear. The effects of CBD on various mitochondrial functions in BV-2 microglial cells indicate that CBD treatment leads to a biphasic increase in intracellular calcium levels and to changes in mitochondrial function and morphology, leading to cell death [11].

Density gradient fractionation analysis by mass spectrometry and western blotting showed colocalization of CBD with protein markers of mitochondria. Single-channel recordings of the outer-mitochondrial membrane protein, the voltage-dependent anion channel 1 (VDAC1) functioning in cell energy, metabolic homeostasis, and apoptosis revealed that CBD markedly decreases channel conductance. Microscale thermophoresis revealed a direct interaction between purified fluorescently labeled VDAC1 and CBD. Thus, VDAC1 serves as a novel mitochondrial target for CBD. The inhibition of VDAC1 by CBD may be responsible for the immunosuppressive and anticancer effects of CBD. The CBD-dependent inhibition of this channel led to a biphasic increase in intracellular calcium levels leading to changes in mitochondrial function and morphology and production of ROS. The studies suggest that CBD interacts with unique mitochondrial sites leading to the modulation of calcium homeostasis and ROS.

ROS can exert different effects according to the basal metabolic rate of the cell, and the high basal metabolic rate of cancer cells makes them more susceptible to redox-directed therapeutics compared to nontransformed cells. The ability of CBD to selectively reduce the viability and proliferation of cancer cells compared to nontransformed cells may be related to differences in metabolic rate, particularly considering that the origin of CBD-dependent production of ROS appears to stem primarily from the mitochondria. Redox-directed therapeutics have been developed

to act as direct cancer inhibitors and to sensitize tumors to first-line agents. However, they are associated with significant toxicity.

Redox dysregulation, originating from metabolic alterations and dependence on mitogenic and survival signaling through reactive oxygen species, represents a specific vulnerability of malignant cells that redox chemotherapeutics can selectively target. Research indicates that numerous oncogenes and tumor suppressor genes exert their functions partially through redox mechanisms amenable to pharmacological intervention by redox chemotherapeutics.

The pleiotropic action of many redox chemotherapeutics that involves simultaneous modulation of multiple redox-sensitive targets can overcome cancer cell drug resistance from the redundancy of oncogenic signaling and rapid mutation. Moreover, some redox chemotherapeutics may function according to synthetic lethality (i.e., drug cytotoxicity confined to cancer cells that display loss of function in tumor suppressor genes or upregulation of oncogene expression). The number of clinical trials examining redox drugs' therapeutic performance in cancer patients demonstrates that redox chemotherapy has made the crucial transition from bench to bedside [12].

Several groups performed a screen of multiple plant and synthetic cannabinoids. They determined that CBD was more potent at reducing cell viability and proliferation than classical CB1 and CB2 receptor agonists, including delta-9-THC; while that holds in culture, two direct comparisons of the antitumor activity of a classical CB1 and CB2 receptor agonist, delta-9-THC, and CBD, have been carried out in vivo. In tumors derived from subcutaneous implanted human GBM, delta-9-THC but not CBD could produce moderate inhibition of tumor progression.

It was demonstrated that the addition of CBD to delta-9-THC enhanced its effects, thereby allowing a lower dose of delta-9-THC to be used to produce equivalent antitumor activity. The study agreed with studies in culture demonstrating that CBD enhanced the inhibitory effects of delta-9-THC on human GBM cell proliferation and survival. CBD's ability to enhance delta-9-THC's activity was associated with ROS production, inhibiting pERK and activating multiple caspases. It was reported in a mouse model of breast cancer metastasis that CBD was more potent than delta-9-THC at inhibiting the formation of metastatic lung foci [13].

Cannabidiol-induced cell death of breast cancer cells, independent of cannabinoid and vanilloid receptor activation, was revealed by electron microscopy of morphologies consistent with the coexistence of autophagy and apoptosis. Western blot analysis confirmed the findings. Cannabidiol induces endoplasmic reticulum stress and inhibits AKT and mTOR signaling, as indicated by decreased phosphorylated mTOR, 4EBP1, and cyclin D1. The cross-talk between the autophagic and apoptotic signaling pathways revealed that beclin1 plays a central role in the induction of CBD-mediated apoptosis in MDA-MB-231 breast cancer cells [3]. Although CBD enhances the interaction between beclin1 and Vps34, it inhibits the association between beclin1 and Bcl-2.

Additionally, CBD reduces mitochondrial membrane potential, triggers the translocation of BID to the mitochondria, the release of Cytochrome C to the cytosol, and, ultimately, the activation of the intrinsic apoptotic pathway in breast cancer

cells. CBD increased the generation of reactive oxygen species, and the reactive oxygen species inhibition blocked the induction of apoptosis and autophagy. The study revealed an intricate interplay between apoptosis and autophagy in CBD-treated breast cancer cells and highlighted the potential use of CBD as an antineoplastic agent.

Another study in culture showed that targeting the human breast cancer cell line, MDA-MB231, with CBD led to endoplasmic reticulum stress, inhibition of the AKT/mTOR pathway, and upregulation of autophagy-mediated cell death. As discussed earlier, targeting the key pathways is also linked to the antitumor activity of CB1 and CB2 receptor agonists such as delta-9-THC.

The striking behavior of CBD homologs treatments was the occurrence of massive cytoplasmic vacuolation in the epithelial MCF-7 cells; vacuoles appeared after 2 h of treatment, and their number and size increased after 24 h. The same changes were less pronounced in mesenchymal MDA-MB-231 cells, while no vacuolation occurred in the epithelial MDA-MB-361 cells.

An increase in oxidative stress has been detected in cancer cells after CBD treatment due to a massive increase in ROS production that leads to the activation of apoptosis and autophagy. ROS levels in MCF-7 cells were treated with 10 μ M nat CBD, syn CBD, CBDB, and CBDP for 24 h using the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) staining. The antioxidant N-acetyl cysteine (NAC) was used to counteract ROS production.

A significant increase in intracellular ROS levels was observed in MCF-7 cells treated with CBD homologs. The NAC treatment in association with CBD homologs revert the relative fluorescence intensity to a value lower than the control due to a remarkable ROS scavenging effect.

Two mitochondria stains, Mitotracker Red and MitoTracker Green, were used to establish the correlation between ROS production and impaired mitochondrial functions. The first is an indicator of mitochondrial membrane potential that selectively stains active mitochondria, while MitoTracker Green allows the detection of the mitochondrial morphology and mass. When MCF-7 cells were exposed to 10 μ M CBDB or CBDP for 24 h, the Mitotracker red fluorescence intensity dramatically dropped compared to the control. Similar results were obtained with nat CBD and syn CBD. The MitoTracker Green fluorescence signal remained similar in control and treated cells.

The observations suggest that the treatment with CBD homologs alters mitochondrial functionality without an appreciable decrease in the mitochondrial mass. The loss of the mitochondrial function was also accompanied by a substantial decrease in ATP, whose levels dropped by approximately 35–40% compared to controls in MCF-7 cells treated with all CBD homologs [57].

Cannabinoids have been reported as palliative adjuvants to conventional chemotherapeutic regimens. Cannabinoids exert a remarkable antitumoral effect on pancreatic cancer cells due to their ability to induce apoptosis of the cells selectively. Studies have described the mechanism of cell death induced by cannabinoids. They offer potential applications, such as being incorporated into chemotherapeutic regimens to prevent nausea and pain and stimulate appetite. Cannabinoids seem

specific, given that cancer cells can be targeted while normal cells can simultaneously be spared.

Although the knowledge concerning the pharmacology of the majority of the cannabinoids is poor, it is accepted that delta-9-tetrahydrocannabinol is the most abundant cannabinoid in the *Cannabis* plant. Other cannabinoids include delta-8-tetrahydrocannabinol, which is almost as potent as delta-9-tetrahydrocannabinol but is found in smaller quantities. Tetrahydrocannabinol affects several biological functions by mimicking endogenous substances that activate cannabinoid receptors.

Cannabinoid receptor is a term to describe receptors that respond to cannabinoid drugs derived from *Cannabis* and its biologically active synthetic analogs. Synthetic cannabinoids, developed in scientific laboratories, have structural similarities to natural and endogenous cannabinoids, such as WIN-55212-2, JWH-133, and (*R*)-methanandamide. Exogenous agonists include synthetic agonists, such as CP55940, and plant-derived natural products, such as tetrahydrocannabinol. In particular, synthetic agonists that bind to cannabinoid receptors include delta-9-tetrahydrocannabinol-like analogs and aminoalkylindole compounds typified by R-WIN-55212.

Research has determined the existence of cannabinoid receptor 1 and cannabinoid receptor 2, numbered in order of their discovery. CB1 and CB2 receptors differ in their predicted amino acid sequence, signaling pathways, and tissue distribution. Studies have shown that potent agonists and antagonists with marked selectivity for CB1 or CB2 receptors can be constructed. Likewise, CB1, CB2, and CB1/CB2 knockout mice have been developed. CB1 has been found in rat, mouse, and human tissues and is identical in 97–99% of its amino acid sequence across species. CB1 mRNA and protein are detected predominately in the brain and neuronal tissues.

When cannabinoid receptor 1 was first discovered in humans, it was revealed to be abundant in the brain. It is expressed in some peripheral presynaptic nerve terminals and many extra-neural locations. CB1 receptor consists of a seven-transmembrane domain receptor. Its function is associated with biological responses and information about the structure–activity relationships of ligands for interaction with this receptor. Stimulation of the CB1 receptors suppresses the release of neurotransmitters, such as gamma-aminobutyric acid, allowing the endocannabinoid system to reduce the activity of the gamma-aminobutyric acid terminal in a retrograde manner back across the synapse.

There is a 48% homology between CB1 and CB2. Mouse CB2 has been cloned, and its sequence is 82% identical to human CB2. The mRNA of CB2 was first detected in immune tissue. Thus, it was thought that CB2 only existed in the immune system. However, it has been described as expressed in many cell types, such as natural killer cells, macrophages, monocytes, β -lymphocytes, microglial cells, and inflammatory cells, in various human body organs, such as the brain and the gastrointestinal tract. Notably, CB2 mRNA is missing from normal nervous tissue. Delta-9-tetrahydrocannabinol-like, aminoalkylindole, and eicosanoid ligands are the basic ligands of expressed CB2 protein. CB2 triggers a sustained activation of ceramide biosynthesis [69].

Both CB1 and CB2 are coupled to toxin-sensitive Gi/o proteins. Gi proteins are heterotrimeric GTP-binding proteins that inhibit plasma membrane adenylyl

cyclase. Binding to an agonist with CB1 or CB2 leads to a signaling cascade in which the activated receptor activates G_i , which inhibits adenylyl cyclase, reducing intracellular cyclic adenosine monophosphate (cAMP). Essentially, the classical theory of receptor occupancy and activation is a conformational induction theory that fits equivalently with Koshland's induced-fit model for enzymes. In particular, when agonist A binds to the resting-state receptor R, conformational changes are provoked, leading to the activation of receptor R.

CB1 is coupled through G_i/o proteins to various potassium and calcium channels. Additionally, in 2007, a new cannabinoid receptor called G protein-coupled receptor 55 (GPR55) was identified, which binds to and is activated by CP55940 with potential action on the brain. This action was confirmed in explaining how GPR55 mediates anxiolytic-like effects in the medial orbital cortex of mice. The effect, in conjunction with analgesia, appetite regulation, and antiemetic actions, is referred to as palliative methods for symptoms of malignancies, with potential use as a new treatment for pancreatic cancer.

The antiproliferative potential of cannabinoids was first reported when it was shown that oral administration of tetrahydrocannabinol in mice inhibits Lewis lung adenocarcinoma tumor growth [45]. Later, studies reported that either endogenous or synthetic cannabinoids induce antitumor effects on a wide spectrum of tumor cells in culture. In particular, it was found that in several types of cancer cells, such as glioma, lymphoma, prostate, breast, pancreatic, and skin cancer cells, cannabinoids reduced tumor size and induced apoptosis.

As various biochemical and pharmacological approaches indicate, the antitumor effects require the efficiency of CB1 and CB2. Elective cannabinoid receptor agonists and antagonists, which ligate to CB1 and CB2, can be used to study their expression and mode of action (apoptotic or stimulatory) in malignant cells. However, at certain doses and in specific cellular contexts, some cannabinoids have been reported to induce the proliferation of cancer cells in vitro. Studies in animal tumor models, such as Wistar rats inoculated with C6 gliomas, and xenografts in athymic nude mice implanted with KiMol or MBA-MD-231 breast cancer cells, revealed that cannabinoid receptor agonists exerted antiproliferative actions.

Cannabinoid receptors, when activated by specific G-protein coupling with cannabinoids, induce several cellular pathways. The cAMP-protein kinase A pathway is inhibited, and the activity of Ca^{2+} and K^+ channels is modulated. The changes lead to the inhibition of neurotransmitter release. CB1 participates in various cellular signaling pathways that control cell fate. In particular, CB1 receptor coupling urges the activation of mitogen-activated protein kinase (MAPK) cascades, such as the extracellular-signal-regulated kinase (ERK), the stress-activated kinases Jun amino-terminal kinase (JNK), and p38 MAPK.

The signaling cascades exert dominant roles in cell proliferation and differentiation regulation. Cannabinoid-induced MAPK stimulation has been reported in neural cell lines, primary neural cells, lymphoid cells, vascular endothelial cells, and Chinese hamster ovary cells transfected with cannabinoid receptor complementary DNAs. An in vitro study in a neuronal-like cell line showed that CB1 receptor activation attenuates ERK [70].

Cannabinoid receptors activate the phosphatidylinositol 3-kinase (PI3K)-AKT serine/threonine kinase 1 (AKT) survival pathway. Particularly, the phosphorylation of AKT induces the inhibition of nuclear translocation of forkhead transcription factors [71]. As a result, the expression of proapoptotic proteins is prevented. A study reported that cannabinoid receptors negatively regulated AKT activation. Some systems showed that PI3K is an upstream component of cannabinoid-induced ERK activation, while others reported contradictory results [72]. Data claim that nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and nitric oxide synthase (NOS) are other targets of CB1 activation, which are involved in the control of cell fate. Nevertheless, it is not clear if the components are activated or inhibited by cannabinoids.

Cannabinoid receptors are also involved in the modulation of sphingolipid-metabolizing pathways. CB1 activation induces sphingomyelin breakdown and increases ceramide levels. Ceramide is a lipid second messenger that stimulates apoptosis and cell-cycle arrest. Interestingly, the actions are not based on the G-protein structure but are cannabinoid receptor-dependent. It is reported that an adaptor factor associated with neutral sphingomyelinase activation (FAN) is involved. Furthermore, cannabinoid receptor activation has the potential to urge a sustained peak of ceramide expression through enhanced de novo synthesis [73].

Overall, cannabinoid receptors are implicated in antitumor effects by various mechanisms, as their activation stimulates transformed-cell death, inhibits transformed-cell proliferation, and limits tumor angiogenesis and metastasis. De novo ceramide accumulation sustained ERK activation, and AKT inhibition induced apoptosis. In glioma cells, ceramide levels are increased after cannabinoid receptor activation. The increase induces the activation of RAF1–mitogen-activated protein kinase (MEK)–ERK signaling cascade and the AKT inhibition. ERK activation stimulates cell proliferation. Many factors can affect ERK activation and cell fate, given that prolonged ERK stimulation mediates cell-cycle arrest and cell death. It has been reported that pharmacological inhibition of de novo ceramide synthesis in prostate tumor cells led to the prevention of cannabinoid-induced cell death [74].

Cannabidiol inhibits cancer cell aggressiveness in various types of cancer through the downregulation of *ID-1* gene expression. Beyond the decrease in *ID-1* gene expression, there is no clear mechanism(s) to explain CBD's antitumor activity in different cancer cells. A CBD-induced gene expression profile was identified consistently across four different cancer types by microarray analysis [60]. They include key regulators of tumor cell proliferation and invasion. Additional genes, such as the transcription factor Forkhead box M1 (*FOXM1*), were revealed as a mediator of CBD antiproliferative effects.

The human breast cancer cell lines MDA-MB231 and MDA-MB436 (ATCC), human glioblastoma (GBM) U251 and SF126 (ATCC), human oral squamous cell carcinoma SAS and human salivary gland cancer cell line ACCM, and human prostate cancer cell lines PC3 and DU145 (ATCC) were studied. The cell populations were first cultured in RPMI-1640 media containing 10% fetal bovine serum (FBS). On the first day of treatment, the media were replaced with vehicle control (Veh) (ethanol) or CBD (2 μ M) in RPMI and 0.1% FBS. The media with the appropriate

compounds were replaced after 24 h, for a total of 2-day treatment, corresponding to the significant effects of CBD.

Cancer cell lines were obtained from the breast, GBM, head, neck (HN), and prostate to evaluate genome-wide changes in gene expression. Total RNA was extracted from the cell lines and hybridized. A total of 12,712 genes with a minimum detection p -value of <0.05 in at least one sample were retained for further analysis. Given the inherent gene expression variation between cell lines, gene expression changes within a cell line between treatment and vehicle were normalized. The log₂ mean of each treatment and control sample was subtracted from individual values to normalize each gene expression value, resulting in positive and negative log₂-folds. Normalized log₂-fold changes for all treatment and control samples were then compared using an empirical Bayes moderated t -test in AltAnalyze version 2.0.8 between all CBD-treated and vehicle-treated lines. Genes with a false discovery rate-adjusted p -value of <0.05 and fold change >2 were analyzed using the GO-Elite module in AltAnalyze to identify and visualize enriched biological pathways, ontologies, and gene sets. Clustering was performed in AltAnalyze using the HOPACH algorithm.

While CBD is broadly used in the self-medication of cancer patients, CBD-based therapies are undergoing clinical evaluation for cancer treatment. Cannabinoids downregulate the expression of the prometastatic gene inhibitor of DNA binding 1 (ID-1) in cancer cells, inhibiting tumor progression in vivo. The study confirmed that ID-1 is a major target downregulated by CBD and also discovered that CBD inhibited FOXM1 (Forkhead box M1), a transcriptional activator involved in cell proliferation, while simultaneously upregulating GDF15 (growth differentiation factor 15), a cytokine associated with tissue differentiation. The results indicated that CBD could represent a promising nontoxic therapeutic for treating tumors of various origins by modulating the expression of shared key cancer-driving genes.

Cannabidiol exerts pharmacological actions, antioxidant and intestinal anti-inflammatory, and mechanisms inhibiting the endocannabinoid enzymatic degradation potentially beneficial for colon carcinogenesis. Its possible chemopreventive effect in the model of colon cancer induced by azoxymethane (AOM) in mice was studied. AOM treatment was associated with aberrant crypt foci (ACF, preneoplastic lesions), polyps, tumor formation, and upregulation of phospho-Akt, iNOS, and COX-2, and downregulation of caspase-3.

Cannabidiol reduced ACF, polyps, and tumors and counteracted AOM-induced phospho-Akt and caspase-3 changes. In colorectal carcinoma cell lines, cannabidiol protected DNA from oxidative damage, increased endocannabinoid levels, and reduced cell proliferation in a CB1-, TRPV1-, and PPAR γ -antagonists sensitive manner. Cannabidiol exerts a chemopreventive effect in vivo and reduces cell proliferation through multiple mechanisms [61].

During apoptosis, the inhibitor of apoptosis (IAP) family of proteins, including X-linked IAP (XIAP), c-IAP1, and c-IAP2, directly inhibit caspases and regulate cell death. XIAP exhibits the most potent antiapoptotic effect. XIAP has three baculoviral IAP repeat (BIR) domains (BIR1, BIR2, and BIR3) and a RING finger domain at the C-terminal. The BIR2 domain is important for inhibiting caspase-3/7

(Cas3) and Cas7, and the BIR3 domain is essential for inhibiting Cas9. The Ring domain induces proteasomal degradation by promoting ubiquitination of other proteins and self-ubiquitination as well [62].

In addition to caspase inhibition, the X-linked inhibitor of apoptosis (XIAP) induces NF-kappaB and MAP kinase activation during TGF- β and BMP receptor signaling and upon overexpression. TAB1 is an upstream adaptor for activating the kinase TAK1, which in turn couples to the NF-kappaB pathway. The BIR1 domain of XIAP directly interacts with TAB1 to induce NF-kappaB activation.

The crystal structures of BIR1, TAB1, and the BIR1/TAB1 complex reveal a butterfly-shaped dimer and the detailed interaction between BIR1 and TAB1. Structure-based mutagenesis and knockdown of TAB1 indicated unambiguously that the BIR1/TAB1 interaction is crucial for XIAP-induced TAK1 and NF-kappaB activation, although not interacting with BIR1, Smac, an antagonist for caspase inhibition by XIAP, also inhibits the XIAP/TAB1 interaction. Disruption of BIR1 dimerization abolishes XIAP-mediated NF-kappaB activation, implicating a proximity-induced mechanism for TAK1 activation. The main negative regulator of XIAP is the second mitochondria-derived activator of caspase (Smac). Smac is normally present in the mitochondria, and when apoptosis occurs, it is released into the cytosol. The signal peptide of the N-terminal region is removed and becomes an active form. Activated Smac then competitively blocks the caspase-binding sites of XIAP and induces the caspase cascade, resulting in further apoptosis [63].

It has been reported that XIAP and Smac are negatively correlated in various cancers, such as renal cell carcinoma and nonsmall cell lung cancer. Usually, XIAP has higher expression in cancer tissues than in normal tissues. In contrast, Smac has lower expression in cancer tissues than in normal tissues, both of which are associated with poor prognosis and survival rate of patients. A WST-1 assay following CBD treatment in various gastric cancer cells, including AGS, MKN45, SUN638, NCI-N87, and normal gastric HFE-145 cells, was conducted to investigate cell proliferation with CBD treatment.

Cell proliferation decreased following CBD treatment but did not affect normal gastric cells. Western blotting analysis was performed to determine the level of apoptosis after CBD treatment in AGS and MKN45 cells. The expression of cleaved-poly (ADP-ribose) polymerase (c-PARP) and Cas3, Cas8, and Cas9, both markers of apoptosis, were increased. Cas3/7 activities were measured using a Caspase-Glo 3/7 Assay kit to confirm the increase of caspases. Caspase activities were elevated in CBD treatment conditions. Annexin V/propidium iodide (PI) staining was measured using flow cytometry to assess apoptosis.

CBD produced apoptosis in AGS and MKN45 cells but not in HFE-145 cells; this was verified by a TdT-mediated dUTP nick-end labeling (TUNEL) assay performed to stain apoptotic cells. The expression of TUNEL-positive cells was increased during CBD treatment in AGS and MKN45 cells.

Apoptotic proteins were screened to determine which were involved in CBD-induced apoptosis. The expression of XIAP was remarkably reduced as measured with western blotting. An immunofluorescence assay was performed in AGS cells treated with CBD to confirm the expression level of XIAP. XIAP expression

decreased in the CBD treatment condition. An experiment conducted to determine whether apoptosis was affected or not in XIAP overexpression conditions confirmed that when XIAP was overexpressed, apoptosis was rescued. Also, the knockdown of XIAP by small interfering RNA (siRNA) exacerbated CBD-induced apoptosis. The modes and molecular mechanisms of programmed cell death by CBD on gastric cancer cells demonstrated that CBD induces apoptotic cell death by XIAP/Smac. CBD induces mitochondrial dysfunction and regulates Smac/XIAP leading to apoptosis, suggesting that Smac/XIAP regulation using CBD can be potentially utilized for treating gastric cancer [64].

The mRNA expression of XIAP did not change in AGS and MKN45 cell lines, indicating that XIAP is regulated by posttranslational modification rather than at the transcriptional level. CBD attenuated the protein stability of XIAP when AGS cells were exposed or not exposed to CBD for indicated periods during cycloheximide (CHX). CHX chase assay revealed that XIAP protein half-life was 10.43 h when AGS cells were incubated with CHX. In contrast, the half-life of XIAP protein was 3.38 h in the CHX and CBD-treated cells, suggesting that CBD reduces the level of XIAP through the ubiquitin-proteasome system (UPS). Ubiquitination was investigated via the treatment of a proteasome inhibitor, MG132. Ubiquitination increased after CBD treatment in AGS cells, and ubiquitination accumulated by MG132 treatment was also further increased after CBD treatment.

Treatment of CBD in the presence of MG132 inhibited the reduction of CBD-induced XIAP. To determine whether the decrease in XIAP expression is an effect of increased proteasomal degradation, coimmunoprecipitation (Co-IP) was conducted in the CBD-treated condition. CBD promoted XIAP ubiquitination, and MG132 significantly blocked CBD-induced XIAP degradation. Protein levels of Smac were assessed by immunoblotting in AGS and MKN45 cells to investigate the expression of Smac.

To reveal the relationship between XIAP and Smac, Western blotting analysis was conducted using siRNA to determine whether Smac affects the apoptosis of gastric cancer cells. The protein expression level of Smac was significantly increased in both cell lines. The interaction between XIAP and Smac binding of the two proteins was elevated by CBD, and MG132 decreased the interaction. Smac downregulation recovered the apoptosis induced by CBD. The CBD-induced Smac increase was attenuated by C/EBP homologous protein (CHOP), a transcription factor, or ER stress knockdown. In contrast, XIAP was increased, indicating that CBD downregulates XIAP by activating Smac through ER stress overproduction and the downregulation is caused by promoting the binding of XIAP and Smac.

Mitochondrial membrane potential (MMP) plays a key role in eliminating dysfunctional mitochondria. As Smac is a mitochondrial protein whose expression can be regulated by CBD, oxygen consumption rate (OCR) was measured to determine whether Smac affects mitochondrial function. The level of basal OCR decreased in CBD-treated AGS cells. Additionally, basal respiration OCR and ATP production levels decreased in the CBD-treated condition.

The effects of CBD on MMP by staining with JC-1 dye indicated that CBD inhibits MMP. Tetramethylrhodamine, ethyl ester, and perchlorate (TMRE) staining

were performed to detect active mitochondria, and CBD reduced the red fluorescence signal. The expression of mitochondrial electron transfer proteins was assessed by western blotting after CBD treatment, and the expression of NADH dehydrogenase ubiquinone 1 α subcomplex subunit 9 (NDUFA9), a protein associated with mitochondria complex I, was significantly reduced. CBD increased Smac secretion to the cytosol. CBD also increased Smac within the mitochondria. Together, CBD affects mitochondrial dysfunction and Smac release in gastric cancer cells.

Four-week-old Balb/c-nude mice were subcutaneously injected with MKN45 gastric cancer cells to establish a mouse gastric cancer model to investigate the anti-tumor effect *in vivo*. After the tumor size reached 100 mm³, the mice were injected intraperitoneally with CBD thrice over 1 week. The effect on tumor growth was monitored for 15 days. After the monitoring period, tumor tissue was extracted from the mice. The tumor size was measured, and it confirmed that tumor size was significantly smaller in the CBD-treated group than in the control group. Tumor growth of the CBD-treated group was remarkably slower than that of the control group. The mean tumor weight of the CBD-treated group was significantly less than that of the control group. There was hardly any difference in body weight between the control and CBD-treated groups. A TUNEL assay was performed using tumor tissue obtained from mice to investigate whether CBD promotes apoptosis. TUNEL-positive cells were significantly increased in the CBD-treated group. The expression of XIAP was also decreased in the CBD-treated group.

CBD induced suppression of viability and apoptosis via caspase cascade in a dose-dependent manner in gastric cancer cell lines, including AGS, MKN45, SNU638, and NCI-N87 cells, but not in the gastric normal epithelial HFE-145 cell line. In addition, CBD inhibited tumor growth and increased apoptosis in *in vivo* mouse models. Administration of 100 mg/kg CBD daily for 5 weeks had no side effects in the xenograft model using pancreatic cells. One important finding is that XIAP downregulation is essential for CBD-induced apoptosis in gastric cancer cells. XIAP is an important target for treatment because of its increased expression in gastric cancer patients. CBD reduced XIAP in a dose- and time-dependent manner and overexpression of XIAP partially restored CBD-induced apoptosis [65].

Conversely, treatment with CBD after XIAP knockdown increased apoptosis. However, flow cytometry data showed more necrosis than apoptosis. This is presumably due to excessive apoptosis leading to necrosis. CBD significantly increased the ubiquitination of XIAP, which Smac regulates. Smac resides in a homodimeric form in the mitochondria, where the N-terminal is truncated by stimulation such as radiation or chemotherapy. The molecule migrates to the cytosol in the form of mature Smac. Interestingly, in our system, Smac showed increased mRNA and protein levels in the mitochondria and the cytosol upon CBD treatment, implying that CBD may promote the cytosolic release of Smac as well as transcription of Smac. In addition, Smac released into the cytosol by CBD treatment showed increased binding with XIAP. Thus, the findings suggest that Smac, when promoted by CBD, translocates to the cytosol and binds to XIAP to increase its ubiquitination, further increasing apoptotic cell death.

The ER is an important organelle for protein synthesis and modification, lipid biosynthesis, and maintenance of intracellular Ca^{2+} homeostasis. ER stress is induced when homeostasis is broken by changes in glycosylation, Ca^{2+} depletion, oxidative stress, or accumulation of misfolded or unfolded proteins in the ER lumen. During the accumulation of misfolded or unfolded proteins, the three receptors in the ER transmembrane, inositol requiring enzyme-1 α (IRE1 α), phospho-PKR-like ER-resistant kinase (PERK), and activating transcription factor 6 (ATF6), are removed from Bip to increase the expression of ER chaperones such as Bip and Glucose Regulated Protein 94 (GRP94), then, maintained their homeostasis by eliminating them. ER stress-related genes are increased in CBD-treated colorectal cancer cells [66].

Consistent with that, CBD increased ER stress-related genes in gastric cancer cells. CHOP, a key transcription factor for ER stress, regulates Smac [67]. CBD elevated CHOP levels and knockdown CHOP using siRNA partially increased CBD-induced XIAP reduction, indicating that CBD inhibits XIAP by stimulating ER stress. XIAP induces mitochondrial damage by regulating the Bcl-2 family proteins. CBD regulates mitochondrial complexes I and IV in hippocampal neurons. Mitochondrial complex I was downregulated by CBD; however, OCR was reduced in CBD-treated gastric cancer cells.

Cell-Cycle Progression

The effects of *Cannabis* extracts and cannabidiol on cell-cycle progression using flow cytometry were assessed. Flow cytometry showed that in the presence of *Cannabis* extracts and camptothecin, SiHa cells exhibited a significant increase ($p < 0.001$) in the sub-G0 population with a decrease in G0/G1, S, and G2/M phase. In butanol, the sub-G0 phase was increased from 4.2% to 20.1%, while decreasing the G0/G1 (from 64.0% to 48.7%), S-phase (from 9.3% to 6.5%), and G2/M (from 18.5% to 17%) in SiHa population. In hexane treated sub-G0 phase was at 39.1% compared to 4.2% in untreated, with a decrease in G0/G1 (from 64.0% to 30.4%), S (from 9.3% to 6.5%), and G2/M (from 18.5% to 13.8%) population. In HeLa cells, butanol extracts reduced G0/G1 to 54.9%, while the S-phase and G2/M significantly increased to 18.4% and 25.7%; with hexane extract, there was an increase in the G2/M phase (20.3%) and a decrease in the S-phase (8.1%). In ME-180, there was an insignificant increase in all cell-cycle stages. Each cell line responded differently to cannabidiol treatment.

Almost 42.2% of SiHa cells were observed in the sub-G0 ($p < 0.001$), while there was a reduction in cells in the G0/G1 phase, from 57.9% to 42.8%. A similar trend was observed in HeLa cells but with much lower sub-G0 from 5.1% to 17.4% and S phase from 4.8% to 11.2%. A similar event was observed during the treatment of ME-180 cells. Cannabidiol significantly increased sub-G0 in ME-180 cells to 34.3%. The data indicate that cannabidiol induced cell death without cell-cycle arrest.

The mode of cell death induced by cannabidiol and the extract of *Cannabis* was that of apoptosis. To determine if the type of apoptosis induced is p53 dependent, protein expression of RBBP6, Bcl-2, Bax, and p53 was performed. In butanol extract, p53 was significantly increased in SiHa and HeLa cells and remained unchanged in ME-180. Similar results were observed in hexane-treated cells. All treatments reduced the level of the p53 negative regulator in cancer development in all cell lines.

Following the treatment of cervical cancer cells, Bax protein was upmodulated, and Bcl-2 was downmodulated. Western blot analysis revealed that cannabidiol effectively increased the expression of proapoptosis proteins, p53 and Bax, while simultaneously decreasing the antiapoptosis proteins, RBBP6 and Bcl-2, in all three of the cervical cancer cell lines (SiHa, HeLa, and ME-180 cells). Caspases play an effective role in the execution of apoptosis. The effector caspase-9 and executor caspase-3 were included in our western blot to determine if they played a role in inducing apoptosis. The *Cannabis* extracts, caspase-3 and caspase-9, were upregulated in all cell lines. Similar results were also observed in cannabidiol-treated cells with upregulation of both caspase-3 and -9.

Autophagy and Apoptosis

Cancer therapy's main principles are cell death induction and cell survival inhibition. Resistance to chemotherapeutic agents is a major problem in oncology, limiting anticancer drug effectiveness. Various factors contribute to drug resistance, including host factors, specifically genetic or epigenetic alterations in the cancer cells. Although multiple mechanisms by which cancer cells become resistant to anticancer drugs in the microenvironment have been well elucidated, how to circumvent the resistance to improve anticancer efficacy remains to be defined.

Autophagy, an important homeostatic cellular recycling mechanism, is a crucial player in response to metabolic and therapeutic stresses, which attempts to maintain and restore metabolic homeostasis through the catabolic lysis of excessive or unnecessary proteins and injured or aged organelles [21]. Recently, several studies have shown that autophagy constitutes a potential target for cancer therapy, and the induction of autophagy in response to therapeutics can be viewed as having a pro-death or a prosurvival role contributing to the anticancer efficacy of the drugs as well as drug resistance.

Pure cannabinoids and extracts from *Cannabis* strain enriched in particular cannabinoids (BDS) were studied on AR-positive (LNCaP and 22RV1) and -negative (DU-145 and PC-3) cells by evaluating cell viability (MTT test), cell-cycle arrest, apoptosis induction, by FACS scans, caspase-3/7 assays, DNA fragmentation, and TUNEL, and size of xenograft tumors induced by LNCaP and DU-145 cells.

Cannabidiol significantly inhibited cell viability. Other compounds became effective in cells deprived of serum for 24 h. Several BDS was more potent than the pure compounds in the presence of serum. CBD-BDS, administered

intraperitoneally, potentiated the effects of bicalutamide and docetaxel against LNCaP and DU-145 xenograft tumors and, given alone, reduced LNCaP xenograft size. CBD (1–10 μ M) induced apoptosis and induced markers of intrinsic apoptotic pathways (PUMA and CHOP expression and intracellular Ca^{2+}). In LNCaP cells, the proapoptotic effect of CBD was only partly due to TRPM8 antagonism and was accompanied by downregulation of AR, p53 activation, and elevation of reactive oxygen species. LNCaP cells differentiated into androgen-insensitive neuroendocrine-like cells were more sensitive to CBD-induced apoptosis [24].

A unique aspect of the study is that the mice were administered CBD orally, whereas most cannabinoid antitumor investigations use systemic administration. As might be expected, there was heterogeneity in response to the treatment. CBD alone inhibited tumor progression in certain models but not in others and enhanced the activity of first-line agents in two of the models but inhibited the efficacy of a first-line agent in one of the *in vivo* models.

Delta-9-tetrahydrocannabinol stimulates an endoplasmic reticulum (ER) stress-related signaling pathway, which activates autophagy via inhibition of the Akt/mTORC1 axis. Activation of the pathway is also necessary for the antitumoral action of cannabinoids *in vivo*. Autophagy is upstream of apoptosis in cannabinoid-induced cancer cell death [99].

The activation of autophagy is highly dependent on the cellular context and the strength and duration of the stress-inducing signals. Thus, besides its role in cellular homeostasis, autophagy can be a form of programmed cell death or play a cytoprotective role, for example, in situations of nutrient starvation. Accordingly, autophagy plays a dual role in cancer. On the one hand, this cellular process may help to overcome the stress evoked at the initial steps of tumorigenesis. On the other, autophagy has been proposed to work as a tumor suppressor.

Moreover, different anticancer treatments activate autophagy in tumor cells, which either enhances cancer cell death or acts as a mechanism of resistance to chemotherapy. The antitumoral actions of cannabinoids are based on their ability to inhibit tumor angiogenesis and activate apoptosis of cancer cells. Studies have unraveled that cannabinoids induce autophagy in different tumor cells, including glioma/astrocytoma and pancreatic cancer cells. In contrast, they do not activate this cellular process in nontransformed cells that are resistant to the cell death-promoting activity of cannabinoids. Pharmacological or genetic inhibition of autophagy prevents cannabinoid-induced cell death and apoptosis, whereas abrogation of apoptosis prevents cell death but not autophagy induced by the agents.

The observations concluded that the induction of autophagy is part of the mechanism by which cannabinoids promote the apoptotic death of cancer cells. The *in vivo* relevance of the findings is demonstrated by the observation that cannabinoid treatment reduces tumor growth and activates autophagy and apoptosis in subcutaneous tumor xenografts derived from human U87MG astrocytoma cells and transformed mouse embryonic fibroblasts (MEFs). Furthermore, autophagy-deficient tumors, generated by subcutaneous injection of transformed *Atg5*^{-/-} MEFs, are resistant to delta-9-THC antitumoral action; strongly supporting the idea that autophagy is essential for the antineoplastic activity of cannabinoids.

In addition, analysis of samples obtained from two glioblastoma multiforme patients indicates that delta-9-THC administration might also trigger autophagy-mediated cell death in human tumors. Molecular investigation of the mechanisms responsible for the activation of autophagy upon delta-9-THC administration indicates that cannabinoids stimulate the process via the endoplasmic reticulum (ER) stress-related signaling pathway. Endoplasmic reticulum stress is an adaptive response activated in the cell when the ER is altered. Delta-9-THC triggers an early accumulation of de novo-synthesized ceramide. This event occurs in the ER, leading to ER dilation and increased eukaryotic initiation translation factor 2 α (eIF2 α) phosphorylation. Activation of the ER stress response induces the upregulation of several genes, including the stress-regulated protein p8, also named the candidate of metastasis 1, Com-1, and its downstream target, the pseudokinase Tribbles homolog 3 (TRB3).

Genetic inhibition of p8 and TRB3 prevents cannabinoid-induced autophagy and cell death, demonstrating that the proteins play a major role in connecting ER stress and autophagy in cannabinoid action. The investigations indicated that a key step in the induction of autophagy by the ER stress-related signaling pathway relies on inhibiting the Akt/mammalian target of the rapamycin complex 1 (mTORC1) axis by TRB3.

One of the important events for the initiation of autophagy in many cellular settings is the inhibition of mTORC1 which plays a central role in the control of protein synthesis, cell growth, and cell proliferation through the regulation of several downstream targets. In addition, mTORC1 is proposed to regulate autophagy by repressing the activity of Atg1. As a result of its central position in the control of cellular homeostasis, mTORC1 integrates signals from different inputs. One of the most important upstream regulators of mTORC1 is the prosurvival kinase Akt, which phosphorylates and inactivates TSC2, an inhibitor of the mTORC1 activator Rheb, as well as PRAS40. Thus, Akt activation stimulates mTORC1 and inhibits autophagy.

Angiogenesis

Angiogenesis is an important process that allows tumors to grow beyond a minimal size. Therefore, the prevention of the angiogenic process is an extremely promising approach in order to eradicate tumors. Studies in mice with glioma or skin carcinoma showed that cannabinoid administration induced the modification of vascular hyperplasia into blood vessels characterized by small and differentiated capillaries [75]. The modifications are associated with reduced expression of proangiogenic cytokines, such as vascular endothelial growth factor. In vascular endothelial cells, cannabinoid receptor stimulation restrained cell migration and survival. In mice injected with lung cancer cells, cannabinoid receptor activation inhibited tumor metastasis [76]. This might result from diminished activity and expression of

matrix metalloproteinase 2, an enzyme that induces tissue proteolysis during angiogenesis and metastasis [75].

In addition to their antiproliferative and proapoptotic actions, it has been shown that cannabinoids can affect other important processes in tumorigenesis, in particular, angiogenesis. Angiogenesis, forming new blood vessels from pre-existing ones, represents an essential part of tumor growth, invasion, and metastasis and constitutes a therapeutic target for cancer therapy.

Various complex cellular actions are implicated in angiogenesis, such as extracellular matrix degradation, migration and proliferation of endothelial cells, and morphological differentiation of endothelial cells to form tubes [19]. The processes require a finely tuned balance between stimulating and inhibitory signals. Stimulating signals include growth factors, such as VEGF, integrins, angiopoietins, and chemokines. Molecules inducing inhibitory signals include thrombospondin, interferons, and other cytokines, as well as other endogenous angiogenesis inhibitory factors, which may target endothelial cells either directly or indirectly.

Cannabinoids that bind to the CB1 and CB2 cannabinoid receptors (WIN5512-2, HU210, JWH-133, and delta-9-THC) have been reported to inhibit vascular endothelial cell survival and migration as part of their antiangiogenic action [20]. Treatment with cannabinoids reduces vascular density in experimental tumors. Met-fluoro-anandamide, a metabolically stable analog of the endocannabinoid anandamide, has been revealed to inhibit the spreading of endothelial cell spheroids, reduce capillary-like tube formation in vitro, and suppress angiogenesis in an in vivo chick chorioallantoic membrane assay. Additionally, cannabinoids can suppress proangiogenic factor production and directly induce apoptosis of endothelial cells.

Cytotoxicity

Cannabis for medical purposes has been legalized in many countries. However, only two cannabinoids, delta-9-THC and CBD, are considered in the legislation. Other substances, such as terpenes and terpenoids, or their specific combinations, could influence the resulting therapeutic effect for specific oncology diagnoses and specific patients. Six different genotypes (Conspiracy Kush, Jilly Bean, Jack Cleaner 2, Jack Skellington, Nordle, and Nurse Jackie) were cultivated indoors at the Czech University of Life Sciences Prague [40]. Ethanol extracts taken from the inflorescences were assayed for their content of main cannabinoids and terpenes/terpenoids. The extracts were used for in vitro cytotoxicity studies on hepatocarcinoma human cell lines Hep-G2 and colorectal carcinoma human cell lines Caco-2 and Ht-29.

Healthy lung fibroblast MRC-5 and healthy intestinal cells FHs-74 Int were used to compare the selectivity of cytotoxicity. The average content of delta-9-THC in the extracts was $59.1 \pm 2.43\%$, and of CBD, $1.84 \pm 0.17\%$. The content of the main cannabinoids in the Nurse Jackie genotype extract was significantly greater than that of the other genotypes. Overall, more than 60 different terpenes/terpenoids

were identified in the extracts. The major terpenes/terpenoids detected in most genotypes were limonene, linalool, α -terpineol, β -caryophyllene, trans- α -bergamotene, α -humulene, β -caryophyllene oxide, guaiol, γ -eudesmol, β -eudesmol, and α -bisabolol. Minor terpenoids, such as β -ocimene, isopulegol acetate, β -elemene, β -selinene, and spathulenol, caused differences in the terpene composition of individual genotypes. All of the extracts were highly cytotoxic to Ht-29 colorectal carcinoma cells and indicated positive selectivity compared to healthy FHs-74 Int colon cells.

The Jack Cleaner 2 extract was cytotoxic to the entire cell lines tested at the lowest concentrations (8.48 ± 2.4 and 16.14 ± 0.07 $\mu\text{g/mL}$) but was positively selective only for colorectal cancer cells, especially Ht-29 and to a lesser extent for Caco-2. Similarly, the Nordle extract showed positive selectivity for Ht-29 and Caco-2 only. Jilly Bean was unique in this study in that its extract functioned on all cell lines at the highest concentrations (20.13 ± 3.05 and 49.88 ± 1.5 $\mu\text{g/mL}$) while also being highly positively selective in all carcinoma lines (Ht-29, Caco-2, and Hep-G2 hepatocarcinoma) compared to healthy cell lines (FHs-74 Int and MRC-5).

The results indicate that delta-9-THC and CBD are responsible for the in vitro cytotoxicity of the extracts, but observed differences in selectivity reveal their synergies with other substances. According to chemical analysis, higher concentrations of myrcene, β -elemene, β -selinene, and α -bisabolol oxide found in the Jilly Bean genotype may positively affect the selectivity of cytotoxic activity.

Pancreatic Cancer

Pancreatic cancer is one of the fatal malignancies and cannabinoids have been reported as palliative adjuvants to conventional chemotherapeutic regimens. Cannabinoid effects are known to be mediated through the activation of cannabinoid receptors. Cannabinoids exert a remarkable antitumoral effect on pancreatic cancer cells due to their ability to induce apoptosis of the cells selectively. Many studies have tried to describe the mechanism of cell death induced by cannabinoids. A review of the effects of cannabinoid receptors in pancreatic cancer provides a brief insight into cannabinoids and their receptors as pancreatic cancer biomarkers and therapeutic strategies. The review strengthens the perception that cannabinoid receptors might be useful in clinical testing to prognosis and treat pancreatic cancer.

A study was conducted to investigate the action of cannabinoids in pancreatic cancer. Cannabinoid receptors are expressed in human pancreatic tumor cell lines and tumor biopsies at much higher levels than in normal pancreatic tissue. Studies conducted with MiaPaCa2 and Panc1 cell lines showed that cannabinoid administration (a) induced apoptosis, (b) increased ceramide levels, and (c) upregulated mRNA levels of the stress protein p8 [99].

The effects were prevented by blockade of the CB2 cannabinoid receptor or by pharmacologic inhibition of ceramide synthesis *de novo*. Knockdown experiments using selective small interfering RNAs showed the involvement of p8 via its

downstream endoplasmic reticulum stress-related targets activating transcription factor 4 (ATF-4) and TRB3 in delta-9-tetrahydrocannabinol-induced apoptosis. Cannabinoids also reduced the growth of tumor cells in two animal models of pancreatic cancer. In addition, cannabinoid treatment inhibited the spreading of pancreatic tumor cells. Moreover, cannabinoid administration selectively increased apoptosis and TRB3 expression in pancreatic tumor cells but not in normal tissue. The results indicated that cannabinoids lead to apoptosis of pancreatic tumor cells via a CB2 receptor and de novo-synthesized ceramide-dependent upregulation of p8 and the endoplasmic reticulum stress-related genes ATF-4 and TRB3. The findings contribute to setting the basis for a new therapeutic approach to treating pancreatic cancer.

Gemcitabine (GEM, 2',2'-difluorodeoxycytidine) is used in advanced pancreatic adenocarcinoma, with a response rate of <20%. Gemcitabine induces cannabinoid receptor-1 and cannabinoid receptor-2 by an NF- κ B-dependent mechanism; its association with cannabinoids synergistically inhibits pancreatic adenocarcinoma cell growth and increases reactive oxygen species induced by single treatments. A study demonstrated that structurally diverse cannabinoids, including a CB1 selective agonist, a CB1 selective antagonist, and an endogenous CB1 and CB2 mixed agonist, could all stimulate the generation of ROS and the production of autophagy in pancreatic tumor cells.

The antiproliferative synergism is stronger in GEM-resistant pancreatic cancer cell lines compared with GEM-sensitive pancreatic cancer cell lines. The radical scavenger N-acetyl-L-cysteine prevents the antiproliferative synergism and the specific NF- κ B inhibitor BAY 11-7085, demonstrating the induction of ROS by GEM/cannabinoids and of NF- κ B by GEM is required for effect. Additionally, neither apoptotic nor cytostatic mechanisms are responsible for the synergistic cell growth inhibition, which is strictly associated with the enhancement of endoplasmic reticulum stress and autophagic cell death.

The combined treatment strongly inhibits the growth of human pancreatic tumor cells xenografted in nude mice without apparent toxic effects. The findings support the key role of the ROS-dependent activation of an autophagic program in the synergistic growth inhibition induced by the GEM/cannabinoid combination in human pancreatic cancer cells [14].

Cannabinoids specific to the CB1 (ACPA) and CB2 (GW) receptors and metabolomics analyses were used to unravel the potential pathways mediating cannabinoid-dependent inhibition of pancreatic cancer cell growth. CB1- and CB2-selective receptor agonists induced autophagic cell death in part through the initial induction of the ROS sensor AMP-activated protein kinase (AMPK). ROS promotes the nuclear translocation of GAPDH, which is further amplified by AMPK, thereby attenuating glycolysis [15]. Panc1 cells treated with cannabinoids show elevated AMPK activation induced by an ROS-dependent increase in AMP/ATP ratio.

Furthermore, ROS determines the accumulation of NADH, suggestive of a blockage in the respiratory chain, inhibiting the Krebs cycle. Concomitantly, inhibition of the Akt/c-Myc pathway leads to decreased activity of the pyruvate kinase isoforms M2 (PKM2), further downregulating glycolysis and glutamine uptake.

The alterations of pancreatic cancer cell metabolism mediated by cannabinoids result in strong autophagy induction and cell growth inhibition.

CBD has been shown to enhance the ability of delta-9-THC to inhibit tumor progression in mice bearing BRAF wild-type melanoma xenografts through activation of autophagy-mediated cell death. Treatment with delta-9-tetrahydrocannabinol resulted in the activation of autophagy, loss of cell viability, and activation of apoptosis, whereas cotreatment with chloroquine or knockdown of Atg7, but not Beclin-1 or Ambra1 prevents delta-9-THC-induced autophagy and cell death *in vitro* [16].

Administration of Sativex-like (a laboratory preparation comprising equal amounts of delta-9-THC and cannabidiol) to mice bearing BRAF wild-type melanoma xenografts substantially inhibited melanoma viability, proliferation, and tumor growth paralleled by an increase in autophagy and apoptosis compared with standard single-agent temozolomide. The findings suggest that delta-9-THC activates the noncanonical autophagy-mediated apoptosis of melanoma cells. Targeting autophagy is, therefore, a means to promote cancer cell death in chemotherapy-resistant tumors.

Anandamide triggers various cellular activities by binding to the cannabinoid receptors (CB1 and CB2) and vanilloid receptor 1 (VR1). A specific inhibitor of cannabinoid receptor 1, SR141716A, did not block anandamide-induced cell death in endogenously cannabinoid receptor 1 expressing cells [17]. Additionally, cannabinoid receptor 1-lacking Chinese hamster ovary (CHO) cells underwent cell death after anandamide treatment.

SR144528, a specific inhibitor of cannabinoid receptor 2, also failed to block anandamide-induced cell death in HL-60 cells. Capsazepine, a specific antagonist of VR1, could not prevent anandamide-induced cell death in constitutively and endogenously VR1-expressing PC12 cells. Moreover, anandamide noticeably triggered cell death in VR1-lacking human embryonic kidney (HEK) cells. In contrast, methyl-beta cyclodextrin (MCD), a plasma membrane cholesterol depletor, completely blocked anandamide-induced cell death in various cells, including PC12, C6, Neuro-2a, CHO, HEK, SMC, Jurkat, and HL-60 cells.

Methyl-beta cyclodextrin also blocked anandamide-induced superoxide generation, phosphatidyl serine exposure, and p38 MAPK/JNK activation. The data imply a role for membrane lipid rafts in anandamide-induced cell death; it demonstrates that the formation of lipid rafts, leading to the production of ROS, was part of the mechanisms for the observed effects. The data suggest that a diverse range of cannabinoids can induce ER stress and ROS generation, regardless of whether they activate or inhibit cannabinoid receptors or, in the case of CBD, do not efficiently target either CB1 or CB2 receptors. However, CBD enhanced the ability of delta-9-THC to induce autophagy, similar to what was reported in a mouse melanoma model.

Pancreatic cancer has evolved as one of the deadliest types of cancer. Pancreatic cancer accounts for about 3% of all cancer in the United States and about 7% of all cancer-related deaths. According to the Surveillance Epidemiology and End Results program, the 5-year survival rate of patients with pancreatic cancer is 4%. This figure is the lowest of all cancer types. However, the high mortality rate results from limited early diagnosis, ineffective chemotherapy, and poor radiotherapy response.

Moreover, 50% of patients with early pancreatic cancer do not show symptoms. After the diagnosis, the 4-year survival rate can rise to 78% after resectioning pancreatic cancer with a size smaller than 2 cm. The antimetabolite gemcitabine (2',2'-difluorodeoxycytidine) is the standard treatment for patients with pancreatic cancer. Gemcitabine is a fluorinated analog of deoxycytidine, which, when incorporated into DNA, inhibits DNA synthesis and repair, culminating in apoptosis.

Unfortunately, the efficacy of this therapy is limited due to the complicated tumor microenvironment, which hinders efficient drug delivery to the targeted cells. The scientific community is trying to target the molecular mechanisms of pancreatic cancer. For instance, activation of KRAS oncoprotein, inactivation of p16INK4A, overexpression of cyclo-oxygenase-2, and loss of p53 effects are the main targets of the molecular pathology of pancreatic cancer [77]. Recent therapeutic strategies include combinations of compounds and gemcitabine to enhance the efficacy of pancreatic cancer treatment [78].

An in vitro study investigated the antitumor effects of CB receptors and their potential role in the human pancreatic cancer cell line MIA PaCa-2 [79]. Cannabinoid derivatives were cytotoxic via a receptor-independent mechanism. Both selective CB1 and CB2 receptor agonists and antagonists induced a significant cytotoxic effect. The absence of CB2 receptors prevented a potential role of the receptors in cannabinoid cytotoxicity. CB1 receptor agonist and antagonist, at nanomolar concentrations, did not significantly alter cell viability in a serum-free medium, which is a condition that avoids nonspecific interactions with serum components. Given that cannabinoids are lipophilic components, it is understandable that cytotoxicity can arise by crossing the cell membrane and rupturing cellular networks, resulting in cell death.

Internucleosomal degradation of DNA by CB1 antagonist *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) showed that apoptosis might contribute to its antitumor effect. AM251 also induced a significant time-response action on caspase-3/7, indicating that the two enzymes of apoptosis were a target of cannabinoids. Apoptosis might be mediated via a caspase-dependent pathway. The gene-expression profile of MIA PaCa-2 cells after AM251 treatment was examined by microarray analysis, and AM251 was found to act on cell-cycle-related pathways, as well as on critical steps in cell proliferation, including Janus kinase (JAK)/signal transducers and activators of transcription (STAT) and MAPK signaling cascades; in that study, AM251 was found to synergistically enhance the anticancer activity of 5-fluorouracil, which is a very common agent used in the treatment of pancreatic cancer. Hence, AM251 is highlighted as a novel compound for pancreatic cancer therapy.

There has been increasing interest in cannabinoids as a therapeutic regimen against pancreatic cancer [79]. About 95% of clinical cases of pancreatic cancer are ductal adenocarcinomas. The immunoreactivity for CB1 and CB2 receptors, as well as for the endocannabinoid-metabolizing enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGLL) in human tissues of pancreatic ductal adenocarcinoma was studied. Significant variation was reported in receptors and FAAH and MGLL levels between healthy people and patients with pancreatic

cancer. As CB1 and CB2 receptor levels were upregulated in pancreatic cancer, it is assumed that cannabinoid receptors have a dominant role in pancreatic carcinogenesis.

In that study, endocannabinoid levels in pancreatic cancer tissues did not change, possibly due to the strong desmoplastic reaction in pancreatic cancer. Researchers found an inverse association between CB1 receptor quantity and survival and an association between FAAH or MGLL levels in cancer cells and survival rate. Studies also claim that cannabinoids are involved in antiproliferative activities on pancreatic cancer growth. In any case, the heterogeneous expression patterns of cannabinoid receptors indicate their potential use as clinical biomarkers [80]. It was shown that CB2R is overexpressed in human pancreatic adenocarcinoma tissues and cell lines. Fluorescent substances such as NIR760-XLP6 binding to CB2R can permit early recognition of pancreatic cancer tissue and earlier diagnosis.

Cannabinoid receptors may be associated with developing pancreatic cancer pain, as CB1 and CB2 receptor expression were inversely correlated with patients' pain symptoms. Nevertheless, given that there was no association between FAAH/MGLL levels and pain scores, endocannabinoid inactivation through FAAH or MGLL is probably not involved in pancreatic cancer pain.

Studies investigating cannabinoid receptors in pancreatic cancer, both in vitro and in vivo, indicated that cannabinoid receptors are more highly expressed in human pancreatic tumor cell lines and tumor biopsies than in normal pancreatic tissue. In vitro studies in MiaPaCa2 and Panc1 cell lines revealed that cannabinoid administration led to apoptosis, reduced cell viability, and augmented the levels of ceramide and caspase-3. The combination of cannabinoid and O2/O3 induced cytotoxicity and augmented chemosensitivity to gemcitabine and paclitaxel [80]. Moreover, mRNA levels of the p8 protein, associated with stress, were upregulated; interestingly, when the CB2 receptor was blocked, all the effects were prevented. The results agree with other observations that had shown that the CB2 receptor is associated with the anticancer effect of cannabinoids in gliomas and lymphomas, as well as skin and prostate carcinomas.

Free radical scavenger *N*-acetyl-cysteine and the specific NF- κ B inhibitor BAY 11-7085 prevented the antitumor synergism, indicating that the stimulation of ROS by gemcitabine and cannabinoid cotreatment and of NF- κ B by gemcitabine were essential for the actions. Combined treatment significantly prevented tumor growth in human pancreatic tumor cells xenografted in nude mice. Similar effects on the tumor size and volume were observed in another study, in which ALAM027 and ALAM108, two new cannabinoid derivatives, were used in human pancreatic female mice bearing tumor cell xenografts; in that study, ALAM108 produced higher cytotoxicity at lower concentrations against pancreatic cancer cell lines [81]. Furthermore, cannabinoid treatment activated transcriptional factor X-box-binding protein-1 (XBP-1) splicing, glucose-regulated protein 78 (GRP78), and CCAAT/enhancer-binding protein (C/EBP) as well as homologous protein (CHOP) gene induction. It is noteworthy that the endoplasmic reticulum stress-related genes were also enhanced by gemcitabine [82]. Those genes are the molecular switch that stimulates autophagic or apoptotic cell death signals.

The evidence shows that the genes might be involved in gemcitabine/cannabinoid cotreatment. Although ACPA or GW induced cell-cycle arrest at the G1 phase, they did not induce apoptosis. Additionally, cannabinoid treatment partially but significantly inhibited apoptosis stimulated by gemcitabine.

Another study of Panc1 cells showed that treatment for 12 h with increasing amounts of ACPA or GW had antiproliferative effects. GW inhibited tumor growth significantly and more intensively than ACPA. Treatment of Panc1 cells with cannabinoids led to the downregulation of phosphorylated alanine aminopeptidase (ANPEP) [83]. ANPEP is a zinc-dependent metalloexopeptidase found on the surface of tumor cells. ANPEP degrades the extracellular matrix and, as a result, it induces tumor invasion, angiogenesis, and metastasis. It constitutes a transcriptional target of RAS signaling pathways. ANPEP inhibitors have been reported to be effective anticancer agents [84]. ANPEP has been proposed as a novel prognostic biomarker for patients with pancreatic cancer.

Proteomic investigation showed that cannabinoid treatment induced several isoforms of phospho-keratins 18 (KRT18) (SSP6411, SSP6402, and SSP6410) [83]. KRT18 is a filament protein associated with mitosis, apoptotic death, signaling pathways, and invasion. GW strongly induced KRT18 phosphorylation at Ser52 and Ser53, while total KRT18 expressed was downregulated. Phosphorylation of KRT18 at Ser52 is necessary for filament reconstruction at cell-cycle arrest, as, during apoptosis, intermediate filaments reorganize into granular structures enriched for phosphorylated KRT18. Phosphorylation of KRT19 at Ser33 is associated with 14-3-3 proteins, but it does not seem essential for apoptosis. Presumably, CB2 receptor activation, by augmenting KRT18 phosphorylation, induces the collapse of the keratin cytoskeleton and cell death.

AMP-activated protein kinase (AMPK) plays an important role in autophagy as it inhibits the mammalian target of rapamycin complex 1 (mTORC1), the major regulator of cell growth [85]. AMPK is a crucial molecule in the autophagic pathway induced by cannabinoids [86]. It was found that ACPA and GW synthetic cannabinoids induced the activation of AMPK in pancreatic adenocarcinoma cells as they increased the cellular AMP/adenosine triphosphate (ATP) ratio. Cannabinoid treatment reduced the phosphorylation level of p70S6K, a direct target of mTORC1, and increased AMPK phosphorylation.

Furthermore, AMPK is reported to be an intracellular energy sensor and plays an active role in maintaining intracellular homeostasis during stress challenges. For instance, oxidative stress has been found to induce AMPK. ROS were essential for the increase in the AMP/ATP ratio, which led to the activation of AMPK by cannabinoids and, as a result, to autophagy. The study reported that treating ACPA or GW elevated the nicotinamide adenine dinucleotide (NADH) level. This increase was prevented in the presence of the radical scavenger *N*-acetyl-L-cysteine.

The results suggest that ROS production induced by cannabinoids might disrupt the electron transport chain, which inhibits the Krebs cycle, leading to NADH accumulation and inhibition of oxidative phosphorylation, which could further elevate the ROS level. The p-21-activated kinase 1 mediated pathway is responsible for the anticancer effects of cannabinoids.

A study was conducted in which the antitumor effects of cannabinoids in cell lines and mouse models were tested. The p-21 pathway is particularly related to the actions of the KRAS pathway. Cannabinoids act through the inhibition of the pathways. Thus, the interaction between pancreatic cells and pancreatic stromal cells was reduced, leading to a reduction in the proliferation of cancer cells and tumor growth; antitumor immunity was restored through the downregulation of programmed death-ligand 1 (PD-L1), leading to a better immune response and reduction of tumor size.

Concomitantly, endocannabinoid treatment increased the expression of phosphatase and tensin homolog (PTEN), a tumor suppressor mutated in different types of human cancer. PTEN is a major regulator of AKT, which, in turn, upregulates c-MYC. It has been shown that c-MYC is involved in the transcription of genes that lead to the expression of kinase isoform M2 (PKM2). PKM2 is expressed in cancer and promotes aerobic glycolysis [87]. Cannabinoids were shown to determine the downregulation of PKM2 via the inhibition of c-MYC16. In pancreatic adenocarcinoma cells treated with cannabinoids, autophagy induced by cannabinoids is highly associated with the inhibition of energetic metabolism, which, in turn, is related to cannabinoid-dependent ROS production.

The studies offer promising results in the field of pancreatic cancer treatment. The studies demonstrated different oncogenic pathways which can be affected through cannabinoid receptors. The action of cannabinoids might be potentiated by using other chemotherapeutic agents or modalities to exert a synergistic antitumor activity. In both in vitro and in vivo studies, the use of cannabinoids against pancreatic malignancy can also be potentiated through the use of radiation and can prolong survival. As a result, the simultaneous use of chemotherapy and radiation can reduce the side effects and improve the clinical outcome.

Metastases are responsible for approximately 90% of breast cancer-related deaths. Cyclooxygenase-2 (COX-2) is involved in inflammatory processes and the metastasis of cancer cells; it is expressed in 40% of human invasive breast cancers. To analyze the effects of cannabidiolic acid (CBDA), a selective inhibitor, on COX-2 expression and the genes involved in metastasis, a DNA microarray analysis of human breast cancer MDA-MB-231 cells was conducted. MDA-MB-231 cells are invasive breast cancer cells that express high levels of COX-2. They were exposed to CBDA for 48 h at 25 μ M.

The results revealed that COX-2 and Id-1, a positive regulator of breast cancer metastasis, were downregulated (0.19-fold and 0.52-fold, respectively), while SHARP1 (or BHLHE41), a suppressor of breast cancer metastasis, was upregulated (1.72-fold) and CHIP (or STUB1) was unaffected (1.03-fold) [88]. The changes were confirmed by real-time RT-PCR analyses. The results demonstrated that CBDA had dual inhibitory effects on COX-2 through downregulation and enzyme inhibition. It may possess the ability to suppress genes that are positively involved in the metastasis of cancer cells in vitro.

The effects of cannabinoids on the expression of tissue inhibitors of metalloproteinases (TIMPs), which play critical roles in the acquisition of migrating and invasive capacities by tumor cells, were evaluated [89]. Administration of

delta-9-tetrahydrocannabinol downregulated TIMP-1 expression in mice bearing subcutaneous gliomas, as determined by Western blot and immunofluorescence analyses. The cannabinoid-induced inhibition of TIMP-1 expression in gliomas was mimicked by JWH-133, a selective CB2 cannabinoid receptor agonist that is devoid of psychoactive side effects; it was abrogated by fumonisin B1, a selective inhibitor of ceramide synthesis *de novo*, and was also evident in two patients with recurrent glioblastoma multiforme (grade IV astrocytoma).

Delta-9-THC also depressed TIMP-1 expression in cultures of various human glioma cell lines and primary tumor cells obtained from a glioblastoma multiforme patient. The action was prevented by pharmacological blockade of ceramide biosynthesis and by knocking down the expression of the stress protein p8.

Three cell lines were exposed to increasing doses of arachidonoyl ethanolamide with and without antagonists to receptors of arachidonoyl ethanolamide to assess the sensitivity of cervical carcinoma cell lines to arachidonoyl ethanolamide [90]. DNA fragmentation and caspase-7 activity were used as apoptosis markers. The expression of receptors to arachidonoyl ethanolamide was analyzed in cervical carcinoma cell lines and cervical carcinoma biopsies. It was revealed that arachidonoyl ethanolamide induced apoptosis of cervical carcinoma cell lines via aberrantly expressed vanilloid receptor-1. In contrast, arachidonoyl ethanolamide binding to the classical CB1 and CB2 cannabinoid receptors mediated a protective effect. A strong expression of the three forms of arachidonoyl ethanolamide receptors was observed in *ex vivo* cervical carcinoma biopsies. The data suggest that targeting vanilloid receptor-1 by endogenous cannabinoids or synthetic molecules offers opportunities to develop potent anticancer medication.

Glioblastoma, Glioma, and Neuroblastoma

Local invasion of cancer cells, followed by invasion to secondary sites, is one of the major hallmarks of metastasis. Glioblastoma multiforme (GBM) is the most common form of primary adult brain tumor. Most glioblastomas grow invasively into distant brain tissue, leading to tumor recurrence, which is ultimately incurable. The transcriptional regulator, Id-1, plays a critical role in modulating the invasiveness of glioblastoma cell lines and primary glioblastoma cells. Id-1 expression levels correlate positively with glioma cell invasiveness in culture and with histopathologic grades in patient biopsies. Id-1 knockdown dramatically reduces glioblastoma cell invasion accompanied by profound morphologic changes, robust reduction in expression levels of mesenchymal markers, and inhibition of self-renewal potential and downregulation of glioma stem cell markers.

Genetic knockdown of Id-1 leads to a significant increase in survival in an orthotopic model of human glioblastoma. Cannabidiol significantly downregulates Id-1 gene expression and associated glioma cell invasiveness and self-renewal. Additionally, cannabidiol significantly inhibits the invasion of glioblastoma cells through an organotypic brain slice and glioma progression *in vivo* [18]. The results

indicated that Id-1 regulates multiple tumor-promoting pathways in glioblastoma and that drugs targeting Id-1 represent a strategy for improving the therapy and outcome of patients with glioblastoma.

In glioma cell lines, treatment with delta-9-THC produced dose-dependent inhibition of cell viability and proliferation. The influence of delta-9-tetrahydrocannabinol and the potent synthetic cannabinoid agonist, WIN 55212-2, were compared using time lapse microscopy. Delta-9-THC decreases cell proliferation and increases cell death of human GBM cells more rapidly than WIN 55212-2. Delta-9-THC was also more potent at inhibiting the proliferation of GBM cells compared to WIN 55212-2. The effects of delta-9-THC and WIN 55212-2 on the GBM cells were partially the result of cannabinoid receptor activation. The same concentration of Delta-9-THC that significantly inhibits proliferation and increases death of human GBM cells has no significant impact on human primary glial cultures [100].

In glioma cells, treatment with delta-9-THC at 3 μ M inhibited cell growth. Delta-9-THC did not affect cell viability in C6 glioma cells when cultured with 10% FBS, while it did have modest activity on inhibiting cell viability in a serum-free culture environment. In C6 glioma cells, delta-9-THC had an IC_{50} of 23 μ M, and delta-9-THC exposure increased cell death due to oxidative stress. On the contrary, other studies have shown that human glioma cells were only sensitive to delta-9-THC at very high, pharmacologically irrelevant concentrations and that it has the potential to stimulate glioma cell growth.

Delta-9-tetrahydrocannabinol induced apoptosis in C6.9 glioma cells, as determined by DNA fragmentation and loss of plasma membrane asymmetry. THC stimulated sphingomyelin hydrolysis in C6.9 glioma cells. THC and N-acetylsphingosine, a cell-permeable ceramide analog, induced apoptosis in several transformed neural cells but not in primary astrocytes or neurons. Although glioma C6.9 cells expressed the CBI cannabinoid receptor, neither delta-9-THC-induced apoptosis nor delta-9-THC-induced sphingomyelin breakdown was prevented by SR141716, a specific antagonist of that receptor.

The results indicated that delta-9-THC-induced apoptosis in glioma C6.9 cells might rely on a CBI receptor-independent stimulation of sphingomyelin breakdown [25]. The effect might be mediated through a CB1 receptor-independent pathway involving the stimulation of sphingomyelin breakdown. It has been shown that delta-9-THC-induced autophagy-mediated cell death in glioma cells results from ceramide accumulation and endoplasmic reticulum stress. Tribbles homolog 3 (TRB3) linked ER stress to autophagy, and autophagy occurred prior to apoptosis in cannabinoid-induced glioma cell death. This sequence of events was required for the in vivo anticancer effects of cannabinoids.

Cannabinoids bind to the G protein-coupled receptors, CB1 and CB2, expressed by neurons and cells of the immune system, respectively. Glioma cells express cannabinoid receptors, and in vitro and in vivo studies in rodents have revealed that apoptosis could be induced by cannabinoids in those cells. A study assessed the effect of cannabinoids on human glioma cell viability with the human astrocytoma cell line U373MG. The U373MG human glioma cells were sensitive only to high

concentrations of cannabinoids (>5 $\mu\text{g/ml}$ for delta-9-THC). Similar concentrations promoted a rapid activation of the extracellular-regulated kinase and c-Jun NH₂-terminal kinase, indicating that cannabinoid receptors are functional in U373MG cells. The kinases were not involved in cannabinoid-induced cell death in U373MG cells, as blocking their activation with specific inhibitors does not reduce cell death. CB1 was expressed in U373MG cells and is involved in cannabinoid-induced cell death. Blocking its activation with a specific antagonist (AM251) almost totally prevented cell death following incubation of the cells with Delta-9-THC [101].

One study found that long-term exposure to delta-9-THC did not stimulate apoptosis and diminished the sensitivity of astrocytes to ceramide accumulation. Stress protein p8 upregulation and endoplasmic reticulum stress were necessary for delta-9-THC-induced apoptosis in glioma cells and cells from human astrocytoma biopsies. In cannabinoid-resistant tumor cells, p8 upregulation did not occur following cannabinoid treatment. They also showed that delta-9-THC treatment upregulated p8 levels in tumors *in vivo* and that tumors deficient in p8 were resistant to the apoptotic effects of cannabinoids [102].

Another study showed that delta-9-THC treatment modified sphingolipid ratios in the endoplasmic reticulum of glioma cells, which resulted in the promotion of autophagy-dependent lysosomal membrane permeabilization and cathepsin release, resulting in the activation of the mitochondrial apoptotic pathway. Administration of delta-9-THC also reduced glioma tumor growth *in vivo* as a result of autophagy-mediated cell death [103].

Intratumoral administration of delta-9-tetrahydrocannabinol and the synthetic cannabinoid agonist WIN-55212-2 induced a considerable regression of malignant gliomas in Wistar rats and in mice deficient in recombination activating gene 2. The cannabinoid treatment did not produce any substantial neurotoxic effect. Experiments with two subclones of C6 glioma cells in culture showed that cannabinoids signal apoptosis by a pathway involving cannabinoid receptors sustained ceramide accumulation and Raf1/extracellular signal-regulated kinase activation [104].

The administration of delta-9-THC (or of delta-9-THC + CBD at a 1:1 ratio) in combination with temozolomide (TMZ), the benchmark agent for the treatment of GBM, synergistically reduces the growth of glioma xenografts. The effect of the combination of TMZ and delta-9-THC/CBD mixtures containing different ratios of the two cannabinoids in preclinical glioma models, including those derived from GICs. The findings show that TMZ + THC/CBD combinations containing a higher proportion of CBD (but not TMZ + CBD alone) produce a similar antitumoral effect as the administration of TMZ together with delta-9-THC and CBD at a 1:1 ratio in xenografts generated with glioma cell lines. In addition, the administration of TMZ + delta-9-THC/CBD at a 1:1 ratio reduced the growth of orthotopic xenografts generated with GICs derived from GBM patients, and enhanced the survival of the animals bearing these intracranial xenografts. Remarkably, the antitumoral effect observed in GICs-derived xenografts was stronger when TMZ was administered together with cannabinoid combinations containing a higher proportion of CBD [105].

The gene expression profile of a large series of human glioma cells with different sensitivities to cannabinoid action was analyzed. A subset of genes specifically associated with delta-9-THC resistance was identified. One of the genes encoding the growth factor midkine (Mdk) is directly involved in the resistance of glioma cells to cannabinoid treatment. Mdk mediates its protective effect via the anaplastic lymphoma kinase (ALK) receptor, and Mdk signaling through ALK interferes with cannabinoid-induced autophagic cell death. Furthermore, *in vivo* Mdk silencing or ALK pharmacological inhibition sensitizes cannabinoid-resistant tumors to delta-9-THC antitumoral action. Altogether, the findings identify Mdk as a pivotal factor in the resistance of glioma cells to delta-9-THC proautophagic and antitumoral action and suggest that selective targeting of the Mdk/ALK axis could help to improve the efficacy of antitumoral therapies for gliomas [106].

Delta-9-tetrahydrocannabinol downregulates MMP-2 expression in gliomas generated in mice, as determined by Western blot, immunofluorescence, and real-time quantitative PCR analyses. The inhibition of MMP-2 expression in gliomas (a) was MMP-2-selective, as levels of other MMP family members were unaffected; (b) was mimicked by JWH-133, a CB2 cannabinoid receptor-selective agonist that is devoid of psychoactive side effects; (c) was abrogated by fumonisin B1, a selective inhibitor of ceramide biosynthesis; (d) was also evident in two patients with recurrent glioblastoma multiforme. Delta-9-THC inhibited MMP-2 expression and cell invasion in cultured glioma cells.

Manipulation of MMP-2 expression by RNA interference and cDNA overexpression experiments proved that downregulation of the MMP plays a critical role in delta-9-THC-mediated inhibition of cell invasion. Cannabinoid-induced inhibition of MMP-2 expression and cell invasion was prevented by blocking ceramide biosynthesis and by knocking-down the expression of the stress protein p8. As MMP-2 upregulation is associated with high progression and poor prognosis of gliomas and many other tumors, MMP-2 downregulation constitutes a hallmark of cannabinoid antitumoral activity [107].

The effect of delta-9-tetrahydrocannabinol and cannabidiol (CBD), both alone and in combination with radiotherapy, in several glioma cell lines (T98G, U87MG, and GL261) were investigated. The cannabinoids were used in two forms, pure (P) and as a botanical drug substance (BDS). The results demonstrated a duration- and dose-dependent reduction in cell viability with each cannabinoid and indicated that delta-9-THC-BDS was more efficacious than delta-9-THC-P, whereas CBD-P was more efficacious than CBD-BDS. Median effect analysis revealed all combinations to be hyperadditive [T98G 48-h combination index (CI) at FU50, 0.77–1.09].

Similarly, pretreating cells with delta-9-THC-P and CBD-P together for 4 h before irradiation increased their radiosensitivity compared to pretreating with either cannabinoid. The increase in radiosensitivity was associated with an increase in markers of autophagy and apoptosis. The *in vitro* results were recapitulated in an orthotopic murine model for glioma, which showed dramatic reductions in tumor volumes when both cannabinoids were used with irradiation (day 21: 5.5 ± 2.2 mm(3) vs. 48.7 ± 24.9 mm(3) in the control group; $p < 0.01$) [23].

Although there are several pharmaceutical preparations that permit the oral administration of delta-9-THC or its analog nabilone or the oromucosal delivery of a delta-9-THC- and CBD-enriched *Cannabis* extract, the systemic administration of cannabinoids has several limitations in part from their high lipophilicity. CBD- and delta-9-THC-loaded poly- ϵ -caprolactone microparticles as an alternative delivery system for long-term cannabinoid administration were examined in a murine xenograft glioma model [108].

In vitro characterization of delta-9-THC- and CBD-loaded microparticles showed that the microencapsulation method facilitates a sustained release of the cannabinoids for several days. Local administration of delta-9-THC-, CBD- or a mixture (1:1 w:w) of delta-9-THC- and CBD-loaded microparticles every 5 days to mice bearing glioma xenografts reduced tumor growth with the same efficacy as a daily local administration of the equivalent amount of the cannabinoids in solution. Moreover, treatment with the cannabinoid-loaded microparticles enhanced apoptosis and decreased cell proliferation and angiogenesis in the tumors [26].

Glioblastoma resistance to therapy is the most common cause of tumor recurrence, which is ultimately fatal in 90% of patients 5 years after initial diagnosis. A subpopulation of tumor cells with stem-like properties, glioma stem cells (GSCs), is designed to resist or adapt to the standard therapies that lead to therapeutic resistance. Several anticancer agents, collectively termed redox therapeutics, act by increasing intracellular levels of reactive oxygen species (ROS).

The mechanisms underlying GSC response and resistance to cannabidiol, a non-toxic, nonpsychoactive cannabinoid and redox modulator, were investigated in primary glioma stem cells. CBD induced a robust increase in ROS, which led to the inhibition of cell survival, phosphorylated (p)-AKT, self-renewal, and a significant increase in the survival of GSC-bearing mice. The activation of the p-p38 pathway and downregulation of key stem cell regulators Sox2, Id-1, and p-STAT3 mediated inhibition of self-renewal. Following CBD treatment, a subset of GSC successfully adapted, leading to tumor regrowth.

Microarray, Taqman, and functional assays revealed that the therapeutic resistance was mediated by enhanced expression of the antioxidant response system Xc catalytic subunit xCT (SLC7A11 (solute carrier family 7 (anionic amino-acid transporter light chain), member 11)) and ROS-dependent upregulation of mesenchymal (MES) markers with concomitant downregulation of proneural (PN) markers, also known as PN–MES transition. This “reprogramming” of GSCs occurred in culture and in vivo and was partially due to activation of the NFE2L2 (NRF2 (nuclear factor, erythroid 2-like)) transcriptional network. Using genetic knockdown and pharmacological inhibitors of SLC7A11, it was revealed that combining CBD treatment with the inhibition of system Xc resulted in synergistic ROS increase leading to robust antitumor effects, that is, decreased GSC survival, self-renewal, and invasion [2].

Delta-9-tetrahydrocannabinol and cannabidiol, combined with radiotherapy, were investigated in several glioma cell lines (T98G, U87MG, and GL261). Cannabinoids were used in two forms, pure (P) and as a botanical drug substance (BDS). The results demonstrated a duration- and dose-dependent reduction in cell

viability with each cannabinoid. They suggested that delta-9-THC-BDS was more efficacious than delta-9-THC-P, whereas CBD-P was more efficacious than CBD-BDS. Median effect analysis revealed all combinations to be hyper-addictive [T98G 48-h combination index (CI) at FU50, 0.77–1.09].

Similarly, pretreating cells with a combination of delta-9-THC-P and CBD-P 4 h before irradiation increased their radiosensitivity compared to pretreating with either cannabinoid [23]. The increase in radiosensitivity was associated with an increase in markers of autophagy and apoptosis. The *in vitro* results were recapitulated in an orthotopic murine model for glioma, which showed dramatic reductions in tumor volumes when both cannabinoids were used with irradiation (day 21: $5.5 \pm 2.2 \text{ mm}^3$ vs. $48.7 \pm 24.9 \text{ mm}^3$ in the control group; $p < 0.01$). The data highlight the possibility that cannabinoids can prime glioma cells to respond better to ionizing radiation and suggest a potential clinical benefit for glioma patients using the two treatment modalities.

A study by Singer et al. showed that CBD-induced apoptosis in glioma stem cells resulted from increased ROS production. An interesting study found that treatment with CBD increased the expression and abundance of heat shock proteins (HSP) in glioma cells due to CBD-induced ROS production. Increases in HSP diminished the cytotoxic effects of CBD; when glioma cells were cultured with CBD and HSP inhibitors, the cytotoxic effects were restored [2].

The stress protein p8 upregulation and endoplasmic reticulum stress were necessary for delta-9-THC-induced apoptosis in glioma cells and cells from human astrocytoma biopsies. In cannabinoid-resistant tumor cells, p8 upregulation did not occur following cannabinoid treatment. They also showed that THC treatment upregulated p8 levels in tumors *in vivo* and that tumors deficient in p8 were resistant to the apoptotic effects of cannabinoids. Another study showed that THC treatment modified sphingolipid ratios in the endoplasmic reticulum of glioma cells, which resulted in the promotion of autophagy-dependent lysosomal membrane permeabilization and cathepsin release, resulting in the activation of the mitochondrial apoptotic pathway [115].

Administration of THC also reduced glioma tumor growth *in vivo* due to autophagy-mediated cell death. THC treatment regresses malignant glioma tumors *in vivo* in murine models. Mice with C6 glioma xenografts were treated for 7 days with THC, which increased survival and reduced tumor progression. THC stimulated apoptosis in glioma cells by accumulating ceramide and Raf1/extracellular signal-regulated kinase activation [103].

The combination of TMZ with both delta-9-THC and CBD together was also looked at, and cannabinoid combinations with higher CBD had a stronger antitumor effect in xenografts derived from GBM patients. The same group looked at the systemic administration of Sativex-like extracts (1:1 THC:CBD) in combination with TMZ, and antitumor effects of glioma cell-derived tumor xenografts were still observed. THC resistance can occur in glioma cells, and the growth factor midkine (Mdk) is involved in the resistance. *In vivo*, silencing Mdk was able to sensitize resistant tumors to the anticancer effects of THC, indicating Mdk as a potential target for improving the effectiveness of cannabinoids in glioma treatment [106].

The local administration of delta-9-THC downregulated the expression of metalloproteinase-2 (MMP-2) in mice bearing glioma tumors. Likewise, in cultured glioma cells, THC exposure inhibited the expression of MMP-2 and reduced invasion, indicating that reduction in MMP-2 plays a fundamental part in the delta-9-THC-induced reduction of cell invasion. Tissue inhibitors of metalloproteinases, for example, MMP-1 (TIMP-1), are downregulated by delta-9-THC exposure, both in vitro and in vivo, which may explain the delta-9-THC-induced inhibition of metalloproteinases in glioma cells [107].

Lung Cancer

Cannabinoids have been shown to inhibit cell growth and induce apoptosis in tumor cells. It has been demonstrated that anandamide, delta-9-tetrahydrocannabinol, HU-210, and Win55212-2 promote mitogenic kinase signaling in cancer cells. Treatment of the glioblastoma cell line U373-MG and the lung carcinoma cell line NCI-H292 with nanomolar concentrations of delta-9-THC led to accelerated cell proliferation that was completely dependent on metalloprotease and epidermal growth factor receptor (EGFR) activity. EGFR signal transactivation was identified as the mechanistic link between cannabinoid receptors and the activation of the mitogen-activated protein kinases extracellular signal-regulated kinase 1/2 and pro-survival protein kinase B (Akt/PKB) signaling. Depending on the cellular context, signal cross-communication was mediated by shedding of proAmphiregulin (proAR) and proHeparin-binding epidermal growth factor-like growth factor (proHB-EGF) by tumor necrosis factor-alpha converting enzyme (TACE/ADAM17). The data indicated that concentrations of delta-9-THC comparable with those detected in the serum of patients after delta-9-THC administration accelerate the proliferation of cancer cells instead of apoptosis and thereby contribute to cancer progression in patients [109].

The antitumor effects of whole *Cannabis* extracts containing significant amounts of cannabinoids were examined on different cancer lines from various tumor origins. The Electrospray ionization liquid chromatography-mass spectrometry method was utilized to analyze the phytocannabinoid contents of 124 *Cannabis* extracts. The effects of 12 extracts were studied on 12 cancer cell lines. The results indicated that specific *Cannabis* extracts impaired the survival and proliferation of cancer cell lines and induced apoptosis. The findings indicated that pure (-)-delta-9-tetrahydrocannabinol did not produce the same effects on the cell lines as the whole *Cannabis* extracts. Furthermore, *Cannabis* extracts with similar amounts of delta-9-THC produced significantly different effects on the survival of specific cancer cells. In addition, specific extracts may selectively and differentially affect cancer cells and different cancer cell lines from the same organ origin. Cannabimimetic receptors were also differentially expressed among various cancer cell lines indicating that the receptor diversity may contribute to the heterogeneous effects of *Cannabis* extracts on each cell line [110].

Delta-9-tetrahydrocannabinol has been shown to either potentiate or inhibit tumor growth, depending on the type of cancer and its pathogenesis. The activities of cannabinoids like delta-9-THC on epidermal growth factor receptor-overexpressing lung cancers, which are often highly aggressive and resistant to chemotherapy, were examined.

The effects of delta-9-THC on the EGF-induced growth and metastasis of human nonsmall cell lung cancer were characterized using the cell lines A549 and SW-1573 as in vitro models. The cells expressed the cannabinoid receptors CB1 and CB2, known targets for delta-9-THC action, and delta-9-THC inhibited EGF-induced growth, chemotaxis, and chemoinvasion. Signaling studies indicated that delta-9-THC might inhibit the EGF-induced phosphorylation of ERK1/2, JNK1/2, and AKT. Delta-9-THC also induced the phosphorylation of focal adhesion kinase at tyrosine 397. Additionally, in in vivo studies in severe combined immunodeficient mice, there was significant inhibition of the subcutaneous tumor growth and lung metastasis of A549 cells in delta-9-THC-treated animals as compared to vehicle-treated controls. Tumor samples from delta-9-THC-treated animals revealed antiproliferative and antiangiogenic effects of delta-9-THC [111].

Patients with nonsmall cell lung cancer (NSCLC) develop resistance to antitumor agents by mechanisms that involve the epithelial-to-mesenchymal transition (EMT). The combined use of delta-9-THC and CBD confers greater benefits, as CBD enhances the effects of delta-9-THC and reduces its psychotropic activity. The relationship between the expression levels of CB1 and CB2 to the clinical features of a cohort of patients with NSCLC and the effect of delta-9-THC and CBD (individually and in combination) on proliferation, EMT, and migration in vitro in A549, H460, and H1792 lung cancer cell lines were evaluated.

Expression levels of CB1, CB2, EGFR, CDH1, CDH2, and VIM were evaluated by quantitative reverse transcription-polymerase chain reaction. Delta-9-THC and CBD (10–100 μ M), individually or in combination (1:1 ratio), were used for in vitro assays. The tumor samples were classified according to the level of expression of CB1, CB2, or both. Patients with high expression levels of CB1, CB2, and CB1/CB2 showed increased survival, reaching significance for CB1 and CB1/CB2 ($p = 0.035$ and 0.025 , respectively).

Both cannabinoid agonists inhibited the proliferation and expression of EGFR in lung cancer cells, and CBD potentiated the effect of delta-9-THC. Delta-9-THC and CBD alone or in combination restored the epithelial phenotype, as evidenced by increased expression of CDH1 and reduced expression of CDH2 and VIM, as well as by fluorescence analysis of cellular cytoskeleton. Both cannabinoids reduced the in vitro migration of the three lung cancer cell lines. The expression levels of CB1 and CB2 have potential use as markers of survival in patients with NSCLC. Delta-9-THC and CBD inhibited the proliferation and expression of EGFR in the lung cancer cells [112].

Delta-9-tetrahydrocannabinol inhibits macrophage cell contact-dependent cytotoxicity of tumor cells. A study examined whether delta-9-THC inhibited macrophage cytotoxic function by targeting tumor necrosis factor (TNF)-dependent pathways versus L-arginine-dependent reactive nitrogen intermediates selectively. An in vitro

system employing RAW264.7 macrophage-like cells as effectors and TNF-sensitive mouse L929 fibroblasts or nitric oxide (NO)-sensitive P815 mastocytoma cells as targets was employed to assess the effect of delta-9-THC on cytotoxicity.

Macrophages were pretreated with delta-9-THC or vehicle for 48 h, subjected to multistep activation with 10 U/ml recombinant mouse gamma-interferon (IFN-gamma) plus 100 ng/ml LPS or to direct activation with 1 µg/ml LPS, and cocultured with tumor cells in the presence or absence of delta-9-THC. Delta-9-THC inhibited TNF-dependent killing by macrophages subjected to either multistep or direct activation. Decreased amounts of TNF-alpha were detected in the medium of macrophage cultures treated with delta-9-THC. In contrast, delta-9-THC inhibited NO-dependent cell contact killing only for macrophages subjected to direct activation. The macrophages produced decreased levels of NO₂⁻, a stable degradation product of the short-lived and highly toxic effector molecule NO. The effect of the enantiomeric pairs (-)-CP55,940/(+)-CP56,667 or (-)-HU-210/(+)-HU-211 on macrophage cell contact-dependent killing was assessed.

Inhibition of macrophage tumoricidal activity against TNF-sensitive L929 cells was affected by both isomers of delta-9-THC analogs. In contrast, both of the enantiomeric pairs affected the killing of NO-sensitive P815 mastocytoma cells only for macrophages subjected to direct activation. The data indicated that cannabinoids inhibit macrophage cell contact-dependent killing of tumor cells by a noncannabinoid receptor-mediated mechanism. However, specific cytotoxic pathways are inhibited differentially by cannabinoids depending on the activation stimuli to which macrophages are exposed [113].

In two weakly immunogenic murine lung cancer models, intermittent administration of delta-9-THC (5 mg/kg, four times/week i.p. for 4 weeks) led to accelerated growth of tumor implants compared with treatment with diluent alone. In immunocompetent mice, delta-9-THC did not affect tumor growth in tumor-bearing SCID mice. The immune inhibitory cytokines, IL-10 and TGF-beta, were augmented, while IFN-gamma was downregulated at both the tumor site and in the spleens of delta-9-THC-treated mice. Administration of either anti-IL-10- or anti-TGF-beta-neutralizing Abs prevented the delta-9-THC-induced enhancement in tumor growth. APC and T cells from delta-9-THC-treated mice showed a limited capacity to generate alloreactivity.

Furthermore, lymphocytes from delta-9-THC-treated mice transferred the effect to normal mice, resulting in accelerated tumor growth similar to that seen in the delta-9-THC-treated mice. Delta-9-THC decreased tumor immunogenicity, as indicated by the limited capacity for tumor-immunized, delta-9-THC-treated mice to withstand tumor rechallenge. In vivo administration of a specific antagonist of the CB2 cannabinoid receptor also blocked the effects of delta-9-THC. The findings indicated that delta-9-THC promotes tumor growth by inhibiting antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway [114].

Urogenital Cancer

To investigate the association between *Cannabis* use and tobacco smoking on the incidence of bladder cancer, the records of 84,170 participants in a multiethnic cohort of men aged 45–69 years were studied [27]. Information on demographic and lifestyle factors, including smoking history and *Cannabis* use, was collected using mailed questionnaires. The study data were linked with clinical records, including cancer data from electronic health records.

Overall, 34,000 (41%) cohort members reported *Cannabis* use, 47,092 (57%) reported tobacco use, 22,500 (27%) reported using both, and 23,467 (29%) used neither. Men were followed over 11 years, and 279 (0.3%) developed incident bladder tumors. Among *Cannabis* users, 89 (0.3%) developed bladder cancer in comparison to 190 (0.4%) men who did not report *Cannabis* use ($p < 0.001$). After adjusting for age, race or ethnicity, and body mass index, using tobacco only was associated with an increased risk of bladder cancer (hazard regression [HR], 1.52; 95% confidence interval [CI], 1.12–2.07), whereas *Cannabis* use only was associated with a 45% reduction in bladder cancer incidence (HR, 0.55; 95% CI, 0.31–1.00). Using both *Cannabis* and tobacco was associated with an HR of 1.28 (95% CI, 0.91–1.80).

To determine the maximal inhibitory concentration for *Cannabis extract* and cannabidiol, an MTT assay was used. Camptothecin, as the positive control, significantly reduced cell viability in SiHa (40.36%), HeLa (47.19%), and ME-180 cells (32.25%), respectively. The IC_{50} was cell type-dependent rather than time-dependent, with SiHa cells at less than 50 $\mu\text{g/mL}$ in butanol (56%) and hexane (48.9%).

Similarly, the IC_{50} in HeLa cells was 100 $\mu\text{g/mL}$, at $p < 0.001$, while ME-180 cells treated with butanoic extract exhibited an IC_{50} of 100 $\mu\text{g/mL}$, reducing viability to 48.6%, and hexane extracts IC_{50} was at 50 $\mu\text{g/mL}$ with 54% death. That was not the case in cannabidiol with SiHa (51%) and HeLa (50); IC_{50} was a much lower dose (3.2 $\mu\text{g/mL}$) and ME-180 cells (56%) at 1.5 $\mu\text{g/mL}$ when compared to *Cannabis* extracts ($p < 0.001$). Dimethyl sulfoxide (DMSO) was included as vehicle control and inhibited between 4% and 11%. Whereas ethanol exhibited between 7.3% and 7.8% since cannabidiol was alcohol extracted.

The IC_{50} obtained during the MTT assay was tested for its ability to alter cell viability in real-time. An impedance-based system was employed to evaluate the effect of *Cannabis* and cannabidiol on SiHa, HeLa, and ME-180 cells. The cells were treated with IC_{50} for 22–24 h, depending on their doubling time. Continuous changes in the impedance were measured and displayed as cell index (CI). Little can be read from xCELLigence except for that cannabidiol in all cell lines has been shown to reduce cell index while the plant extract had mixed results, sometimes showing the reduction. On the other hand, it remained unchanged, indicating that cannabidiol is the most effective compound.

Cannabidiol was also tested for its ability to induce apoptosis in all three cell lines. Flow cytometry revealed a significant increase in SiHa cells undergoing apoptosis during treatment with butanol (from 2% to 28.5%) and hexane (from 2% to

17.2%) compared to camptothecin with 30.4%. In HeLa cells, apoptosis was increased to 31.9% in butanol extract and only 15.3% in hexane cells. A similar event was observed following the treatment of ME-180 cells with butanol extract 44.8% apoptosis and 43.2% in hexane-treated cells. The results confirmed that the type of cell death induced was apoptosis. Cannabidiol was more effective in inducing apoptosis than both extracts of *Cannabis*. In SiHa cells, cannabidiol induced 51.3% apoptosis, 43.3% in HeLa, and 28.6% in ME-180 cell lines.

To characterize the cell death type following treatment with the test compounds, cells were stained with DAPI and Annexin V to show if apoptosis was taking place. Treatment of SiHa and HeLa cells with IC_{50} of butanol and hexane extracts confirmed the type of cell death as apoptosis since they picked a green color from Annexin V that binds to phosphatidyl molecules that appear in the early stages of apoptosis.

Another feature that is representative of cell death is the change in morphology. The morphological appearance of live cells displayed round blue nuclei following staining with DAPI. Exposure of SiHa and HeLa cells to IC_{50} of *Cannabis* extracts caused a change in morphology coupled with the uptake of Annexin. Loss of shape, nuclear fragmentation, reduction in cell size, and blebbing of the cell membrane were among the observed morphological features implicated as associated with apoptosis. Similar results were also observed in cannabidiol-treated cells.

Since Adenosine 5'-triphosphate acts as a biomarker for cell proliferation and cell death, an ATP assay was conducted to determine whether *Cannabis* and cannabidiol deplete ATP levels in cervical cancer cells. ATP levels were first detected after 2 h. SiHa, HeLa, and ME-180 cells were treated at different time points, between 2 and 24 h. In general, ATP depletion was cell type-dependent. In HeLa cells treated with crude extracts from butanol and hexane, ATP was reduced by 74% (from 627,621 to 164,208 RLU) and 78% (from 627,621 to 133,693 RLU), respectively.

In SiHa cells, there was a reduction of 31% (from 4,719,589 to 3,221,245 RLU) and 22.5% (4,719,589 to 3,655,730 RLU), respectively (figure). In ME-180, there was no change between treatments and untreated. Similar results were observed in cannabidiol-treated cells. At 2 h, treatment with IC_{50} led to a reduction in ATP levels by ~61% (from 4,704,419 to 1,802,508 RLU), 93% (from 627,621 to 40,371 RLU), and 8% (from 798,688 to 734,039 RLU) in SiHa, HeLa, and ME-180 cells, respectively. A 24 h incubation period of cells with IC_{50} led to a further decrease in the ATP levels by ~66% (from 4,486,150 to 1,497,648 RLU), 97% (from 601,694 to 13,426 RLU), and 8.5% (from 790,757 to 723,039 RLU) in SiHa, HeLa, and ME-180 cells, respectively; an indication that cannabidiol depletes ATP levels more than *Cannabis* extracts and is the main component responsible for cell death in cancer cells treated with *Cannabis* extract.

Increased caspase-3/7 activity in all three cell lines following treatment was observed with 0.3 μ M of camptothecin. Similar results were observed in cells treated with crude extracts: 25% (SiHa) and 40% (HeLa) in butanol extract and 50% (SiHa) and 100% (HeLa) in hexane extract. There was no significant change in ME-180 cells. When the cells were treated with cannabidiol, caspase-3/7 activity

increased in all three cell lines. SiHa cells show an increase from 200,000 RLU to 2,500,000, while HeLa increased to 900,000 from 800,000 RLU. ME-180 has also fairly increased to 230,000 from 200,000 RLU; all increases were significant and in line with other increases in apoptosis.

Hodgkin's Lymphoma

Hodgkin's lymphoma (HL) is pregnant females' fourth most frequent cancer diagnosis. A 21-year-old female patient was diagnosed with HL stage IIB. The patient started first-line chemotherapy and radiotherapy; with incomplete remission, she refused any other treatment. Five years later, the patient became pregnant and was offered chemotherapy in the second trimester, which she refused and delivered by C-section at 37 weeks. In the same year, the patient became pregnant again and was proposed termination of pregnancy, which she also refused.

The MRI scan revealed the progression of HL, and she was admitted to the hospital several times for altered general condition, respiratory infections, and increased need for painkillers, including opioids. At 26 weeks of pregnancy, the patient began, on her own, a treatment with pure *Cannabis*. Her pain and general status got better, and the tumor tissue decreased. She delivered by C-section at 34 weeks a boy that presented in the first 24 h postpartum a withdrawal syndrome and intestinal invagination, requiring care in NICU and surgery with bowel resection [28].

The extent of medical *Cannabis* use among Hodgkin's lymphoma patients and its efficacy in controlling chemotherapy-related adverse events were evaluated [38]. Patterns of medical *Cannabis* use and efficacy were evaluated using physician-completed application forms, medical files, and patient-completed questionnaires for all consecutive adult Hodgkin's lymphoma patients. One hundred and thirty-three patients met the inclusion criteria. The cohort's median age was 37 years; 53% were male, 46% were diagnosed early, and 88% received a complete treatment response. Fifty-one patients (38%) used *Cannabis*.

There were no significant differences in baseline characteristics between *Cannabis* users and nonusers. *Cannabis* users reported improvement in pain, general well-being, appetite, and nausea in 94%, 87%, 82%, and 79% of cases, respectively. Importantly, 81.5% reported high overall efficacy of *Cannabis* in relieving symptoms. Adverse events related to *Cannabis* use were mild.

Prostate Cancer

The most commonly mutated genes observed in primary prostate cancers include SPOP, TP53, FOXA1, and PTEN [34]. The retinoblastoma tumor suppressor gene RB1 is more commonly mutated in metastatic and ADT-recurrent prostate cancer than in primary tumors. In contrast, the expression of the ST6GalNAc1 gene is

upregulated in primary prostate cancer cells and repressed in patients undergoing androgen deprivation therapy [35].

The prostate-specific antigen (PSA), an androgen receptor widely employed as a marker in early prostate cancer detection, is regulated by androgens. Increased PSA levels are a biomarker of prostate disorders, including prostate cancer, prostatitis, and benign prostatic hypertrophy. In LNCaP prostate cancer cells, androgens work through the androgen receptor to regulate PSA mRNA and glycoprotein levels. It was observed that *Cannabis* extracts reduced both intracellular PSA mRNA expression and secreted PSA levels, implying that cannabinoid receptor agonists may be exploited to prevent prostate cancer progression [36].

The expression of cannabinoid receptors on the surface of prostate cancer cells is greater than that seen in noncancerous cells, which suggests that the endocannabinoid system may play a crucial role in the growth of cancer cells. It has also been suggested that CB1 and CB2 play a role in the development of prostate cancers.

Prostate cancer is a major cause of death in men. Recent preclinical evidence implicates cannabinoids as robust cell growth and differentiation regulators and potential anticancer agents. Six studies that report on the effect of cannabinoids on prostate cancer based on in vivo/xenograft animal models were evaluated, and the effect of cannabinoids was evaluated.

The studies have reported that the treatment of prostate cancers in in vivo/xenograft models with various cannabinoids decreased tumor size, the outcomes of which depended on the dose and length of treatment. In PC3 and DU145 xenografts, WIN55212-2 reduced cell proliferation dose-dependently. In LNCaP xenografts, WIN55212-2 reduced cell proliferation by 66–69%. PM49, a synthetic cannabinoid quinone, was also found to significantly inhibit tumor growth of up to 90% in xenograft models of LNCaP and 40% in xenograft models of PC3 cells, respectively [37].

Cannabinoid receptor activation induces prostate carcinoma cell (PCC) apoptosis. Some of the compounds antagonize transient receptor potential melastatin type-8 (TRPM8) channels, the expression of which is necessary for androgen receptor (AR)-dependent PCC survival.

Pure cannabinoids and extracts from *Cannabis* strains enriched in particular cannabinoids (BDS) were tested on AR-positive (LNCaP and 22RV1) and -negative (DU-145 and PC-3) cells, by evaluating cell viability (MTT test), cell-cycle arrest and apoptosis induction, by FACS scans, caspase-3/7 assays, DNA fragmentation, and TUNEL, and size of xenograft tumors induced by LNCaP and DU-145 cells.

Cannabidiol significantly inhibited cell viability. Other compounds became effective in cells deprived of serum for 24 h. Several BDS were more potent than the pure compounds in the presence of serum. CBD-BDS (i.p.) potentiated the effects of bicalutamide and docetaxel against LNCaP and DU-145 xenograft tumors and, given alone, reduced LNCaP xenograft size. Cannabidiol (1–10 μ M) induced apoptosis and induced markers of intrinsic apoptotic pathways (PUMA and CHOP expression and intracellular Ca^{2+}). In LNCaP cells, the proapoptotic effect of CBD was only partly due to TRPM8 antagonism and was accompanied by downregulation of AR, p53 activation, and elevation of reactive oxygen species. LNCaP cells

differentiated to androgen-insensitive neuroendocrine-like cells were more sensitive to CBD-induced apoptosis [95].

Exosomes and microvesicles (EMV) are lipid bilayer-enclosed structures released by cells and involved in intercellular communication through the transfer of proteins and genetic material. Exosomes and microvesicles release is also associated with various pathologies, including cancer, where increased EMV release is, among others, associated with chemo-resistance and active transfer of pro-oncogenic factors. Studies indicated that EMV-inhibiting agents could sensitize cancer cells to chemotherapeutic agents and reduce cancer growth *in vivo*. Cannabidiol has anti-inflammatory and antioxidant properties and displays antiproliferative activity. The role of CBD as a potent inhibitor of EMV release from three cancer cell lines: prostate cancer (PC3), hepatocellular carcinoma (HEPG2), and breast adenocarcinoma (MDA-MB-231), was examined.

Cannabidiol significantly reduced exosome release in all three cancer cell lines and inhibited microvesicle release significantly, albeit more variably. The EMV-modulating effects of CBD were dose-dependent (1 and 5 μ M) and cancer cell type-specific. Moreover, the evidence that this may be associated with changes in mitochondrial function, including modulation of STAT3 and prohibitin expression, and that CBD can be used to sensitize cancer cells to chemotherapy [116].

Myeloma

Cannabidiol has been noted to induce cell death in multiple myeloma (MM), both alone and in synergy with the proteasome inhibitor bortezomib. In other types of cancers, the combination of CBD with delta-9-tetrahydrocannabinol was found to act synergistically with other chemotherapeutic drugs suggesting their use in combination therapy. A study evaluated the effects of delta-9-THC alone and in combination with CBD in MM cell lines. The CBD-THC combination reduced MM cell migration by downregulating the expression of the chemokine receptor CXCR4 and the CD147 plasma membrane glycoprotein. Cannabidiol and delta-9-THC, mainly in combination, reduced cell viability by inducing autophagic-dependent necrosis [120].

Furthermore, since the immunoproteasome is considered a target in MM and also since carfilzomib (CFZ) is a promising immunoproteasome inhibitor that creates irreversible adducts with the β 5i subunit of immunoproteasome, the effect of CBD and delta-9-THC in regulating the expression of the β 5i subunit and their effect, were evaluated in combination with CFZ. The CBD and delta-9-THC combination reduced the expression of the β 5i subunit and acted in synergy with CFZ to increase MM cell death and inhibits cell migration.

Multiple myeloma is a plasma cell malignancy characterized by accumulating a monoclonal plasma cell population in the bone marrow (BM). Cannabidiol has antitumoral activities, and the transient receptor potential vanilloid type-2 (TRPV2) channel has been reported as a potential CBD receptor. TRPV2 activation by CBD

decreases proliferation and increases susceptibility to drug-induced cell death in human cancer cells. However, no functional role in MM has been ascribed to CBD and TRPV2.

A study identified the presence of heterogeneous CD138⁺TRPV2⁺ and CD138⁺TRPV2⁻ PC populations in MM patients, whereas only the CD138⁺TRPV2⁻ population was present in RPMI8226 and U266 MM cell lines. Since bortezomib (BORT) is commonly used in MM treatment, the effects of CBD and BORT were examined in CD138⁺TRPV2⁻ MM cells and MM cell lines transfected with TRPV2 (CD138⁺TRPV2⁺). The results demonstrated that CBD or synergy with BORT strongly inhibited growth, arrested cell-cycle progression, and induced MM cell death by regulating the ERK, AKT, and NF- κ B pathways with major effects in TRPV2⁺ cells [121].

Delta-9-THC has been demonstrated to exert anticancer effects in multiple myeloma (MM) cells. Delta-9-THC inhibits cell viability and proliferation at concentrations ranging between 30 and 40 μ M and induces autophagic-dependent necrosis of MM cells. The combination of delta-9-THC and CBD had the most potent effects in MM cells compared to each compound. Delta-9-THC and CBD synergized with carfilzomib, increasing cell death and decreasing migration. Delta-9-THC treatment alone also reduced the migration of MM cells by decreasing the expression of CXCR4 and the plasma membrane glycoprotein CD147 [121].

MDSCs are potent immunosuppressive cells induced during inflammatory responses and by cancers to evade antitumor immunity. Cannabinoids are potent inducers of MDSCs. In the current study, the epigenetic mechanisms by which delta-9-THC induces MDSCs were examined and compared with the naïve MDSCs found in the bone marrow of BL6 (WT) mice.

The administration of delta-9-THC to WT mice produced increased methylation at the promoter region of DNMT3a and DNMT3b in delta-9-THC-induced MDSCs, which correlated with reduced expression of DNMT3a and DNMT3b. The promoter region methylation was decreased at Arg1 and STAT3 in the delta-9-THC-induced MDSCs, and consequently, the MDSCs expressed higher levels of Arg1 and STAT3. Delta-9-THC-induced MDSCs secreted elevated levels of S100A8, a calcium-binding protein associated with the accumulation of MDSCs in cancer models. Neutralizing S100A8 by using anti-S100A8 (8H150) *in vivo* reduced the ability of delta-9-THC to trigger MDSCs. The elevated S100A8 expression also promoted the suppressive function of MDSCs. The study demonstrates that delta-9-THC mediates epigenetic changes to promote MDSC differentiation and function, and S100A8 plays a critical role in the process [122].

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous, immature myeloid cell population that can suppress immune responses. Infections, inflammatory diseases, and solid tumors have characterized MDSCs. The presence, frequency, and functional characteristics of MDSCs in patients with newly diagnosed relapsed and relapsed/refractory multiple myelomas (MM) were compared with healthy donors. The immunomodulatory effects of lenalidomide and bortezomib were evaluated on MDSCs in MM.

Due to the phenotypic heterogeneity of MDSCs in humans, it was essential to characterize MDSCs not only by their distinct phenotype but also by their immune-suppressive activity in patients; the CD11b⁺CD14⁻HLA-DR⁻/lowCD33⁺CD15⁺ MDSC population was analyzed for immune-suppressive function in the MM-BM and PB microenvironment. CD11b(+)CD14(-)HLA-DR(-/low)CD33(+)CD15(+) MDSCs were significantly increased in both the peripheral blood and the bone marrow of patients with active MM compared with healthy donors.

Furthermore, MDSCs induced MM growth while suppressing T-cell-mediated immune responses. Conversely, MM cells induced the development of MDSCs from healthy donor peripheral blood mononuclear cells, confirming a bidirectional interaction between MDSCs and MM cells and immune effector cells. The results indicate that MDSCs may be associated with the activity of the disease in MM [123].

Oral Cancer

In human oral cancers resistant to anticancer drugs, exposure to delta-9-THC significantly inhibited mitochondrial oxygen consumption and exhibited strong toxicity in the highly malignant cells. Delta-9-THC and delta-8-THC disturb mitochondrial functions and possess antitumor activities. The effects on mitochondrial oxygen consumption were evaluated in human oral cancer cells (Tu183), an epithelial cell line that overexpresses bcl-2 and is highly resistant to anticancer drugs.

A rapid decline in respiration rate was observed when delta-9-THC or delta-8-THC was added to the cells. The inhibition was concentration-dependent, and delta-9-THC was the more potent of the two compounds. Anandamide was ineffective, indicating that the cannabinoid receptors did not mediate the effects of delta-9-THC and delta-8-THC. Inhibition of oxygen consumption by cyanide confirmed the oxidations that occurred in the mitochondrial respiratory chain. Delta-9-THC inhibited the respiration of isolated mitochondria from beef heart [124].

Colorectal Cancer

Colorectal cancer (CRC) is among the primary malignant tumors with a high incidence and mortality rate. Nearly two million new cases were diagnosed, and one million deaths were registered in 2018. The figures represent 9.7% of all cancer diagnoses and 8.9% of all deaths due to cancer in both genders. Despite the rapid progress in diagnostic approaches and treatment protocols, no other cancer kills more men and women than CRC after lung and prostate cancer in the case of men and after breast cancer among women. The World Health Organization predicts 77% more new CRC cases in the coming years, resulting in 80% more deaths by 2030.

Natural products can stop or slow down multiple pathways of carcinogenesis, for example, angiogenesis and apoptosis, which are the main driving forces in regulating solid tumors' development. It is accepted that CRC is an angiogenesis-dependent tumor. High vascular density and stimulation of the vascular endothelial growth factor (VEGF) are linked to cancer cell proliferation, migration, local tissue invasion, and distant metastasis, which threaten the patient's chances of survival.

Beta-Caryophyllene (BCP), a naturally occurring sesquiterpene abundant in *Cannabis*, is an active candidate for vascular-inhibiting compounds that block existing tumor blood vessels. A study examines the effects of BCP on in vitro, ex vivo, and in vivo models of antiangiogenic assays and evaluates its anticancer activity in ectopic and orthotopic xenograft tumor mice models of human colorectal cancer. Computational structural analysis and an apoptosis antibody array were performed to understand the molecular mechanisms underlying the effect.

Beta-Caryophyllene exhibits strong antiangiogenic activity by blocking the migration of endothelial cells, tube-like network formation, suppression of vascular endothelial growth factor secretion from human umbilical vein endothelial cells, and sprouting of rat aorta microvessels. Moreover, BCP significantly deformed the vascularization architecture in a chick embryo chorioallantoic membrane assay compared to the negative control. Beta-Caryophyllene has a binding at Site#0 on the surface of VEGFR2.

Beta-Caryophyllene showed a remarkable reduction in tumor size and fluorescence molecular tomography signal intensity in all the mice treated with BCP, in a dose-dependent relationship, in ectopic and orthotopic tumor xenograft models, respectively. The histological analysis of the tumor from BCP-treated mice revealed a clear reduction of the density of vascularization. Additionally, BCP induced apoptosis through the downregulation of HSP60, HTRA, survivin, and XIAP, along with the upregulation of p21 expressions. The results indicated that BCP acts at multiple stages of angiogenesis and could be useful as a therapeutic product to halt the growth of colorectal tumor cells [125].

Deregulation of cell survival pathways and resistance to apoptosis are widely accepted as fundamental aspects of tumorigenesis. As in many tumors, the aberrant growth and survival of colorectal tumor cells depend upon a small number of highly activated signaling pathways, the inhibition of which elicits potent growth inhibitory or apoptotic responses in tumor cells. Accordingly, there is considerable interest in therapeutics that can modulate survival signaling pathways and target cancer cells for death. There is evidence that cannabinoids, especially delta-9-THC, may represent anticancer agents due to their ability to regulate signaling pathways critical for cell growth and survival.

CB1 and CB2 cannabinoid receptors are expressed in human colorectal adenoma and carcinoma cells, indicating that delta-9-THC induces apoptosis in colorectal cancer cells. Delta-9-THC-induced apoptosis was rescued by pharmacological blockade of the CB1, but not CB2, cannabinoid receptor. Delta-9-THC treatment resulted in CB1-mediated inhibition of RAS-MAPK/ERK and PI3K-AKT survival signaling cascades, two key cell survival pathways frequently deregulated in colorectal tumors. The inhibition of ERK and AKT activity by delta-9-THC was

accompanied by activation of the proapoptotic BCL-2 family member BAD. Reduction of BAD protein expression by RNA interference rescued colorectal cancer cells from delta-9-THC-induced apoptosis. The data suggest an important role for CB1 receptors and BAD in regulating apoptosis in colorectal cancer cells [126].

Purified cannabinoids have been shown to prevent proliferation and induce apoptosis in colorectal carcinoma cell lines. The cytotoxic effect of cannabinoid extracts and purified cannabinoids were assessed on colorectal polyps and normal colonic cells and their synergistic interaction. Biopsies from polyps and healthy colonic tissue were obtained from 22 patients undergoing colonic polypectomies. Various blends were tested to identify the optimal synergistic effect. The toxicity of various cannabinoid extracts and purified cannabinoids at different concentrations was evaluated, and the synergistic effect was calculated based on the cells' survival.

Isolated cannabinoids illustrated different toxic effects on the viability of cells derived from colorectal polyps. Delta-8-THC and delta-9-THC were the most toxic and exhibited persistent toxicity in all the polyps tested. Cannabidiol was more toxic to polypoid cells than normal colonic cells at a concentration of 15 μ M. The combinations of the cannabinoids CBDV, THCV, CBDVA, CBCA, and CBGA exhibited a synergistic inhibitory effect on the viability of cells derived from colon polyps of patients. Isolated cannabinoid compounds interacted synergistically against colonic polyps, and some also possessed a differential toxic effect on the polyp and adjacent colonic tissue, suggesting possible future therapeutic value [127].

The role of the CB2 receptor during melanoma and colorectal cancer aggressiveness and metastatic growth in the liver was examined using the synthetic cannabinoid URB447, a known CB2 agonist and CB1 antagonist drug. The prometastatic ability of mouse B16 melanoma and MCA38 CRC cells were evaluated through proliferation, apoptosis, cell cycle, migration, and matrix degradation in vitro upon URB447 treatment.

A dose-dependent viability decrease in both tumor types was observed, a result partly mediated by apoptotic cell death and cell-cycle arrest in G1/G0 phase, as observed through flow cytometry. Melanoma and CRC cell migration was affected in a dose-dependent manner, observed through scratch assay. The secretion of matrix-degrading metalloprotease 2 (MMP2) and 9 (MMP9) in tumor cells did not change. Daily treatment of tumor-bearing mice with URB447 decreased the development of liver metastasis in a melanoma model in vivo [128].

Cannabidiol exerts antioxidant and intestinal anti-inflammatory actions, and the inhibition of endocannabinoid enzymatic degradation is potentially beneficial for colon carcinogenesis. Its chemopreventive effect in a model of colon cancer induced by azoxymethane (AOM) in mice was investigated. Cannabidiol reduced ACF, polyps, and tumors and counteracted AOM-induced phospho-Akt and caspase-3 changes. Azoxymethane treatment was associated with aberrant crypt foci, preneoplastic lesions, polyps, tumor formation, and upregulation of phospho-Akt, iNOS, COX-2, and downregulation of caspase-3.

In colorectal carcinoma cell lines, cannabidiol protected DNA from oxidative damage, increased endocannabinoid levels, and reduced cell proliferation in a CB1-,

TRPV1- and PPAR γ -antagonists sensitive manner. It is concluded that cannabidiol exerts a chemopreventive effect *in vivo* and reduces cell proliferation through multiple mechanisms [117].

Cannabigerol interacts with specific targets involved in carcinogenesis. Specifically, CBG potently blocks transient receptor potential (TRP) M8 (TRPM8), activates TRPA1, TRPV1, and TRPV2 channels, blocks 5-hydroxytryptamine receptor 1A (5-HT1A) receptors and inhibits the reuptake of endocannabinoids. Cell growth was evaluated in colorectal cancer (CRC) cells using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide and 3-amino-7-dimethylamino-2-methylphenazine hydrochloride assays; apoptosis was examined by histology and by assessing caspase 3/7 activity; reactive oxygen species (ROS) production by a fluorescent probe; CB receptors, TRP and CCAAT/enhancer-binding protein homologous protein (CHOP) messenger RNA (mRNA) expression were quantified by reverse transcription-polymerase chain reaction; small hairpin RNA-vector silencing of TRPM8 was performed by electroporation.

The *in vivo* antineoplastic effect of CBG was assessed using mouse models of colon cancer. Colorectal cancer cells expressed TRPM8, CB1, CB2, 5-HT1A receptors, TRPA1, TRPV1 and TRPV2 mRNA. CBG promoted apoptosis, stimulated ROS production, upregulated CHOP mRNA, and reduced cell growth in CRC cells. Cannabigerol's effect on cell growth was independent of TRPA1, TRPV1, and TRPV2 channels activation; it was increased by a CB2 receptor antagonist and mimicked by other TRPM8 channel blockers but not by a 5-HT1A antagonist. The effect of CBG on cell growth and CHOP mRNA expression was reduced in TRPM8-silenced cells. *In vivo*, CBG inhibited the growth of xenograft tumors and chemically induced colon carcinogenesis. Cannabigerol hampers colon cancer progression *in vivo* and selectively inhibits the growth of CRC cells, an effect shared by other TRPM8 antagonists [68].

The effects of cannabidiol on angiogenesis and cell death were investigated in mice with experimental colon cancer induced by injection of CT26 cell line. Fifty male BALB/c mice were assigned randomly to five study groups, including (1) negative control, (2) cancer control, (3) cancer vehicle control, (3) cancer treatment (1 mg/kg cannabidiol), and (5) cancer treatment (5 mg/kg cannabidiol).

Treatment responses were evaluated based on histopathological examination, the expression of the vascular endothelial growth factor gene, measurement of interleukins (ILs 6 and 8), oxidative stress parameters (glutathione peroxidase, glutathione reductase, superoxide dismutase serum activities, total antioxidant capacity, and malondialdehyde levels).

Cannabidiol reduced VEGF gene expression, decreased IL6, IL8, and malondialdehyde serum levels, and increased antioxidant enzyme activity in mice with colon cancer. Moreover, cannabidiol induces apoptosis and reduced cellular pleomorphism [118].

Tumor cell migration and adhesion constitute essential features of metastasis. G protein-coupled receptor 55 has been shown to play an important role in carcinogenesis and involvement in the migration and metastasis of colon cancer cells.

Adhesion and migration assays were performed using the highly metastatic colon cancer cell line HCT116 and an *in vivo* assay of liver metastasis. The GPR55 antagonist CID16020046, cannabidiol, a putative GPR55 antagonist, and GPR55 siRNA were used to block GPR55 activity in HCT116 colon cancer cells. HCT116 cells significantly decreased adhesion to endothelial cells and migration after blockade with CID16020046 or cannabidiol. The inhibitory effects of CID16020046 or cannabidiol were averted by GPR55 siRNA knockdown in cancer cells. The integrity of endothelial cell monolayers was increased after pretreatment of HCT116 cells with the antagonists or after GPR55 siRNA knockdown. In contrast, pretreatment with lysophosphatidylinositol (LPI), the endogenous ligand of GPR55, decreased the integrity of the monolayers. Lysophosphatidylinositol also induced migration in GPR55 overexpressing HCT116 cells that GPR55 antagonists blocked. In the mouse metastasis model, the arrest of HCT116 cancer cells in the liver was reduced after treatment with CID16020046 or cannabidiol. Increased levels of LPI (18:0) were found in colon cancer patients compared to healthy individuals [119].

The apoptotic action of CBD in colorectal cancer cells and its effects on the proapoptotic Noxa-reactive oxygen species signaling pathway was examined. Cannabidiol experiments were performed using the CRC cell lines HCT116 and DLD-1. Cannabidiol induced apoptosis by regulating many pro- and antiapoptotic proteins, of which Noxa showed significantly higher expression. To understand the relationship between Noxa and CBD-induced apoptosis, Noxa levels were down-regulated using siRNA, and the expression of apoptosis markers decreased. After ROS production was blocked, the level of Noxa also decreased, suggesting that ROS is involved in the regulation of Noxa, which along with ROS, is a known proapoptotic signaling agent. As a result, CBD induced apoptosis in a Noxa- and ROS-dependent manner [66].

Melanoma

Melanoma causes the greatest number of skin cancer-related deaths. The efficacy of cannabinoid receptor agonists as antitumoral compounds for skin melanoma was investigated in human melanomas and melanoma cell lines that express CB1 and CB2 cannabinoid receptors.

Activation of the receptors decreased growth, proliferation, angiogenesis, and metastasis and increased apoptosis of melanomas in mice. Cannabinoid antimelanoma activity was independent of the animal's immune status and could be achieved without overt psychoactive effects, and was selective for melanoma cells vs. normal melanocytes. Cannabinoid antiproliferative action on melanoma cells was due, in part, to cell-cycle arrest at the G1-S transition via inhibition of the prosurvival protein Akt and hypophosphorylation of the pRb retinoblastoma protein tumor suppressor [129].

Although the global incidence of cutaneous melanoma is increasing, survival rates for patients with the metastatic disease remain <10%. Autophagy is a means to

promote cancer cell death in chemotherapy-resistant tumors, and the hypothesis that cannabinoids promote autophagy-dependent apoptosis in melanoma has been examined. Treatment with delta-9-Tetrahydrocannabinol resulted in the activation of autophagy, loss of cell viability, and activation of apoptosis. In contrast, cotreatment with chloroquine or knockdown of Atg7, but not Beclin-1 or Ambra1, prevented delta-9-THC-induced autophagy and cell death in vitro.

The administration of Sativex-like preparation to mice bearing BRAF wild-type melanoma xenografts substantially inhibited melanoma viability, proliferation, and tumor growth, paralleled by an increase in autophagy and apoptosis compared with standard single-agent temozolomide. The treatment indicates that delta-9-THC activates noncanonical autophagy-mediated apoptosis of melanoma cells [16].

Patients with malignant melanoma often relapse after treatment with BRAF and mitogen-activated protein kinase (MEK) inhibitors (MEKi) due to the development of drug resistance. Immunohistochemistry for CD271 and p62 was performed on human nevi and primary malignant melanoma tumors to establish the temporal pattern of CD271 regulation during the development of resistance by melanoma to trametinib and determine the association between the development of resistance to trametinib and the induction of prosurvival autophagy. Western blotting was used to analyze the expression of CD271, p62, and LC3 in melanoma subpopulations. Flow cytometry and immunofluorescence microscopy were used to evaluate trametinib-induced cell death and CD271 expression. MTS viability assays and zebrafish xenografts were used to evaluate the effect of CD271 and autophagy modulation on trametinib-resistant melanoma cell survival and invasion, respectively.

In vitro studies demonstrate MEKi of BRAF-mutant melanoma-induced cytotoxic autophagy, followed by the emergence of CD271-expressing subpopulations. CD271 and autophagic signaling were increased in stage III primary melanomas vs. benign nevi. Trametinib-induced CD271 reduced autophagic flux, leading to the activation of prosurvival autophagy and the development of MEKi resistance. Treatment of CD271-expressing melanoma subpopulations with RNA interference and small-molecule inhibitors to CD271 reduced the development of MEKi resistance. In contrast, clinically applicable autophagy modulatory agents, including delta-9-tetrahydrocannabinol and Vps34, reduced the survival of MEKi-resistant melanoma cells. Combined MEK/autophagy inhibition also reduced the invasive and metastatic potential of MEKi-resistant cells in an in vivo zebrafish xenograft [130].

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is one of the most common solid tumors and the third leading cause of cancer-related death. The survival of patients with HCC has not significantly improved in the past two decades. Current treatments only apply at the early stages of tumor development and include tumor resection, liver transplantation, chemoembolization, and sorafenib administration. However, approximately

half of the patients suffer tumor recurrence. The most important mechanism of liver cancer progression is cell proliferation.

It has been indicated that delta-9-THC triggers human glioma cell death by stimulating an ER stress pathway that activates autophagy and promotes apoptosis. Autophagy is a cellular self-digestive process whereby bulk cytoplasmic components and intracellular organelles are sequestered into double-membrane vesicles named autophagosomes and delivered for degradation to the lysosomes. In the liver, autophagy may have an important role in regulating energy balance for basic cell functions. Although functional autophagy acts as a metabolic stress buffer, many lines of evidence support the role of autophagy in antagonizing cell survival and promoting cell death and apoptosis. Autophagy has an important role in cancer, and inhibition of this cellular process has been proposed to contribute to HCC progression; therefore, it is a potentially very important target for liver cancer prevention and treatment.

Delta-9-THC and JWH-015, a cannabinoid receptor 2-selective agonist, reduced the viability of the human hepatocellular liver carcinoma cell line (HepG2) and hepatocellular carcinoma cells (HuH-7); an effect that relied on the stimulation of CB2 receptor. Delta-9-THC- and JWH-015-induced autophagy relies on tribbles homolog 3 (TRB3) upregulation and subsequent inhibition of the serine-threonine kinase Akt/mammalian target of rapamycin C1 axis and adenosine monophosphate-activated kinase (AMPK) stimulation. Pharmacological and genetic inhibition of AMPK upstream kinases supported that calmodulin-activated kinase β was responsible for cannabinoid-induced AMPK activation and autophagy. In vivo studies revealed that delta-9-THC and JWH-015 reduced the growth of HCC subcutaneous xenografts, an effect that was not evident when autophagy was genetically or pharmacologically inhibited in those tumors. Moreover, cannabinoids also inhibit tumor growth and ascites in an orthotopic model of HCC xenograft [86].

Delta-9-THC inhibits tumor growth in animal models of cancer, an effect that relies on, in part, the upregulation of several endoplasmic reticulum stress-related proteins, including the pseudokinase tribbles homolog-3 (TRIB3), which leads in turn to the inhibition of the AKT/mTORC1 axis and the subsequent stimulation of autophagy-mediated apoptosis in tumor cells. Cells derived from Trib3-deficient mice were used to investigate the precise mechanisms by which TRIB3 regulates the anticancer action of delta-9-THC.

RasV(12)/E1A-transformed embryonic fibroblasts derived from Trib3-deficient mice are resistant to delta-9-THC-induced cell death. Genetic inactivation of this protein also abolishes the ability of delta-9-THC to inhibit the phosphorylation of AKT and several of its downstream targets, including those involved in the regulation of the AKT/mammalian target of rapamycin complex 1 (mTORC1) axis. The data indicate that delta-9-THC-induced TRIB3 upregulation inhibits AKT phosphorylation by regulating the accessibility of AKT to its upstream activatory kinase, the mammalian target of rapamycin complex 2; mTORC2. Finally, Tumors generated by inoculation of Trib3-deficient cells in nude mice are resistant to delta-9-THC anticancer action. The observations support the crucial role that TRIB3 plays in delta-9-THC antineoplastic activity [131].

Cannabinoids exert antiproliferative effects in hepatocellular carcinoma (HCC) cells. The cannabinoids increased the activity and intracellular level of PPAR γ mRNA and protein, which was abolished by the PPAR γ inhibitor GW9662. The PPAR γ -activated pathway contributed to the antitumor effect of delta-9-tetrahydrocannabinol and JWH-015 against HepG2 and HUH-7 HCC cells.

Genetic ablation with small interfering RNA (siRNA) and pharmacological inhibition of PPAR γ decreased the cannabinoid-induced cell death and apoptosis. Likewise, GW9662 blocked the antitumoral action of cannabinoids in xenograft-induced HCC tumors in mice. In addition, PPAR γ knockdown with siRNA caused accumulation of the autophagy markers LC3-II and p62, indicating that PPAR γ is necessary for the autophagy flux promoted by cannabinoids. Interestingly, down-regulation of the endoplasmic reticulum stress-related protein tribbles homolog 3 (TRIB3) markedly reduced PPAR γ expression and induced p62 accumulation, which was counteracted by overexpression of PPAR γ in TRIB3-knocked down cells [132].

The influence of delta-9-THC to modulate irinotecan (CPT-11)-induced toxicity in Male Wistar rats were treated either with a single irinotecan intraperitoneal dose, 100 mg/kg body-weight (b.w.) or with irinotecan in combination with delta-9-THC (7 mg/kg b.w., p.o., administered repeatedly for one, three and 7 days) was examined. Serial blood samples were obtained up to 7 days after dosing and were analyzed for complete blood count, liver enzymes, creatinine, inflammatory markers, and lipid status. Serial urine samples were collected in the first 24 h to monitor the time course of delta-9-THC metabolite 11-nor-9-carboxy-delta-9-THC (THC-COOH) excretion with concomitant irinotecan treatment or without.

Both irinotecan and irinotecan + delta-9-THC administration produced moderate leukopenia. However, a greater decrease in leukocyte count was observed in the irinotecan + delta-9-THC treated compared to irinotecan, indicating higher cytotoxic effects in combined treatment. Irinotecan treatment induced elevation of aspartate aminotransferase (AST) in rats without diarrheal symptoms and without an increase in circulating proinflammatory mediators. The elevation of AST was not observed in the irinotecan + delta-9-THC group. The median creatinine-corrected urinary THC-COOH concentration was higher in the irinotecan + delta-9-THC group compared to the delta-9-THC-only group in a time-dependent manner, suggesting a possible early interaction between cannabinoids and irinotecan [133].

Both cholangiocarcinoma cell lines and surgical specimens from cholangiocarcinoma patients expressed cannabinoid receptors. Delta-9-THC inhibited cell proliferation, migration, invasion, and induced cell apoptosis; decreased actin polymerization and reduced tumor cell survival in anoikis assay pMEK1/2 and pAkt demonstrated a lower extent than untreated cells [134].

Endometrial and Cervical Cancers

The main elements of the endocannabinoid system are expressed in human female reproductive tissues, such as the ovaries, Fallopian tubes, uterus, and placenta. They have also been localized to areas of the hypothalamus responsible for producing hormones, which act through the hypothalamic–pituitary–gonadal (HPG) axis to control many reproductive functions. In the human ovaries, CB1 and CB2 are expressed in the granulosa cells of primordial, primary, secondary, and tertiary follicles, as well as in theca cells of secondary and tertiary follicles, with the highest expression at the time of ovulation. Additionally, both receptors are expressed in the corpus luteum and corpus albicans, even in the absence of pregnancy. Moreover, FAAH is present within theca cells, but NAPE-PLD appears only in the granulosa of secondary and tertiary follicles, the corpus luteum, and corpus albicans.

Tissue samples were collected from 18 patients undergoing surgical treatment for endometrial adenocarcinoma and 16 healthy age-matched controls to test the expression of the endocannabinoid system in endometrial cancer. A human endometrial cancer cell line (AN3CA) was used and transiently transfected with a plasmid containing the cDNA for the endocannabinoid receptor CB2 to evaluate the physiological role of the endocannabinoid system. The cells were incubated for 48 h with the agonist JWH133 or the antagonist SR144528 of CB2 24 h after transfection. Cell proliferation was measured by the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide formazan assay.

The endocannabinoid system plays an important role in human endometrial carcinoma, and modulation of CB2 expression accounts for a tumor-suppressive effect. In human endometrial carcinoma biopsies, the expression of the CB2 receptor and the levels of its ligand, 2-arachidonoylglycerol, increased, whereas monoacylglycerol lipase, an enzyme responsible for 2-arachidonoylglycerol degradation, was downregulated. Immunohistochemical analysis revealed that CB2 was overexpressed only in malignant endometrial cells. CB2-overexpressing AN3CA cells produced a significant reduction in cell vitality compared with parental AN3CA cells. Incubation with the selective CB2 antagonist SR144128 restored the viability of CB2-overexpressing cells to that of untransfected cells [135].

Delta-9-tetrahydrocannabinol has been reported to impact proliferative and migratory activities during the impairment of cancer progression. A study examined cell migration in response to delta-9-THC using Transwell assays. Using western blot analysis, the levels of cannabinoid receptors in endometrial cancer (EC) tissues and the pathways leading to the inhibition of cell migration by delta-9-THC on human EC cells were detected. Furthermore, delta-9-THC inhibited EC cell viability and motility by inhibiting epithelial-mesenchymal transition (EMT) and down-regulating matrix metalloproteinase-9 (MMP-9) gene expression in aggressive human EC cells.

Endometrial cancer can be classified into types I and II tumors in general. Moreover, with regard to different disease stages, the therapies of EC are various. At the early stage of most EC patients, only surgery is considered the effective

treatment method. As a result of tumor recurrence and metastasis, even under surgical resection plus systemic chemotherapy, the prognosis is still poor for newly diagnosed EC patients.

Cannabinoids exert most of their actions by binding to specific G protein-coupled receptors. The CB1 receptor is widely distributed in the central nervous system, where they mediate psychoactive effects, although it has also been detected in reproductive organs such as the uterus and testis. Cannabinoids act as anti-inflammatory agents and suppress the antitumor immune response. Several preclinical studies suggest delta-9-THC shows anticancer performance *in vitro* against breast cancer, lung carcinoma, skin carcinoma, pancreatic cancer, and prostate carcinoma [136].

Cannabinoid receptors are overexpressed in skin, breast, and lung cancers. The expression of cannabinoid receptors in EC was investigated in normal endometrium and paired adjacent normal tissues using PCR and western blot. The data indicated that CB1R and CB2R were overexpressed in EC tissues compared with the normal endometrium. Moreover, the expressions of CB1R and CB2R were positively correlated with VIM, the marker of mesenchymal cells.

ATP-binding cassette (ABC) transporters are highly implicated in the resistance of cancers to anticancer drugs. Delta-9-THC increased the accumulation of Fluo3 and Vincristine in ovarian cancer cells that over-expressed ABCC1, both of which are substrates for the transporter.

The ATP-binding cassette transporter ABCC1 or multidrug resistance-related protein 1 (MRP1) is implicated in Phase II metabolism and multidrug resistance as it effluxes substrate anticancer drugs. As cannabinoids inhibit two related ABC transporters, P-glycoprotein and ABCG2, an investigation was conducted on whether they also inhibit ABCC1. The cannabinoids enhanced the intracellular accumulation of two ABCC1 substrates, Fluo3, and vincristine, in ovarian carcinoma cells over-expressing ABCC1 (2008/MRP1) with a rank order of potency: cannabidiol > cannabinol > delta-9-tetrahydrocannabinol. Cannabinoid inhibition of ABCC1 was confirmed using insect cell membrane MRP1 ATPase assays [137].

In another study, a different multidrug transporter, ABCG2, was shown to be inhibited by delta-9-THC, where delta-9-THC exposure increased the accumulation of mitoxantrone, an ABCG2 substrate. CBN, CBD, and delta-9-THC increased the intracellular accumulation of the Abcg2/ABCG2 substrate, mitoxantrone, in an over-expressing cell line. The delta-9-THC metabolite, (–)-11-nor-9-carboxy-delta-9-THC, was much less potent. The cannabinoids inhibited both basal and substrate-stimulated ATPase activity of human ABCG2. Cannabinoid cytotoxicity occurred without known cannabinoid cell surface receptors and only at concentrations higher than those required for Abcg2/ABCG2 inhibition. Subtoxic concentrations of the cannabinoids resensitized the overexpressing cell line to the cytotoxic effect of Abcg2/ABCG2 substrates, mitoxantrone, and topotecan; this occurred in the absence of any effect on ABCG2 expression [138].

In cervical cancer cells, one study found that delta-9-THC decreased invasion via the increased expression of tissue inhibitor of MMP-1 (TIMP-1). Matrigel-coated and uncoated Boyden chambers were used to quantify invasiveness and migration,

respectively, of human cervical cancer (HeLa) cells that had been treated with cannabinoids (the stable anandamide analog R(+)-methanandamide [MA] and the phytocannabinoid delta-9-tetrahydrocannabinol) in the presence or absence of antagonists of the CB1 or CB2 cannabinoid receptors or of transient receptor potential vanilloid 1 or inhibitors of p38 or p42/44 mitogen-activated protein kinase pathways. Reverse transcriptase-polymerase chain reaction and immunoblotting were used to assess the influence of cannabinoids on the expression of matrix metalloproteinases (MMPs) and endogenous tissue inhibitors of MMPs (TIMPs). The role of TIMP-1 in the anti-invasive action of cannabinoids was analyzed by transfecting HeLa, human cervical carcinoma (C33A), or human lung carcinoma cells (A549) cells with siRNA targeting TIMP-1. All statistical tests were two-sided [97].

Without modifying migration, MA and delta-9-THC caused a time- and concentration-dependent suppression of HeLa cell invasion through Matrigel accompanied by increased expression of TIMP-1. At the lowest concentrations tested, MA (0.1 μ M) and THC (0.01 μ M) led to a decrease in invasion (normalized to that observed with vehicle-treated cells) of 61.5% (95% CI = 38.7–84.3%, $p < 0.001$) and 68.1% (95% CI = 31.5–104.8%, $p = 0.0039$), respectively. The stimulation of TIMP-1 expression and suppression of cell invasion was reversed by pretreatment of cells with antagonists to CB1 or CB2 receptors, with inhibitors of MAPKs, or, in the case of MA, with an antagonist to TRPV1. Knockdown of cannabinoid-induced TIMP-1 expression by siRNA led to a reversal of the cannabinoid-elicited decrease in tumor cell invasiveness in HeLa, A549, and C33A cells.

Breast Cancer

Data from preclinical studies suggest cannabinoids may be beneficial for treating the best-known breast cancer subtypes. Taking into account the expression of specific molecular biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), breast cancer can be divided into four subtypes, luminal A, luminal B, human epidermal growth factor receptor 2 positive (HER2⁺) or triple-negative (TNBC). Luminal tumors are the most common, representing 60–73% of all cases.

Luminal A is a low-risk breast cancer subtype, being low grade and presenting the most favorable prognosis. This subtype has a high ER-regulated gene expression and a high PR expression and does not express HER2, being responsive to endocrine therapy. On the other hand, luminal B breast cancers are high grade and, thus, more aggressive and associated with a worse prognosis than luminal A tumors. HER2 expression is generally associated with this subtype, and the tumors are usually positive for the expression of ER and PR. In turn, HER2⁺ or nonluminal breast tumors represent 12–20% of all breast tumors. This subtype is typically high grade, associated with high aggressiveness and poor prognosis, and characterized by an overexpression of HER2 and the lack of expression of ER and PR.

TNBC accounts for approximately 15–20% of all breast cancers, is typically high grade, presents a high proliferation index, and is associated with a high rate of local and distant recurrence and, consequently, with a poor prognosis, usually being the most aggressive; the absence of ER, PR, and HER2 expression characterizes it. Around 80% of all TNBCs are basal-like, which means that the tumors are enriched in cells expressing characteristic genes of normal basal cells.

A study on breast cancer cells found that CB1 receptor activation induced cell-cycle arrest at the G1-S transition by inhibiting adenylyl cyclase and the cAMP signaling cascade. Given that the phosphorylation of protein kinase A reduces RAF1 expression, cannabinoid receptors block the inhibition of RAF1. As a result, the RAF1–MEK–ERK pathway is stimulated. Corresponding research in thyroid epithelioma cells transformed with the Kirsten rat sarcoma (*KRAS*) oncogene showed that CB1 receptor activation also blocks the cell cycle at the G1-S transition. Similar results of cell-cycle arrest following cannabinoid receptor activation have been reported in skin and prostate cancer cells. In those studies, growth-factor-receptor signaling was inhibited.

Evidence has revealed that the ECS is altered in breast cancer cases and intimately associated with tumor aggressiveness. Endocannabinoid concentrations, expression levels of CBs, and enzymes responsible for endocannabinoid metabolism are typically associated with cancer aggressiveness, reinforcing their involvement in cancer development. It is known that CB2 is overexpressed in breast cancer and that CB1 is present in significantly lower quantities. CB2 expression is mainly observed in HER2⁺ tumors and detected in 90% of all HER2⁺ tumors [46]. Overexpression of CB2 is linked to a poor prognosis.

A correlation has been established between CB2 expression and tumor aggressiveness, as mRNA CB2 levels were higher in ER[−]/PR[−] tumors than in ER⁺/PR⁺ tumors and in HER2⁺ tumors than in HER2[−] and high-grade histological tumors than in low-grade histological tumors [47]. Conversely, CB2 expression on ER⁺ and ER[−] tumors is associated with a better prognosis. Concerning endocannabinoids, in breast cancer cases, the levels of N-acylphosphatidylethanolamine, the AEA precursor, and MAGL levels have been observed, mainly in ductal carcinomas.

Most studies on breast cancer were performed on TNBC models; it has been established that the sensitivity of human breast cancer cell cultures to cannabinoids is correlated with their aggressiveness, being ER[−] cell lines more sensitive than ER⁺ cells. CBD is the most studied cannabinoid in TNBC. It has been reported that it reduces the proliferation of MDA-MB-231 cells through the direct activation of TRPV1 receptors and possibly through other yet uncharacterized CBD targets. Cannabidiol also induces apoptosis in this cell model by involving CB1, CB2, and TRPV1 receptors. The effect is mediated by endoplasmic reticulum stress and inhibition of the AKT/mTOR pathway, which ultimately culminates in autophagy and mitochondria-driven apoptosis [3].

The induction of apoptosis by CBD in MDA-MB-231 cells was verified, where CBD inhibited cell survival and induced apoptosis, favored by the interplay among PPAR, mTOR, and cyclin D1. In contrast, regarding the MDA-MB-231 cell line, a study showed that delta-9-THC promotes the proliferation of the cells [48].

Cannabidiol's antiproliferative, antimigratory, and anti-invasive properties through the inhibition of EGF/EGFR signaling in several TNBC cell lines, including SUM159, 4T1.2, and SPC2 cells, also demonstrated; the effects were mediated by downstream inhibition of the Raf-1/MEK/ERK, NF- κ B, and AKT signaling pathways.

Cannabidiol effects on cell proliferation were also verified in xenografts generated from MDA-MB-231 cells in immune-deficient mice and orthotopic xenografts generated from 4T1 cells in syngeneic BALB/c mice. In both cases, CBD reduced tumor growth but acquired resistance to CBD was developed for the latter. CBD also impairs the metastatic potential of MDA-MB-231 and 4T1 cells, probably by downregulation of Id-1, which ERK mediates. The effects exerted by ERK signaling depend on the duration of the stimulus since sustained ERK activation causes cell growth inhibition, while short-term stimulation promotes cell growth.

ROS plays a key role in the breast cancer microenvironment, promoting the differentiation of neighboring cells, including fibroblasts that secrete growth factors, cytokines, and metalloproteinases, leading to tumor development and growth [49]. It has been demonstrated in MCD-MB-231 cells that CBD induces the production of reactive oxygen species, which can downregulate Id-1 activity, corroborating previously mentioned apoptotic effects. A dependence on increased ROS levels for the survival of TNBC cell models has been verified.

The dependence on ROS levels is particularly high in TNBC. Several antioxidant and mitochondria-targeted therapies have been suggested for breast cancer. The minor phytocannabinoids CBN and CBG also showed interesting results in TNBC. In MCD-MB-231 and MDA-MB436 cells, both compounds reduced cell viability and cell migration through a process probably involving a decrease in Id-1 expression. Combinations of minor and major cannabinoids have also been evaluated in TNBC [50].

Besides the actions of the phytocannabinoids, synthetic cannabinoids have also demonstrated interesting effects on TNBC. In two *in vivo* models, a xenograft-based and a PyMT genetically engineered model of TNBC, the synthetic cannabinoid JWH-133 provoked a significant reduction in tumor growth and inhibited angiogenesis. Antimigration effects were induced by several synthetic cannabinoids, Met-F-AEA, WIN 55212-2, JWH-133, and JWH-015, in MDA-MB-231 cells. The effects were mediated by CB1, with inhibition of the focal adhesion kinase (FAK)/Src and RhoA-ROCK pathways, and by CB2, along with COX-2/PGE2 axis inhibition, or through inhibition of the ERK pathway and cytoskeletal adhesion and stress fiber formation. Met-F-AEA, WIN 55212-2, and JWH-133 induce cell-cycle arrest.

The studies suggest that the interaction of synthetic cannabinoids with CBs reduces the metastatic potential of TNBCs, a behavior already identified for lung metastasis formation [51]. Cannabinoids exert antiproliferative and antimetastatic actions on TNBC models *in vivo* and *in vitro*, mainly by the induction of apoptosis and autophagy via CB activation and modulation of several signaling pathways involved in cell proliferation, such as AKT/mTOR and EGF/EGFR.

In relation to HER2⁺ breast cancer, overexpression of CB2 has been verified, which is associated with a poor prognosis. In HER2⁺ breast cancer models, the anti-tumor effects are mediated by CB2 activation, which is why CB2-directed therapy should be effective in the growth inhibition of those tumors.

Cannabinoids represent an attractive potential therapy for this subtype of breast cancer. The most prominent study regarding HER2⁺ breast cancer and cannabinoids was conducted in MMTV-neu mice, a model for the study of HER2⁺ tumors. In that study, it was revealed that delta-9-THC impaired tumor growth, angiogenesis, and the formation of metastasis through the induction of apoptotic cell death and inhibition of AKT, effects that were reproduced with JWH-133, a selective CB2 agonist [52].

Regarding luminal tumors, the most frequently diagnosed are ER⁺ tumors. A study involving MCF-7 and EFM-19 cells demonstrated that AEA inhibits basal-, prolactin- and nerve growth factor (NGF)-induced proliferation through CB1 activation. Cell-cycle arrest and activation of the Raf-1/ERK/MAPK pathway through the inhibition of adenylyl cyclase have been verified since the sustained activation of the ERK pathway leads to the downregulation of prolactin and NGF receptors. It was shown that AEA reduces cell proliferation via CB1 activation in MCF-7 cells. On the other hand, delta-9-THC induced a similar effect on EVSA-T cells, though the CB2 receptor mediated this effect through CDK1 inhibition and activation of the transcription factor JunD [53].

In relation to minor phytocannabinoids, CBG decreased MCF-7 cell proliferation, while CBN stimulated proliferation in the same cell line. There are studies on MCF-7 cells showing that delta-9-THC is also able to promote cell proliferation, an effect that might involve Act1 and Erb2. Regarding CBD, it was demonstrated in the T-47D cell line that this phytocannabinoid inhibits cell survival and promotes apoptosis due to interplay among PPAR, mTOR, and cyclin D1, probably through a mechanism similar to the one observed in TNBC cases [54]. It was also verified that CBD could induce endoplasmic reticulum stress, which leads to cell death, in MCF-7 cells through the activation of TRPV1 and the consequent increase in Ca²⁺ and ROS levels.

In silico study demonstrated that delta-9-THC analogs can bind to ER β and probably lead to its activation; this represents an important advantage for ER⁺ breast cancer treatment since the receptor is associated with antitumor effects [55]. It has been verified that delta-9-THC prevents estradiol-induced proliferation in MCF-7 cells in an ER α -independent manner. In MCF-7aro cells, AEA, CBD, and delta-9-THC reduce cell viability and disrupt cell-cycle progression; AEA and delta-9-THC caused apoptotic cell death, while CBD induced autophagy to promote apoptosis. Additionally, AEA and CBD inhibit the enzyme aromatase; data are in line with studies where it was demonstrated that cannabinoids inhibit aromatase in human placental microsomes [56].

It was demonstrated that tamoxifen and other SERMs act as CB1 and CB2 modulators and that tamoxifen in combination with delta-9-THC or a *Cannabis* preparation caused additive antiproliferative responses in T47D cells. It was also demonstrated that tamoxifen binds to CBs, acting as an inverse agonist. Furthermore,

it was shown that delta-9-THC upregulates ER β , a beneficial effect for ER $^{+}$ breast cancer treatment. In MCF-7aro cells, the three cannabinoids reduced aromatase and ER α levels while only AEA and CBD upregulated ER β levels – data that reinforces the interplay between the endocannabinoid system and estrogen signaling.

Additionally, combinations of major and minor cannabinoids evaluated on MCF-7 cells revealed proapoptotic effects. In ER $^{+}$ breast cancer, cannabinoids can inhibit cell growth and avoid metastatic development. The effects are typically associated with cell-cycle arrest, apoptosis, and autophagy through the involvement of CB1 and CB2 and the modulation of survival pathways, such as the mTOR and Raf-1/ERK/MAPK pathways.

Cannabidiol of both synthetic and natural origin, syn CBD and nat CBD, respectively, are commercially available, while cannabidibutol (CBDB) and cannabidiphlorol (CBDP) have been isolated from the *Cannabis* plant. The stereoselective synthesis of the compounds allowed absolute stereochemistry by comparison of their spectroscopic and optical properties. Nat CBD and syn CBD are identical molecules; however, high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) analyses have shown that nat CBD contains small amounts (0.1–0.5%, w/w) of two impurities corresponding to CBD propyl and butyl homologs, cannabidivarin (CBDV) and CBDB, respectively [50].

The CBD homologs differ only by the length of the alkyl side chain on the resorcinyl moiety. The alkyl side chain length could dramatically affect the affinity for the biological targets, as has been demonstrated for delta-9-THC, a CBD isomer. CBDB has a linear C4 (butyl) side chain, both nat CBD and syn CBD have a C5 (pentyl) chain, and CBDP has a C7 (heptyl) chain. The four cannabinoids present similar physicochemical and spectroscopic properties; UV and FT-IR spectra are perfectly superimposable, while Nuclear Magnetic Resonance (NMR) spectroscopy shows a difference only in the signal corresponding to the alkyl chain; HRMS data highlight a perfect match of all m/z of the fragments with the difference only in the number of methylene units.

The biological effects of phytocannabinoids are due to their affinity to CB1 and CB2 receptors. The expression was investigated in vitro using a panel of breast cancer cell models estrogen and progesterone receptor positive: MCF-7, MDA-MB-361, and estrogen and progesterone receptor-negative MDA-MB-231. Qualitative analysis of cannabinoid targets in vitro showed that the three breast cancer cells express both CB1 and CB2 receptors.

Before investigating the activity of the CBD homologs on cancer cell lines, their cytotoxicity was screened on two different epithelial and one mesenchymal breast cancer cell line, MCF-7, MDA-MB-361, and MDA-MB-231, using the metabolism-dependent MTT viability assay. The cells were treated with concentrations (from 1 to 100 μ M) of nat CBD, syn CBD, CBDB, and CBDP, for 24 h. All of the CBD homologs inhibited the viability of breast cancer cell lines with lower activity in MCF-7 and MDA-MB-361 cells, and a higher effect was observed in MDA-MB-231.

The striking behavior of CBD homologs treatments was the occurrence of massive cytoplasmic vacuolation in the epithelial MCF-7 cells; vacuoles appeared after 2 h of treatment, and their number and size increased after 24 h. The same changes

were less pronounced in mesenchymal MDA-MB-231 cells, while no vacuolation occurred in the epithelial MDA-MB-361 cells.

An increase in oxidative stress has been detected in cancer cells after CBD treatment due to a massive increase in ROS production that leads to the activation of apoptosis and autophagy. ROS levels in MCF-7 cells were treated with 10 μ M nat CBD, syn CBD, CBDB, and CBDP for 24 h using the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) staining. The antioxidant N-acetyl cysteine (NAC) was used to counteract ROS production.

A significant increase in intracellular ROS levels was observed in MCF-7 cells treated with CBD homologs. The NAC treatment in association with CBD homologs revert the relative fluorescence intensity to a value lower than the control due to a remarkable ROS scavenging effect.

Two mitochondria stains, Mitotracker Red and MitoTracker Green, were used to establish the correlation between ROS production and impaired mitochondrial functions. The first is an indicator of mitochondrial membrane potential that selectively stains active mitochondria, while MitoTracker Green allows the detection of the mitochondrial morphology and mass. When MCF-7 cells were exposed to 10 μ M CBDB or CBDP for 24 h, the Mitotracker red fluorescence intensity dramatically dropped compared to the control. Similar results were obtained with nat CBD and syn CBD. The MitoTracker Green fluorescence signal remained similar in control and treated cells.

The observations suggest that the treatment with CBD homologs alters mitochondrial functionality without an appreciable decrease in the mitochondrial mass. The loss of the mitochondrial function was also accompanied by a substantial decrease in ATP, whose levels dropped by approximately 35–40% compared to controls in MCF-7 cells treated with all CBD homologs [57].

Cannabidiolic acid downregulates the mRNA expression of cyclooxygenase-2 (COX-2) in highly aggressive MDA-MB-231 human breast cancer cells. The molecular mechanism(s) underlying the cannabidiolic acid suppression of COX-2 has yet to be elucidated thoroughly. In MDA-MB-231 cells, COX-2 expression is regulated by the transcriptional activity of activator protein-I (AP-1); composed of a heterodimer of c-Fos and c-Jun. AP-1-mediated transcriptional activity was inhibited by cannabidiolic acid in a dose-dependent manner. The expression of c-fos was maintained at markedly lower levels (0.035) than basal c-jun expression levels (1.0), implicating c-fos as a limiting factor in the regulation of COX-2. Analyses indicated that cannabidiolic acid abrogated the expression of c-fos mRNA without affecting c-jun, indicating that cannabidiolic acid abolishes the expression of COX-2 by interfering with AP-I activity in MDA-MB-231 cells [91].

Bone Cancer

Skeletal complications are common causes of morbidity in patients with primary bone cancer and bone metastases. The type 2 cannabinoid (CB2) receptor is implicated in cancer, bone metabolism, and pain perception. Studies have revealed the role of CB2 in regulating tumor-bone cell interactions. They suggest that agents that target CB2 in the skeletal system have potential efficacy in reducing skeletal complications associated with cancer [58]. Development and testing of peripherally acting tumor-, CB2-selective ligands in preclinical models of metastatic cancer will advance knowledge about the basic mechanism(s) by which the endocannabinoid system regulates cancer metastasis, stimulate the development of a safer *Cannabis*-based therapy for the treatment of bone cancer and provide powerful tools to assess the science and therapeutic potential of cannabinoid-based therapy.

Cannabinoid receptors play an important role in regulating bone mass and bone turnover. Studies have shown that young mice lacking type 1 cannabinoid receptor (CNR1^{-/-}) had increased bone mass and were resistant to ovariectomy-induced bone loss. Other reports that type 2 cannabinoid receptor knockout mice (CNR2^{-/-}) develop age-related osteoporosis. CNR2^{-/-} mice had normal bone mass and bone turnover at 3 months of age. However, following ovariectomy, the mice were partially protected from bone loss because of a mild osteoclast formation and bone resorption defect. In keeping with this, studies in vitro showed that RANKL-stimulated bone marrow cultures from CNR2^{-/-} mice had fewer osteoclasts than cultures from wild-type littermates. The CNR2-selective antagonist/inverse agonist AM630 inhibited osteoclast formation in wild-type bone marrow cultures in vitro and prevented ovariectomy-induced bone loss in wild-type mice in vivo.

In contrast, osteoclast cultures from CNR2^{-/-} mice were resistant to the inhibitory effects of AM630 at low concentrations, and CNR2^{-/-} ovariectomized mice did not respond to its protective effects at low doses, consistent with a CNR2-mediated effect. The results indicate that CNR2 regulates bone loss under conditions of increased bone turnover, such as ovariectomy, by affecting osteoclast differentiation and function.

CNR2-deficient mice developed accelerated age-related osteoporosis, and by 12 months of age, they significantly reduced osteoblast numbers and bone formation. In contrast, osteoclast numbers remained comparable to wild-type littermates. In agreement with this, osteoblasts derived from the bone marrow of CNR2^{-/-} mice had reduced PTH-stimulated alkaline phosphatase activity and the ability to form bone nodules compared with wild-type cultures. The CNR2-selective agonist, HU308, stimulated bone nodule formation in wild-type calvarial osteoblast cultures in vitro and reversed the ovariectomy-induced bone loss in wild-type mice in vivo. HU308 had blunted effects on bone nodule formation in cultures from CNR2^{-/-} mice and no significant effects on ovariectomy-induced bone loss in CNR2^{-/-} mice, indicating a CNR2-mediated effect.

The studies demonstrate that CNR2 protects against age-related bone loss by enhancing osteoblast differentiation and bone formation. Type 2 cannabinoid

receptors protect from bone loss by maintaining bone remodeling at balance. Additionally, type 2 cannabinoid receptor agonists produce evidence of anabolic activity, whereas antagonists/inverse agonists show evidence of antiosteoclastic activity in vitro and in vivo.

Leukemia

In a leukemia model, delta-9-THC had an IC_{50} of 13 μ M, and CBD had an IC_{50} of 8 μ M. When delta-9-THC and CBD were combined in a 1:1 ratio, the IC_{50} was decreased to 4 μ M. They then combined delta-9-THC/CBD combinations with other anticancer agents and, in some cases, observed synergistic effects. Most importantly, they observed that equivalent anticancer effects can still result from lower concentrations of combined agents compared to each agent alone at a higher concentration. Combinations of delta-9-THC and CBD also slightly sensitized leukemic cells to anticancer agents, vincristine, and cytarabine. The beneficial effects of combined therapy with cannabinoids and chemotherapeutic agents depended on the administration sequence; increased cell death was observed when cannabinoids were administered after chemotherapy [139].

The interactions between delta-9-THC and antileukemia therapies revealed synergistic interactions between delta-9-THC and the cytotoxic agents in leukemic cells. Exposure of cells to sublethal delta-9-THC (1 μ M) sensitized cells to the cytotoxic agents by reducing $IC(50)$ values by approximately 50%. Sensitization appeared to depend upon the ability of delta-9-THC to downregulate phosphorylated ERK, as cells dominantly expressive of MEK were not sensitized to the cytotoxic drugs by equimolar amounts of delta-9-THC [140].

Inhibition of the differentiation blockage in acute myeloid leukemia is the most successful target in leukemia therapy. Dronabinol, the enantiomer (–)-*trans*- Δ^9 -tetrahydrocannabinol approved by the FDA for conditions like HIV/AIDS-induced anorexia and chemotherapy-induced nausea and vomiting, was found to inhibit the differentiation blockage in acute leukemia cells in vitro and that O-linked- β -*N*-acetyl glucosamine transferase was fundamental to this process. This data supports the notion that cannabinoids combined with other therapeutic agents may enhance the overall anticancer effects.

Dronabinol also reduced cell viability and proliferation and induced apoptosis in an array of acute leukemia cell lines and native leukemic cells cultured ex vivo. Dronabinol's proapoptotic effects in patient-derived leukemic cells correlated with the expression of CB1 and CB2 receptors, where the presence of the receptors was necessary to see apoptotic effects. The response to delta-9-THC treatment was higher in leukemia blasts derived from a lymphatic lineage and expressed lymphatic markers [141].

The effect of delta-9-THC on the upstream and downstream events that modulate the extracellular signal-regulated kinase (ERK) module of mitogen-activated protein kinase pathways, primarily in human Jurkat leukemia T cells, was investigated.

The data indicated that delta-9-THC downregulated Raf-1/mitogen-activated protein kinase/ERK kinase (MEK)/ERK/RSK pathway leading to translocation of Bad to mitochondria. Delta-9-THC also decreased the phosphorylation of Akt. However, no significant association of Bad translocation with phosphatidylinositol 3-kinase/Akt and protein kinase A signaling pathways was noted when the treated cells were examined in relation to the phosphorylation status of Bad by Western blot and localization of Bad to mitochondria by confocal analysis. Furthermore, THC treatment decreased the Bad phosphorylation at Ser(112). However, it failed to alter the phospho-Bad on-site Ser(136) level that has been reported to be associated with the phosphatidylinositol 3-kinase/Akt signal pathway. Jurkat cells expressing a constitutively active MEK construct were found to be resistant to delta-9-THC-mediated apoptosis and failed to exhibit decreased phospho-Bad on Ser(112) as well as Bad translocation to mitochondria. The use of Bad small interfering RNA reduced the expression of Bad in Jurkat cells leading to increased resistance to delta-9-THC-mediated apoptosis [142].

Human Jurkat leukemia cell lines with defects in intrinsic and extrinsic signaling pathways were used to elucidate the mechanism of apoptosis induced by Delta-9-tetrahydrocannabinol. Jurkat cells deficient in FADD or caspase-8 were partially resistant to apoptosis, while dominant-negative caspase-9 mutant cells were completely resistant to apoptosis. The use of caspase inhibitors confirmed this. Furthermore, overexpression of Bcl-2 rendered the cells resistant to delta-9-THC at early times but not upon prolonged exposure. Delta-9-THC treatment led to the loss of Deltapsi(m) in both wild-type and FADD-deficient Jurkat cells, thereby indicating that the delta-9-THC-induced intrinsic pathway was independent of FADD. Delta-9-THC treatment of wild-type Jurkat cells caused cytochrome C release and cleavage of caspase-8, -9, -2, -10, and Bid. Caspase-2 inhibitor blocked delta-9-THC-induced caspase-3 in wild-type Jurkat cells but not loss of Deltapsi(m) [143].

The transporter, P-glycoprotein (P-gp), confers multiple drug resistance (MDR) by effluxing a diverse array of anticancer agents. A study examined the effect of cannabinoids on P-gp. Unlike the known P-gp inhibitor, PSC833, short 1-h exposure to three plant-derived cannabinoids, cannabinol, cannabidiol, and delta-9-tetrahydrocannabinol and the synthetic cannabinoid receptor agonist, WIN55212-2 (WIN), did not inhibit the efflux of the P-gp substrate Rhodamine 123 (Rh123) in either a drug-selected human T lymphoblastoid leukemia cell line (CEM/VLB(100)) or a mouse fibroblast MDR1-transfected cell line (77.1). However, in CEM/VLB(100) cells, prolonged 72 h of exposure to the cannabinoids, delta-9-THC and CBD decreased P-gp expression to a similar extent as the flavonoid curcumin. This correlated with an increase in intracellular accumulation of Rh123 and enhanced sensitivity of the cells to the cytotoxic actions of the P-gp substrate, vinblastine [144].

Cannabinoids increase the apoptotic response in leukemia, supporting the concept that the compounds, particularly CBD, can be valid therapeutic agents. CBD also targets mitochondria and perturbs calcium homeostasis in acute lymphoblastic leukemia. The activity of CBD appears to be mediated by the interaction with G

protein-coupled receptor 55 (GPR55) and the transient receptor potential cation channel subfamily V member 1 (TRPV1), but not with the classical cannabinoid receptors CB1 and CB2.

Extracts from *Cannabis* varieties were characterized utilizing ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS), and their antiproliferative activity was assessed in vitro. The human chronic myelogenous leukemia cell line K562 was chosen to study the mechanism of cell death. The physical properties of the cells were assessed using the Micropipette Aspiration Technique (MAT) [59]. The effects on the cell cycle and cell death were analyzed by flow cytometry. Proteins related to apoptosis were studied by western blotting.

The results indicated that the cannabidiol-rich extract inhibited cell proliferation of the K562 cell line in a dose-dependent manner and induced apoptosis via caspase-3 and -7 activations. A significant decrease in the mitochondrial membrane potential was detected, together with the release of Cytochrome C into the cytosol. The main apoptotic markers were not involved in the mechanism of cell death.

The potential ability of the CBD-rich extracts R1 and D1 to interfere with cell viability was assessed at 24 and 48 h on different human cancer cell lines, including the colon carcinoma HT-29, the glioblastoma U87MG, and the myeloid chronic leukemia K562. After 24 h of treatment, the antiproliferative activity of the R1 extract from raw CBD-type *Cannabis* inflorescences did not alter cell viability except at the highest concentration of 100 µg/mL. The D1 extract obtained from the decarboxylated plant material was active in all cell lines, reaching a significant inhibition of cell viability on K562 and U87MG at a concentration of 70–80 µg/mL. The effect was pronounced after 48 h of treatment for both extracts on all cell lines. K562 cells were the most sensitive to the treatment with R1 and D1. D1 reduced the K562 cell viability by about 50% and 80% at 10 µg/mL and 20 µg/mL, respectively. Imatinib, used as a positive control, produced an IC_{50} of 1 µM on K562 cells after 48 h of exposure. The findings led to the chronic myelogenous leukemia K562 as a suitable model to further characterize the antiproliferative activity of *Cannabis* extracts with a different phytochemical composition.

Other extracts, D2 and D3, with different cannabinoid profiles, were tested for their ability to modulate the K562 cell viability after 48 h of incubation. D1 was more effective than D2 and D3. D1 reached an IC_{50} of 11.6 µg/mL, whereas D2 and D3 produced an IC_{50} of 40.9 and 66.7 µg/mL, respectively. The effect of D1 on K562 cell viability was time-dependent, reaching the maximal effect after 72 h of incubation with an IC_{50} of 9.8 µg/mL. The activity of the D1 extract was assessed on human peripheral blood mononuclear cells (PBMC) to exclude a cytotoxic effect on non-cancer cells. A concentration of 50 µg/mL did not affect the viability of PBMCs after 48 h of exposure.

The activity of pure CBD on K562 decreased the cell viability by 50% at a concentration of 28 µM, whereas the IC_{50} of D1 was 11.6 µg/mL, corresponding to 16 µM in CBD-equivalents. Even if CBD is the main component to which the activity of the extract could be ascribed, other minor compounds can work in a synergic way to enhance the overall antiproliferative activity of D1.

In the presence of oxygen, CBD may be oxidized to monomeric and dimeric hydroxyquinones. The monomeric CBD-hydroxyquinone is known to have high antiproliferative activity. K562 cell lysates treated with D1 were analyzed in UHPLC-HRMS to identify CBD-related compounds formed under oxidative conditions. Only CBD was found in the D1-treated cell lysates, both in the positive and negative ion modes, at a retention time of 16.7 min. No other compound derived from CBD oxidation was detected.

K562 cell-cycle distribution was evaluated to investigate cell-cycle arrest at different time points. At a concentration of 20 µg/mL, flow cytometry analysis revealed that the K562 cell cycle was not affected by the treatment with the CBD-rich decarboxylated extract D1 at 16 and 24 h of exposure. The study indicated that the cannabidiol-rich extract inhibited cell proliferation of the K562 cell line in a dose-dependent manner and induced apoptosis via caspase-3 and -7 activations. A significant decrease in the mitochondrial membrane potential was detected, together with the release of Cytochrome C into the cytosol. The main apoptotic markers were not involved in the mechanism of cell death.

Cannabis and Tobacco

Cannabis smoke is qualitatively similar to tobacco smoke, although it contains up to twice the concentration of carcinogenic polyaromatic hydrocarbons. *Cannabis* is less densely packed than tobacco cigarettes and tends to be smoked without filters to smaller butt sizes, leading to higher concentrations of smoke inhaled. Smokers of *Cannabis* inhale more deeply and hold their breath for longer, facilitating the deposition of the carcinogenic products in the lower respiratory tract. Those factors are likely to be responsible for the fivefold greater absorption of carbon monoxide from a *Cannabis* joint compared with a tobacco cigarette of similar size despite similar carbon monoxide concentrations in the smoke inhaled.

Studies have revealed precancerous histological and molecular abnormalities in the respiratory tracts of *Cannabis* smokers, and the carcinogenic effects of *Cannabis* smoke have been demonstrated in vitro and in vivo animal models [29]. Conversely, there is also evidence that delta-9-tetrahydrocannabinol may have anticarcinogenic effects. Epidemiological evidence for an association between *Cannabis* and lung cancer has not provided a clear answer. Case series have suggested a causative role of *Cannabis* in lung cancer in young adults.

Published case-control studies have indicated both the presence and absence of an association. However, they have been limited by the inability to quantify use, confounding with combined *Cannabis* and tobacco use, studies being undertaken in populations in which users may have serious legal consequences resulting in potential information bias and poor response rates.

New Zealand represents a unique country in which to study the association between *Cannabis* and respiratory tract cancer. New Zealand has a high rate of *Cannabis* use, and it is rarely mixed with tobacco within the joint, as is the custom

in other countries. New Zealand has among the highest lung cancer rates worldwide; with the indigenous Maori population having the highest incidence of any ethnic group [30]. Those circumstances provided the opportunity to undertake a case-control study investigating the association between lung cancer and *Cannabis* use in young people.

Patients with confirmed lung cancer aged 55 years and under at the time of diagnosis were identified from hospital databases and the New Zealand Cancer Registry. Cases were a mixture of prevalent and incident cases of lung cancer. Subjects were excluded if they had lung metastasis from a distant primary other than the lung, carcinoid, or melanoma histological diagnosis. Age at diagnosis, anatomical location of the malignancy, and histological type were collected for cases. Controls without lung cancer were randomly selected from the electoral roll, and frequency was matched in 5-year age groups and district health boards. Subjects came from eight district health board regions serving urban and rural populations. The eight District Health Boards serve approximately 1.8 million people, representing just under half of the New Zealand population.

Questionnaires were administered face-to-face by trained interviewers, usually at the participant's home. Information on demographics (including ethnic group), smoking history, passive smoking exposure, recreational drug use, diet, occupation, income, education, alcohol consumption, and family history of malignancy was collected. A family history of lung cancer was defined as having a sibling or parent with lung cancer. Occupations associated with a higher risk of lung cancer were identified from the literature, and subjects were assigned a 'duration at risk' value in years. Ethnicity was derived by priority coding the responses into three groups; Maori, Pacific Islander, and "other." The highest level of educational attainment was recorded, as was income at diagnosis (or reference age). Alcohol consumption was calculated using a semiquantitative score based on the amount and frequency of consumption.

The patients who reported having smoked 20 or more joints of *Cannabis* were then asked to complete a more detailed exposure questionnaire regarding their *Cannabis* smoking. This questionnaire obtained information about the age of starting *Cannabis* use, the amount, frequency, and duration of use, and the characteristics of their smoking.

The information was collected for each period of their lives when the use pattern changed and was then summed to estimate total lifetime use. If subjects smoked *Cannabis* in a form other than a joint, e.g., pipes or bongs, they were asked to estimate the number of *Cannabis* joints to which that would equate. This conversion allowed *Cannabis* use for all participants to be quantified in joint years of use, with one joint year equivalent to one joint per day for 1 year. Subjects who had smoked <20 and ≥ 20 *Cannabis* joints in their lifetime were classified as nonsmokers and smokers of *Cannabis*, respectively. A similar approach to determining the lifetime use of cigarettes was employed to calculate pack-years of cigarette smoking, with one pack-year equivalent to 20 cigarettes per day for 1 year. The patients who reported smoking tobacco occasionally but never daily or daily at some stage were classified as nonsmokers and smokers of cigarettes, respectively.

Tertiles of *Cannabis* use were determined by the marginal distribution of use for all subjects to reduce the chance of zero cell counts if just the control group was used. Relative risks were estimated by calculating odds ratios by logistic regression using SPSS version 11.0 for Mac OSX and adjusted for confounding variables. Adjustment for age, joint years of *Cannabis* smoking, and pack years of cigarette smoking was made by including them as continuous variables in the regression models. The effects of categories of pack-years of cigarette smoking (quintiles of smoking for all subjects interviewed) and joint-years of *Cannabis* smoking (tertiles of use for all subjects interviewed) were also assessed. The relative risks were also calculated based on *Cannabis* use up to 5 years prior to diagnosis or reference date in the controls on the basis that exposure after that time was unlikely to have caused the malignancy. The age at which *Cannabis* smoking started was categorized, and the relative risk associated with starting at 16 years of age, compared with 21 years and older, was estimated.

The 79 cases of lung cancer included 16 (20%) small-cell and 63 (80%) non-small cell cancers, consistent with the established distribution of histological type seen in the general population [31]. There were 39 (49%) female cases of lung cancer, similar to the female proportion (50%) of all lung cancer cases aged under 55 years in New Zealand between 1998 and 2003. The risk of lung cancer did not vary with age due to cases and controls being frequently matched on the 5-year age group to improve the efficiency of the study.

A family history of lung cancer was associated with an increased risk of lung cancer (RR = 3.9, 95% CI 1.8–8.4). There was no significant association between lung cancer risk and passive smoking, diet, occupation, income, educational level, and alcohol use after adjustment for age, sex, ethnicity, cigarette smoking, and a family history of lung cancer. The proportion of controls (aged 35–55 years) who had ever smoked *Cannabis*, with age adjustment for the age distribution of the general population, was 36%, similar to the 40–52% reported among the younger 15–45-year age group in the New Zealand population.

While *Cannabis* smoking (defined as lifetime use of ≥ 20 joints) was not associated with a significantly increased risk of lung cancer, those with the highest tertile of use (>10.5 joint-years of exposure) had a significantly increased risk, RR = 5.7 (95% CI 1.5–21.6), after adjustment for age, sex, ethnicity, pack-years of cigarette smoking and a family history of lung cancer. Using the prevalence of the highest tertile of *Cannabis* smoking of the controls, we estimated that about 5% of lung cancer in those aged 55 years and under in New Zealand may be attributable to *Cannabis* smoking.

***Cannabis* in Immunotherapy**

Studies examining the effects of cannabinoid-based drugs on immunity have shown that various cellular and cytokine mechanisms are suppressed by the agents, mainly by four mechanisms: induction of apoptosis of T cells, macrophages, splenocytes,

and thymocytes; inhibition of cell proliferation, inhibition of production of chemokines and cytokines, and the induction of T-regs. Knowing the potential immunomodulatory effects of cannabinoids, which may work in the same immunosuppressive mechanism as glucocorticosteroids, there is no contraindication for using them in combination with immunotherapy.

The influence of *Cannabis* use during immunotherapy treatment on response rate (RR), progression-free survival (PFS), and overall survival (OS) was evaluated in a retrospective, observational study. Data were collected from the files of patients treated with nivolumab at a hospital and *Cannabis* from six *Cannabis*-supplying companies. In the study of patients with advanced melanoma, nonsmall cell lung cancer, and renal clear cell carcinoma were 140 patients, 89 on nivolumab alone and 51 on nivolumab plus *Cannabis*. The groups were homogenous regarding demographic and disease characteristics [32].

In the multivariate model, *Cannabis* was the only significant factor that reduced RR to immunotherapy (37.5% RR in nivolumab alone compared with 15.9% in the nivolumab/*Cannabis* group ($p = 0.016$, odds ratio = 3.13, 95% confidence interval 1.24–8.1). *Cannabis* use was not a significant factor for PFS or OS. Factors affecting PFS and OS were smoking (adjusted hazard ratio [HR] = 2.41 and 2.41, respectively) and brain metastases (adjusted HR = 2.04 and 2.83, respectively). Low-performance status (adjusted HR = 2.83) affected OS alone. Delta-9-tetrahydrocannabinol and cannabidiol percentages did not affect RR in any group ($p = 0.393$ and 0.116, respectively). The use of *Cannabis* during immunotherapy treatment decreased RR without affecting PFS or OS and without relation to *Cannabis* composition.

***Cannabis* and Radiation Therapy**

The potential antitumor activity of cannabinoid receptor agonists, such as the aminoalkylindole WIN55212-2 (WIN2), has been studied on the antiproliferative activity of radiation in human MCF-7, MDA-MB231, and murine 4T1 breast cancer cells. The antiproliferative effects produced by a combination of WIN2 and radiation were more effective than either agent alone [22]. The stereoisomer of WIN2, WIN55212-3 (WIN3), failed to inhibit growth or potentiate the growth-inhibitory effects of radiation, indicative of stereospecificity.

Two other aminoalkylindoles, pravadoline and JWH-015 [(2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenyl-methanone], also enhanced the antiproliferative effects of radiation, but other synthetic cannabinoids (i.e., nabilone, CP55,940 [(+)-rel-5-(1,1-dimethylheptyl)-2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol], and methanandamide) or delta-9-tetrahydrocannabinol and cannabidiol did not. The combination treatment of WIN2 plus radiation promoted autophagy and senescence but not apoptosis or necrosis. WIN2 also failed to alter radiation-induced DNA damage or the apparent rate of DNA repair.

Although the antiproliferative actions of WIN2 were mediated through noncannabinoid receptor-mediated pathways, the observation that WIN2 interfered with growth stimulation by sphingosine-1-phosphate (S1P) implicates the potential involvement of S1P/ceramide signaling pathways. In addition to demonstrating that aminoalkylindole compounds could potentially augment the effectiveness of radiation treatment in breast cancer, the study suggests that delta-9-THC and nabilone are unlikely to interfere with the effectiveness of radiation therapy, which is of particular relevance to patients using cannabinoid-based drugs to alleviate the toxicity of cancer therapies.

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Chapter 11

Cannabis and Parkinson's Disease



Parkinson's disease is a slowly progressive, degenerative disorder characterized by resting tremors, stiffness (rigidity), slow and decreased movement (bradykinesia), and eventually, gait and postural instability. Treatment aims to restore dopaminergic functions in the brain with levodopa/carbidopa and other drugs, e.g., dopamine agonists, monoamine oxidase type B [MAO-B] inhibitors, and amantadine. For refractory, disabling symptoms in patients without dementia, stereotactic deep brain stimulation or lesional surgery, levodopa, and an apomorphine pump may help.

Juvenile Parkinsonism, which is rare, begins during childhood or adolescence up to 20 years of age. Onset between the ages of 21 and 40 is sometimes called young or early-onset Parkinson's disease. Genetic causes are more likely in juvenile and early-onset Parkinson's disease; the forms may differ from later-onset Parkinson's disease because they progress more slowly; they are very responsive to dopaminergic treatments and most disability results from non-motor symptoms such as depression, anxiety, and pain.

Secondary Parkinsonism is a brain dysfunction characterized by basal ganglia dopaminergic blockade similar to Parkinson's disease. However, it is caused by something other than Parkinson's disease, e.g., drugs, cerebrovascular disease, trauma, or postencephalitic changes.

Atypical Parkinsonism refers to a group of neurodegenerative disorders that have some features similar to those of Parkinson's disease but have some different clinical features, a worse prognosis, a modest or no response to levodopa, and a different pathology, e.g., neurodegenerative disorders such as multiple system atrophy, progressive supranuclear palsy (PSP), dementia with Lewy bodies, and corticobasal ganglionic degeneration.

Parkinson's disease affects about 0.4% of people from 40 years of age, 1% of people from 65, and 10% of people 80 years and older. The mean age at onset is approximately 57 years of age. Parkinson's disease is usually idiopathic. The pathologic hallmark of sporadic or idiopathic Parkinson's disease is synuclein-filled Lewy bodies in the nigrostriatal system. However, synuclein can accumulate in

many other parts of the nervous system. Lewy bodies appear in a temporal sequence, and many experts believe Parkinson's disease is a relatively late development in systemic synucleinopathy. Other synucleinopathies include dementia with Lewy bodies and multiple system atrophy. Parkinson's disease may share features of other synucleinopathies, such as autonomic dysfunction and dementia. Rarely Parkinson's disease occurs without Lewy bodies, e.g., in a form due to a mutation in the *PARK 2* gene.

In Parkinson's disease, there is degeneration of the pigmented neurons of the substantia nigra, locus ceruleus, and other brain stem dopaminergic cell groups. Loss of substantia nigra neurons results in the depletion of dopamine in the dorsal aspect of the putamen and causes many of the motor manifestations of Parkinson's disease. A genetic predisposition is likely in some cases of Parkinson's disease. About 10% of patients have a family history of Parkinson's disease. Several abnormal genes have been identified. Inheritance is autosomal dominant for some genes and autosomal recessive for others. A mutation in leucine-rich repeat kinase 2 (*LRRK2*; also known as *PARK8*) is a gene that codes for the dardarin protein. It is the most prevalent mutation in sporadic cases of Parkinson's disease worldwide in patients ≥ 50 (in about 2%), and it is the most prevalent autosomal dominant mutation of the inherited forms of the disease. In genetic forms, age at onset tends to be younger, but the course is typically more benign than that of later-onset, presumably non-genetic Parkinson's disease.

Usually, the hands or feet are affected first, most often asymmetrically. The jaw and tongue may also be affected, but not the voice. However, speech may become hypophonic, with characteristic monotonous, sometimes stuttering dysarthria. Tremor may become less prominent as rigidity progresses. In predominantly rigid-akinetic forms of Parkinson's disease, resting tremor is subtle or absent. Rigidity develops independently of tremors in many patients. When a clinician moves a rigid joint, semi-rhythmic jerks occur because the intensity of the rigidity varies, causing a ratchet-like effect. Slow movements (bradykinesia) are typical in Parkinson's disease. Repetitive motor activity results in a progressive or sustained decrease in movement amplitude (hypokinesia), and movement becomes hard to initiate (akinesia).

Rigidity and hypokinesia may contribute to muscle aches and sensations of fatigue. The face becomes masklike (hypomimic), with an open mouth and reduced blinking. Excessive drooling (sialorrhea) may contribute to disability. Hypokinesia and impaired control of distal muscles are responsible for micrographia (writing in very small letters) and make daily living increasingly difficult. Patients have difficulty starting to walk, turning, and stopping. They shuffle, taking short steps, holding their arms flexed to the waist and swinging their arms little or not at all with each stride. Steps may inadvertently quicken while stride length progressively shortens; this gait abnormality, called festination, is often a precursor to freezing of gait (when, without warning, walking and other voluntary movements may suddenly halt). There is a tendency to fall forward (propulsion) or backward (retropulsion) when the center of gravity is displaced, resulting from loss of postural reflexes.

Posture becomes stooped. Postural instability may develop later in Parkinson's; if present at disease onset, alternative diagnoses should be suspected.

Dementia develops in about one-third of patients, usually late in Parkinson's disease. Early predictors of its development are visuospatial impairment and decreased verbal fluency. Sleep disorders are common. Insomnia may result from nocturia or from the inability to turn in bed. Sleep deprivation may exacerbate depression and cognitive impairment and contribute to excessive daytime sleepiness. Rapid eye movement (REM) sleep behavior disorder may develop; in this disorder, verbalization and uncontrollable, possibly violent limb movements occur during REM sleep because the paralysis that normally occurs during REM sleep is absent. REM sleep behavior disorder is often accompanied by early neurodegenerative signs that occur primarily in patients with alpha-synucleinopathies, which can precede and increase the risk of developing Parkinson's disease, multiple system atrophy, or dementia with Lewy bodies.

Neurologic symptoms unrelated to Parkinsonism commonly develop because synucleinopathy occurs in other areas of the central, peripheral, and autonomic nervous systems. The following are examples: almost universal sympathetic denervation of the heart, contributing to orthostatic hypotension; esophageal dysmotility, contributing to dysphagia and increased risk of aspiration; lower bowel dysmotility, contributing to constipation; urinary hesitancy and urgency, potentially leading to incontinence. Seborrheic dermatitis is also common. In some patients, some symptoms occur before the motor symptoms of Parkinson's disease and frequently worsen over time.

Levodopa is the most effective treatment. However, when Parkinson's disease becomes severe, sometimes soon after diagnosis, response to levodopa can wear off, causing fluctuations in motor symptoms and dyskinesia. Levodopa is the metabolic precursor of dopamine. It crosses the blood–brain barrier into the basal ganglia, where it is decarboxylated to form dopamine. The co-administration of the peripheral decarboxylase inhibitor carbidopa prevents levodopa from being decarboxylated into dopamine outside the brain (peripherally), thus lowering the levodopa dosage required to produce therapeutic levels in the brain and minimizing adverse effects due to dopamine in the peripheral circulation.

Neurodegenerative diseases affect millions of people, and the burden of the diseases has significantly increased over the past 20 years, mostly due to population aging and the environmental stress associated with contemporary societies. The disease has become the leading cause of disability and death. Despite decades of research, there is no cure for them or to slow down their progression. Neurodegenerative diseases include Alzheimer's, amyotrophic lateral sclerosis, Huntington's, Lewy body disease, and Parkinson's. The two most prevalent neurodegenerative diseases are Alzheimer's and Parkinson's diseases.

About 5% of the population over 85 years suffers from Parkinson's disease, overwhelming the healthcare support systems and families. The mechanisms underlying the particular conditions are related to the accumulation of misfolded and aggregated proteins, which may promote the disease either by a gain of the harmful activity or by loss of biological function [1]. The misfolded protein may aggregate into

amyloid fibrils which deposit in the form of extracellular amyloid plaques, neurofibrillary tangles, and other intracytoplasmic or intranuclear inclusions [2].

Parkinson's disease is the most rapidly growing neurodegenerative disease. Over the past generation, the global burden has more than doubled due to human life span increment, better health conditions, and environmental factors, such as exposure to the anthropogenic pollutant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and pesticides [3]. The onset of the disease usually occurs at an average of 60 years of age. However, in some cases, Parkinson's disease occurs much earlier, between 21 and 50 years of age, as early-onset Parkinson's disease.

Symptomatically, the initial phases of the disease are characterized by movement disorders, such as shaking, rigidity, slowness, and aberrant gait. Other symptoms, such as cognitive and behavioral problems, appear as dementia in the later phases of the disease. The onset of Parkinson's disease and early-onset Parkinson's disease can arise due to genetic and environmental factors, but most cases are sporadic. It is generally accepted that known genetic causes may account for about 5% of the total Parkinson's disease population.

A study explained 16–36% of Parkinson's disease as heritability; prevalence estimates 0.5–2.0%. The heritability in early-onset Parkinson's disease is higher than in later-onset Parkinson's disease. The low values agree with clinical reports describing most Parkinson's disease cases as having an unknown etiology. Studies highlight that the combination of genetic and environmental factors, such as the consumption of substances and the variability of brain vulnerability, may also increase the risk of Parkinson's disease onset.

Scientific reports suggest that the incidence of Parkinson's disease and early-onset Parkinson's disease is increasing. Studies indicate that variability in genes associated with the cellular response and metabolism of xenobiotics or toxins increases the predisposition to Parkinson's disease [4]. The neurotoxic effect of MPTP changes the expression of genes associated with Parkinson's disease, which may alter the resilience of neurons to toxins.

The onset of Parkinson's disease is tightly associated with the neuronal protein α -synuclein. Alpha-Synuclein's physiological function promotes N-ethylmaleimide-sensitive factor (NSF) attachment to the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) protein complex assembly during synaptic exocytosis. Thus, α -synuclein seems to regulate the synaptic vesicle release from presynaptic cells [5]. However, in pathological conditions, the overexpression and modification of α -synuclein forms neurotoxic aggregates, which promote the selective loss of dopaminergic neurons in the *substantia nigra pars compacta* [6].

Most studies associate Parkinson's disease with several defective cellular and physiological mechanisms, such as neuroinflammation, excitotoxicity, mitochondrial dysfunction, reduced trophic support, abnormal kinase activity, disruption of calcium homeostasis, and proteostasis dysfunction [7]. Nevertheless, there is still much to clarify, such as the precise molecular mechanism of amyloid fibrils formation and its relationship with glial activation and peripheral immune cell infiltration of inflammatory responses.

It has been hypothesized that the increase in the risk of Parkinson's disease might be associated with the consumption of drugs of abuse, such as stimulants. The supporting evidence suggests that the neurotoxic effects of amphetamine-like stimulants on the nigrostriatal pathway are intriguingly similar to those in neurodegeneration observed in Parkinson's disease [8]. Parkinsonism was also described as a consequence of heroin consumption, which is a depressor of the central nervous system [9].

Conversely, scientific data supports that other abused substances, such as some phytocannabinoids, which interact with the endocannabinoid system, may play a neuroprotective role in Parkinson's disease [10]. Interestingly, both neurotoxic and neuroprotective effects are described as the outcomes of the consumption of different drugs of abuse. Recently, a new trend of synthetic molecules with similar effects to traditional illicit drugs, the novel psychoactive substances, has emerged, and little is known about their toxicological impact. Consumption of novel psychoactive substances among young people may be a promoter of the initial stages of neurodegeneration and may well increase the future incidence of Parkinson's disease.

The Endocannabinoid System and Parkinson's Disease

The endocannabinoid system is constituted of endocannabinoids, biosynthesizing (N-arachidonoyl-phosphatidylethanolamine [NAPE]-specific phospholipase D and diacylglycerol [DAG] lipase- α) and degrading (fatty acid amide hydrolysis [FAAH] and monoacylglycerol lipase [MAGL]) enzymes, and cannabinoid receptors. The endocannabinoid system is highly represented in the basal ganglia and is altered in several movement disorders, including Parkinson's disease. Preclinical research suggests that modulating cannabinoid signaling could improve motor symptoms. Levodopa-induced dyskinesia dramatically complicates long-term pharmacological treatment in Parkinson's patients [58]. Levodopa-induced dyskinesia is thought to arise from pulsatile stimulation of dopamine receptors with progressive sensitization of dopamine receptor-associated striatal signaling.

The best-characterized endocannabinoids (N-arachidonylethanolamine [AEA] or anandamide and 2-arachidonoylglycerol [2-AG]) interact with the two main CBRs subtypes (CB1R and CB2R) and with other receptors, including the transient receptor potential vanilloid type 1 (TRPV1) cation channel, the GTP-binding protein-coupled receptor GPR55, the abnormal-cannabidiol receptor, and the peroxisome proliferator-activated receptor (PPAR) [59]. Endocannabinoids regulate synaptic transmission producing a physiological feedback mechanism aimed at preventing an excess of excitation or inhibition. The "retrograde signaling" results in depolarization-induced suppression of inhibition (DSI) at γ -aminobutyric acid (GABA)ergic synapses and depolarization-induced suppression of excitation (DSE) at glutamatergic synapses. The presynaptic location of CB1R also allows endocannabinoids to directly modulate other neurotransmitters, including opioid peptides, acetylcholine, and 5-hydroxytryptamine (5-HT).

CB1R can form heteromers with other metabotropic receptors, including the dopamine D1 and D2 receptors. Although nigrostriatal dopaminergic neurons seem not to express CB1R, they are significantly affected by either the activation or the blockade of the endocannabinoid system. The effects are likely mediated by CB1R located in other neuronal subpopulations (i.e., GABAergic, glutamatergic, and opioidergic neurons) near and connected with dopaminergic neurons [60]. Dopaminergic neurons may, in turn, produce endocannabinoids from their somata and dendrites, thus facilitating the retrograde signaling at excitatory and inhibitory synapses. Additional direct mechanisms have been proposed to explain the modulation of the endocannabinoid system on dopamine transmission. Some endocannabinoids, including AEA, have been found to interact with TRPV1 receptors, which are expressed in dopaminergic neurons. CB2R has been identified in human nigrostriatal dopaminergic neurons; this may support the direct role of endocannabinoids in modulating dopaminergic transmission.

Activation of endocannabinoids has been associated with motor inhibition and reduced dopaminergic activity. In hyperkinetic conditions, reduced endocannabinoid tone accompanies increased dopaminergic activity, whereas, in hypokinetic movement disorders, the opposite pattern is observed. In experimental models of Parkinson's disease, endocannabinoids can enhance the hypokinetic effects of dopamine-depleting agents and reduce the effects of drugs producing hyperstimulation of dopamine receptors [61]. In particular, it has been proposed that motor inhibition produced by CB1R stimulation is mediated by the regulation of the phosphorylation state of a critical mediator of dopamine action in striatal neurons, dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32).

At the cellular level, CB–DA interactions seem much more complex. Dopaminergic transmission can influence the endocannabinoid levels in the striatum, as shown by the increase of AEA levels after D2-like receptor stimulation [62]. This effect depends on both stimulation of its synthesis and inhibition of its degradation, as suggested by the ability of D2-like receptor agonists to modulate the activity of NAPE-phospholipase D and FAAH. Such DA-stimulated endocannabinoid activity can counter the action of D2 receptor activation in the striatum, suggesting an inhibitory feedback mechanism aimed at limiting the hyperkinetic effect of dopamine. Cooperative action of CB1 and D2 receptors has also been proposed by the findings that AEA produced by dopamine stimulation can enhance the effects of D2 receptor activation. Inhibition of GABA transmission via D2-like receptors can be partly prevented by cannabinoid receptor blockade, suggesting that the endocannabinoid system may act as a downstream effector of D2 receptors. Accordingly, both D2 and CB1 receptors are expressed on the GABA terminals of the striatum.

The complex interaction between DA and endocannabinoids explains the reorganization of the systems in both idiopathic and experimental Parkinson's disease. Studies in experimental Parkinson's disease showed enhanced endocannabinoid activity in the basal ganglia, including increased CB1 mRNA levels, CB1 activity, AEA levels, and decreased CB clearance [63]. Accordingly, an increased level of AEA has been shown in the cerebrospinal fluid of untreated Parkinson's disease patients. Also, increased expression of CB1 receptors in the basal ganglia has been

reported. The changes are associated with movement suppression and may be reversed by chronic levodopa treatment.

Cannabinoids and Parkinson's Disease

The neuroprotective properties of phytocannabinoids apart from cannabidiol and delta-9-tetrahydrocannabinol, including delta-9-tetrahydrocannabinolic acid, delta-9-tetrahydrocannabivarin, cannabidiolic acid, cannabidivarin, cannabichromene, cannabichromenic acid, cannabichromevarin, cannabigerol, cannabigerolic acid, cannabigerivarin, cannabigerovarinic acid, cannabichromevarinic acid, cannabidivarinic acid, and cannabinol, were evaluated in 2341 studies with 31 that met inclusion criteria. Cannabigerol (range 5–20 mg/kg) and cannabidivarin (range 0.2–400 mg/kg) displayed efficacy in models of Huntington's disease and epilepsy, Cannabichromene (10–75 mg/kg), delta-9-tetrahydrocannabinolic acid (20 mg/kg), and tetrahydrocannabivarin (range 0.025–2.5 mg/kg) showed promise in models of seizure and hypomobility, Huntington's, and Parkinson's diseases. The mechanistic data showed cannabigerol, its derivatives VCE.003 and VCE.003.2, and delta-9-tetrahydrocannabinolic acid mediated some of their effects through PPAR- γ , but no other receptors were detected [44].

Cannabis has been successfully utilized as an adjunctive treatment for malignant brain tumors, Parkinson's and Alzheimer's diseases, multiple sclerosis, neuropathic pain, childhood seizure disorders, and Lennox-Gastaut and Dravet syndromes. Animal and human research data have been presented on the clinical and neurological uses for cannabidiol alone or with delta-9-THC, revealing its neuroprotective, anti-inflammatory, and immunomodulatory benefits when applied to various clinical situations.

Cannabinoid receptor pharmacology began in the late 1960s when delta-9-THC was isolated and synthesized and found to be the primary psychoactive constituent of *Cannabis*. The discovery of specific membrane receptors for delta-9-THC in the early 1990s led to the identification of an endogenous signaling system, the endocannabinoid system. The endogenous cannabinoids, *N*-arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol, were subsequently identified. The endocannabinoid system consists of two major types of endogenous G protein-coupled cannabinoid receptors (CB1 and CB2) located in the mammalian brain and throughout the central and peripheral nervous systems, including tissues associated with the immune system.

Cannabinoid receptors 1 and 2 can co-exist in various concentrations in the same locations. The phytocannabinoids and endogenous cannabinoids function as retrograde messengers that provide feedback inhibition of both excitatory and inhibitory transmission in the brain by activating presynaptic CB1 receptors. Manipulations of endocannabinoid derivative enzymes, CB1 and CB2 receptors, and their endogenous ligands have shown promise in modulating numerous processes associated with neurodegenerative diseases, cancer, epilepsy, and traumatic brain injury. In

addition, the endocannabinoid system is known to influence neuroplasticity, apoptosis, excitotoxicity, neuroinflammation, and cerebrovascular breakdown associated with stroke and trauma.

Cannabidiol and Delta-9-THC

Phytocannabinoid compounds and extracts can come from both hemp and *Cannabis* subspecies. Cannabidiol does not elicit the same psychoactive effects as seen with delta-9-THC (i.e., users of cannabidiol do not feel euphoric). The various psychoactive effects generally associated with delta-9-THC are attributed to the activation of the CB1 cannabinoid receptor found abundantly in the brain. CB1 receptors have the highest densities on the outflow nuclei of the basal ganglia, substantia nigra pars reticulata (SNr), and the internal and external segments of the globus pallidus (a portion of the brain that regulates voluntary movement). The hippocampus, particularly within the dentate gyrus (DG) and cerebellum, also has higher CB1 receptor densities. Very few CB1 receptors are found in the brainstem. The locations suggest CB1 receptor involvement in the modulation of memory, emotion, pain, and movement. Delta-9-THC, which targets CB1 receptors, has been shown to reduce nociception in animal models of acute, visceral, inflammatory, and chronic pain. In patients with chronic and neuropathic pains, using *Cannabis* or cannabinoid extracts produced positive and improved symptoms.

The activation of the signaling pathways by CB1 receptors and the high levels of the receptors on presynaptic terminals indicate that endocannabinoid stimulation of CB1 receptors suppresses neuronal excitability and inhibits neurotransmission. Activation of neuronal CB1 receptors resulted in the inhibition of adenylyl cyclase and decreased neurotransmitter release through the blockade of voltage-operated calcium channels. The effects have led to the study of phytocannabinoids to treat epilepsy. Pharmaceutical companies are attempting to develop synthetic high-affinity CB1 antagonists and inverse agonists as therapeutic drugs for diabetes, metabolic syndrome, and drug dependence.

The CB2 receptor, unlike CB1, is not highly expressed in the central nervous system. Effects of delta-9-THC on immune function have been attributed to the CB2 cannabinoid receptor interaction found predominantly in immune cells. CB2 receptors are distributed widely in the major tissues of immune cell production and regulation, including the spleen, tonsils, and thymus. The cell lines include B and T lymphocytes, natural killer cells, monocytes, macrophages, microglial cells, and mast cells. Like CB1 receptors, endocannabinoid stimulation inhibits the neurotransmission of CB2 receptors.

Activation of CB2 has been implicated in several neurodegenerative diseases such as Huntington's and Alzheimer's. A study of cultured microglial cells showed c-interferon and granulocyte macrophage-colony stimulating factor (GM-CSF), known as inflammatory response activators of the microglial cell, was accompanied by significant CB2 receptor upregulation, suggesting that the CB2 receptors play an

important role in microglial cell function in the central nervous system inflammatory response. Increased expression of CB2 in the brain was confirmed with CB2-selective positron emission tomography (PET) tracers in Alzheimer's mice models. The increased expression was concomitant with the formation of amyloid-beta plaques, suggesting a potential utility for CB2 PET tracers as a diagnostic modality for detecting the onset of neuroinflammation.

Cannabidiol's action with the CB2 receptor is just one of several pathways by which cannabidiol can affect neuroinflammation. The interaction of cannabidiol with CB2 receptors is more complex, but like delta-9-THC, cannabidiol reduces the inflammatory response. Because cannabidiol and delta-9-THC modulate the activity of G protein-coupled receptors associated with the endocannabinoid system, cannabidiol can function as a partial agonist and antagonizes delta-9-THC, and cannabidiol at higher doses may counter to some extent the psychoactive effects of delta-9-THC. Anecdotally, the effect is noted by many *Cannabis* users who co-ingest cannabidiol.

Molecular targets of cannabidiol, including cannabinoid and non-cannabinoid receptors, enzymes, transporters, and cellular uptake proteins, help to explain cannabidiol's low-binding affinity to both CB1 and CB2 cannabinoid receptors. In animal models, cannabidiol has demonstrated an ability to attenuate brain damage associated with neurodegenerative and ischemic conditions outside the endocannabinoid system. Cannabidiol stimulates synaptic plasticity and facilitates neurogenesis, which may explain its positive effects on attenuating psychotic, anxiety, and depressive behaviors.

The mechanisms underlying the effects involve multiple cellular targets to elevate brain-derived neurotrophic factor (BDNF) levels, reduce microglia activation, and decrease levels of proinflammatory mediators. Unlike the psychoactive properties associated with delta-9-THC, cannabidiol has been shown to have very low toxicity in humans and other species. Ingested and absorbed cannabidiol is rapidly distributed and can easily pass the blood-brain barrier due to its lipophilic nature. The terminal half-life of cannabidiol is about 9 h and is preferentially excreted in the urine as it is free and glucuronide forms.

Cannabidiol research in animal models and humans has shown therapeutic properties for brain function and protection, directly affecting the endocannabinoid system and influencing endogenous cannabinoids. Cannabidiol decreases the production of inflammatory cytokines, influences microglial cells to return to a ramified state, preserves cerebral circulation during ischemic events, and reduces vascular changes and neuroinflammation. Cannabidiol has demonstrated anxiolytic, antidepressant, neuroprotective, anti-inflammatory, and immunomodulatory benefits.

Cannabidiol increases brain adenosine levels by reducing adenosine reuptake. Increased adenosine is associated with neuroprotection and decreased inflammation after brain trauma. Other effects of cannabidiol include the inhibition of calcium transport across membranes, the inhibition of anandamide uptake and enzymatic hydrolysis, and the inhibition of inducible *nitrous oxide synthase* protein expression and nuclear factor (NF)- κ B activation.

Cannabidiol is also known to exert vascular effects, producing vasodilation and hypotension that may hold promise as a protectant against cerebrovascular damage associated with stroke. Cannabidiol has several features that may be exploited to treat Alzheimer's disease, including the prevention of glutamate-induced excitotoxicity, reduction of proinflammatory mediators, and the ability to scavenge reactive oxygen species and reduce lipid peroxidation.

Experimentally, *in vitro*, cannabinoid receptor interactions with cannabidiol and delta-9-THC, together and separately, have demonstrated neuronal protection from excitotoxicity, hypoxia, and glucose deprivation; *in vivo*, cannabinoids decrease hippocampal neuronal loss and infarct volume after cerebral ischemia, acute brain trauma, and induced excitotoxicity. The effects have been ascribed to the inhibition of glutamate transmission, reduction of calcium influx, reduced microglial activation, and subsequent inhibition of noxious cascades, such as tumor necrosis factor- α generation and oxidative stress. Delta-9-THC can mediate the effects of the neurotransmitter serotonin by decreasing 5-HT₃ receptor neurotransmission; this can contribute to the pharmacological action to reduce nausea. The effect can be reversed at higher doses or with chronic use of delta-9-THC.

Synthetic analogs of delta-9-THC, nabilone (cesamet), and dronabinol have been prescribed to suppress nausea and vomiting produced by chemotherapy. There is limited use for synthetic delta-9-THC due to multiple side effects mediated through the activation of CB1 in the central nervous system of patients.

Delta-9-THC has been shown to protect the brain from various neuronal insults and improve the symptoms of neurodegeneration in animal models of multiple sclerosis, Parkinson's and Huntington's diseases, amyotrophic lateral sclerosis, and Alzheimer's disease. Like cannabidiol, delta-9-THC can offer non-endocannabinoid system protection by directly affecting neuronal cells and non-neuronal elements within the brain. The mechanisms include modulation of excitatory glutamatergic transmissions and synaptic plasticity, modulation of immune responses, the release of anti-inflammatory mediators, modulation of excitability of *N*-methyl-D-aspartate receptors, and its effect on gap junctions, calcium, and antioxidants.

Cannabidiol is effective in an experimental model of Parkinsonism (6-hydroxydopamine-lesioned rats) by acting through antioxidant mechanisms independently of cannabinoid receptors; it attenuates Parkinson's disease-related dystonia, but not tremor, in agreement with a positive correlation between cannabidiol levels measured in the putamen/globus pallidus of recreational users of *Cannabis* [45].

Cannabis has been receiving attention due to changing legislation and reports of benefits in various conditions. The mechanism of action of the interaction of the compounds with the endocannabinoid receptors CBR1 and CBR2, both G protein-coupled receptors, with the former highly expressed in the central nervous system, particularly the olfactory bulb, hippocampus, thalamus, and basal ganglia, and the latter expressed more peripherally.

There is consistent evidence that delta-9-THC binds actively to CBR1, and cannabidiol interacts with endocannabinoid receptors that provide neuroprotection. While there is great interest in this link, clinical trials are lacking in Parkinson's

disease and other neurological conditions. To date, only Epidiolex, which is a purified form of cannabidiol, has been FDA approved for the treatment of seizures associated with Lennox-Gastaut syndrome or Dravet syndrome.

In a Web-based survey completed by 454 patients with Parkinson's disease, two-thirds had tried *Cannabis* at some point, and one-third continued to use *Cannabis* to treat various symptoms of Parkinson's disease. On the self-report scales, patients noted improved memory, mood, and fatigue. In a survey of 339 patients with Parkinson's disease in the Czech Republic, a quarter of respondents had tried *Cannabis* at some point, and nearly half reported some benefit to a variety of motor symptoms, including the cardinal features of Parkinson's disease and dyskinesia.

In a smaller study of 47 patients, most reported improvement after *Cannabis* use, including a reduction in falls, stiffness, and tremor, as well as non-motor symptoms, including pain, mood, and sleep quality. More than half of the patients reported adverse events that included confusion, anxiety, hallucinations, amnesia, psychosis, dizziness, unsteadiness, and inhalation side effects of cough and dyspnea. In a 2015 survey conducted in Colorado shortly after the legalization of recreational *Cannabis*, 4.3% of the patients reported using *Cannabis* for Parkinson's disease symptoms and 78% found it to be useful.

In an open-label, observational study of 22 patients with Parkinson's disease, mUPDRS scores were significantly improved 30 min after smoking 0.5 g of *Cannabis*. Another group of five patients with Parkinson's disease noted no improvement in tremors shortly after smoking 1 g of *Cannabis*. In a randomized, placebo-controlled trial, a Brazilian group studied two dosages of cannabidiol, 75 mg/day and 300 mg/day, administered in a capsule for 6 weeks. There was no significant difference in UDPRS scores, plasma brain-derived neurotrophic factor (BDNF) levels, or proton magnetic resonance spectroscopy scans compared to placebo. A significant improvement in quality of life was reported in the higher dose of PDQ-39.

In 2009, the results of an open-label study of cannabidiol for patients with Parkinson's disease and psychosis, starting with 150 mg/day and titrating up to 400 mg/day, were reported. Researchers from the UK reported in a randomized, double-blind study of cannador (an oral capsular formulation with a 2:1 delta-9-THC/Cannabidiol ratio) using a crossover design aimed specifically at measuring improvements in levodopa-induced dyskinesia. Nineteen patients were randomized to receive either a placebo followed by *Cannabis* or vice versa; there were no reported improvements in measures of dyskinesia or subjective reports. The result was incongruent with previous data, where a smaller placebo-controlled trial of seven patients with Parkinson's disease demonstrated that the cannabinoid receptor agonist nabilone could decrease levodopa-induced dyskinesia compared to placebo.

The results indicated a significant improvement from baseline to 4 weeks on all subscales of the Brief Psychiatric Rating Scale (BPRS), Parkinson's Psychosis Questionnaire (PPQ), UPDRS total score, and Clinical Global Impression-Improvement (CGI-I) scale, without worsening of the Mini-Mental State Examination (MMSE) or Frontal Assessment Battery (FAB). The group later described four patients with Parkinson's disease and rapid eye movement sleep behavior disorder (RBD) who were treated with cannabidiol up to 300 mg per day

for 6 weeks and found that they had “prompt and substantial” reduction in the frequency of RBD-related events without appreciable side effects. No other studies have included measures of RBD to corroborate the finding. Importantly, the compounds did not include delta-9-THC, which is more likely to cause psychotropic side effects.

An open-labeled trial of 20 patients with Parkinson's disease reported significant pain at baseline and found that the *Cannabis* improved the UDPRS scale, Pain Rating Index (PRI), and Visual Analog Scale (VAS) of the McGill Pain Questionnaire. They evaluated temperature thresholds after short- and long-term exposures. The mean cold pain threshold decreased significantly in the more affected limb after short-term exposure, and the mean heat pain threshold decreased significantly after long-term exposure. It was deduced that *Cannabis* might improve motor function and lessen pain perception in patients with Parkinson's disease.

Open-label studies of inhaled *Cannabis* cited common side effects of dizziness and drowsiness, plus smoking-related complications of cough or a foul taste in the mouth. A study that used a delta-9-THC-containing product reported more than double the adverse effects in the experimental group, including physical symptoms such as dry mouth, altered taste, gastrointestinal symptoms, and dizziness, as well as psychological symptoms such as drowsiness, detached sensation, paranoia, poor concentration and memory, and vivid dreams.

Pure cannabidiol, however, is non-psychotropic and generally well tolerated up to 1500 mg/day doses. Open-label studies reported few side effects. One open-label study reported various adverse effects in all 13 patients included in the trial. Symptoms were generally mild but included diarrhea (85%), somnolence (69%), fatigue (62%), weight gain (31%), dizziness (23%), abdominal pain (23%), and headache, weight loss, nausea, anorexia, and increased appetite (each 5%) [46]. Though many patients with Parkinson's disease reported using *Cannabis* at some point during their disease course, the available data show mixed results, likely due in part to the heterogeneous administration methods employed in various studies and the lack of standardized products. *Cannabis* also has a greater side effect profile than other interventions, though some side effects can be mitigated by ingesting, rather than inhaling, the drug.

The Parkinson's disease community's perception of medical *Cannabis* and patients' view on treatment with medical *Cannabis* was assessed through a questionnaire-based survey of the general knowledge and interest in medical *Cannabis* and the frequency, modalities, efficacy, and tolerability of application [47]. Questionnaires were distributed nationwide via the membership journal of the German Parkinson's Association and locally in clinics to control for report bias. One hundred and twenty-three questionnaires (1.123 nationwide, 225 local) were analyzed. Fifty-one percent of the participants were aware of the legality of medical *Cannabis* application, 28% of various routes of administration, and 9% of the difference between delta-9-THC and cannabidiol.

Parkinson's disease-related *Cannabis* use was reported by 8.4% of the patients and associated with younger age, living in large cities, and better knowledge about the legal and clinical aspects of medical *Cannabis*. Reduction of pain and muscle

cramps was reported by more than 40% of *Cannabis* users. Stiffness/akinesia, freezing, tremor, depression, anxiety, and restless legs syndrome subjectively improved by more than 20%, and overall tolerability was good. Improvement of symptoms was reported by 54% of the users taking oral cannabidiol and 68% inhaling delta-9-THC-containing *Cannabis*. Compared to cannabidiol intake, inhalation of delta-9-THC was more frequently reported to reduce akinesia and stiffness (50.0% vs. 35.4%; $p < 0.05$). Interest in using medical *Cannabis* was reported by 65% of the non-users.

The basal ganglia are subcortical structures that regulate the initiation, execution, and orientation of the movement. Cannabinoid receptors are highly expressed in the basal ganglia circuit of both animals and humans. Cannabinoid receptors regulate dopaminergic transmission in the nigrostriatal pathway and, thus, the basal ganglia circuit. The functioning of the basal ganglia is affected in pathologies related to movement disorders, especially those occurring in Parkinson's disease, which produces motor and non-motor symptoms involving GABAergic, glutamatergic, and dopaminergic neural networks. The most effective medication for Parkinson's disease is levodopa; however, long-term levodopa treatment causes long-term dyskinesia, levodopa-induced dyskinesia.

With neuromodulation offering a novel treatment strategy for Parkinson's patients, research has focused on the endocannabinoid system, as it participates in the physiological neuromodulation of the basal ganglia to control movement. Cannabinoid receptors have been shown to inhibit neurotransmitter release, while endocannabinoids play a key role in the synaptic regulation of the basal ganglia. Cannabidiol has been shown to have compensatory effects on the endocannabinoid system and as a neuromodulator and neuroprotector in models such as 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and reserpine, and other Parkinson's disease models [48].

The cannabidiol-induced neuroprotection observed in animal models of Parkinson's disease has been attributed to the activation of the CB1 receptor. Research conducted at a molecular level has indicated that cannabidiol can activate other receptors, such as CB2 and the TRPV-1 receptors, which are expressed in the dopaminergic neurons of the nigrostriatal pathway. The findings open new lines of scientific inquiry into the effects of cannabidiol at the level of neural communication. Cannabidiol activates the PPAR γ , GPR55, GPR3, GPR6, GPR12, and GPR18 receptors, causing various biochemical, molecular, and behavioral effects due to the broad range of the receptors it activates in the central nervous system. Given the low number of pharmacological treatment alternatives for Parkinson's disease, searching for molecules with the therapeutic potential to improve neuronal communication is crucial. Investigation of cannabidiol and the mechanisms involved in its function is required to ascertain whether receptor activation could be a treatment alternative for Parkinson's disease and levodopa-induced dyskinesia.

Neurodegeneration leading to Parkinson's and Alzheimer's diseases has become a major health burden. Treatments mainly target controlling symptoms, and there are no clinical practice therapeutics to prevent neurodegeneration or induce neuronal repair. Parkinson's disease is frequently treated with l-DOPA and deep brain

stimulation. A literature review was conducted to provide a collection of published work on Parkinson's and Alzheimer's diseases and current uses of the endocannabinoid system as a potential drug target for neurodegeneration.

Recent gene modification and remodeling techniques, such as CRISPR through human embryonic stem cells and induced pluripotent stem cells, have shown promising strategies for personalized medicine. Alzheimer's disease, characterized by extracellular deposits of amyloid β -senile plaques and neurofibrillary tangles of tau protein, commonly uses choline acetyltransferase enhancers as therapeutics. The endocannabinoid system has been evaluated as a Parkinson's and Alzheimer's disease drug target where overexpression of the endocannabinoid system receptors exerted neuroprotection against Parkinson's disease and reduced neuroinflammation in Alzheimer's disease.

The delta-9-tetrahydrocannabinoid and cannabidiol have shown neuroprotection against Parkinson's and Alzheimer's diseases in animal models but triggered toxic effects on patients when administered directly. Therefore, understanding the precise molecular cascade following cannabinoid treatment is suggested, focusing especially on gene expression to identify drug targets for preventing and repairing neurodegeneration.

Due to its potential neuroprotective effects, the endocannabinoid system has drawn significant attention among researchers. A review of published literature was conducted to examine the use of the endocannabinoid system and its components as a drug target for the most common neurodegenerative disorders, namely, Parkinson's and Alzheimer's diseases. Among the neurodegenerative disorders, 1.8% account for Parkinson's disease, while Alzheimer's disease accounts for 12% [49]. The review also discussed using cannabinoids, mainly delta-9-THC and cannabidiol, to target endocannabinoid systems in developing therapeutics for the two diseases.

An open-label observational study was conducted to assess the clinical effect of *Cannabis* on motor and non-motor symptoms of Parkinson's disease on 22 patients attending the motor disorder clinic of a tertiary medical center [50]. The patients were evaluated at baseline and 30 min after smoking *Cannabis* using the following battery: Unified Parkinson's Disease Rating Scale, Visual Analog Scale, present pain intensity scale, Short-Form McGill Pain Questionnaire, and Medical *Cannabis* Survey National Drug and Alcohol Research Center Questionnaire. The mean (SD) total score on the Motor Unified Parkinson's Disease Rating Scale score improved significantly from 33.1 (13.8) at baseline to 23.2 (10.5) after *Cannabis* consumption ($t = 5.9$; $p < 0.001$).

Analysis of specific motor symptoms revealed significant improvement after treatment in tremors ($p < 0.001$), rigidity ($p = 0.004$), and bradykinesia ($p < 0.001$). There was a significant improvement in sleep and pain scores. No significant adverse effects of the treatment were observed. The study indicated that *Cannabis* might have a place in the therapeutic armamentarium of Parkinson's disease.

A study was conducted to evaluate the impacts of acute cannabidiol administration, at a dose of 300 mg, on anxiety and tremors induced by a Simulated Public Speaking Test (SPST) in individuals with Parkinson's disease. The randomized, double-blinded, placebo-controlled, crossover clinical trial was conducted on 24

individuals [51]. They underwent two experimental sessions within a 15-day interval. After taking cannabidiol or a placebo, the participants underwent the SPST. The following data were collected during the test: heart rate, systemic blood pressure, and tremor frequency and amplitude. The Visual Analog Mood Scales (VAMS) and Self-Statements during Public Speaking Scale were applied. Statistical analysis was performed by repeated-measures analysis of variance (ANOVA) while considering the drug, SPST phase, and interactions between the variables.

There were statistically significant differences in the VAMS anxiety factor for the treatment; cannabidiol attenuated the anxiety experimentally induced by the SPST. Repeated measures by ANOVA indicate significant differences in the drug for the variable related to tremor amplitude as recorded by the accelerometer. Acute cannabidiol administration at a dose of 300 mg significantly decreased anxiety in patients with Parkinson's disease, and there was also decreased tremor amplitude in an antigenic situation.

To evaluate the usefulness of *Cannabis*-derived phytocannabinoids and resveratrol as neuroprotective agents for Parkinson's disease, based on their anti-oxidative and anti-inflammatory properties, a systematic review of the scientific literature was conducted [52]. A total of 1034 publications were analyzed, of which 18 met the eligibility criteria for the review. Collectively, the majority of Parkinson's disease rodent studies demonstrated that treatment with *Cannabis*-derived phytocannabinoids or resveratrol produced a significant improvement in motor function and mitigated the loss of dopaminergic neurons.

Biochemical analysis of rodent brain tissue suggested that neuroprotection was mediated by anti-oxidative, anti-inflammatory, and anti-apoptotic mechanisms. The review highlights the neuroprotective potential of *Cannabis*-derived phytocannabinoids and resveratrol for in vivo models of Parkinson's disease. It suggests their potential translation to human clinical trials to either ameliorate Parkinson's disease progression or be implemented as a prophylactic means to reduce the risk of development of Parkinson's disease.

There is evidence that *Cannabis* causes hallucinations and psychosis, especially with heavy and prolonged use. Parkinson's disease patients have an increased risk of developing hallucinations and psychosis [53]. It is, therefore, possible that *Cannabis* may exacerbate the risk and result in earlier and greater amounts of hallucinations and psychosis in the vulnerable population. In a literature review, two articles listed the incidence of hallucinations and delusions during the use of *Cannabis* in Parkinson's disease patients. Ten out of 47 (21.3%) of the patients reported the development of hallucinations while treated with *Cannabis*, and 2.8% developed delusions. While the numbers are within the range of the prevalence of hallucinations and psychosis in Parkinson's disease, the number of studies and patients evaluated is too small to make a definite conclusion.

In a review conducted to describe the main advances in the development of the experimental and clinical use of cannabidiol in neuropsychiatry, cannabidiol was shown to have anxiolytic, antipsychotic, and neuroprotective properties. Clinical investigations on the effects of cannabidiol have been carried out in the context of many other health conditions, including its potential use in epilepsy, substance

abuse and dependence, schizophrenia, social phobia, post-traumatic stress, depression, bipolar disorder, sleep disorders, and Parkinson's disease [54]. Cannabidiol is a good and promising treatment for patients with several clinical conditions. Controlled clinical trials with different neuropsychiatric populations currently under investigation should bring important answers and support the translation of research findings to clinical settings.

Parkinson's disease has a progressive course and is characterized by the degeneration of dopaminergic neurons. Although no neuroprotective treatments for Parkinson's disease have been found, the endocannabinoid system has emerged as a promising target. From a sample of 119 patients consecutively evaluated in a specialized movement disorders outpatient clinic, 21 Parkinson's patients without dementia or comorbid psychiatric conditions were selected [55]. The participants were assigned to three groups of seven subjects, each treated with a placebo, cannabidiol 75 mg/day, or cannabidiol 300 mg/day. One week before the trial and in the last week of treatment, participants were assessed concerning (i) motor and general symptoms score (UPDRS), (ii) well-being and quality of life (PDQ-39), and (iii) possible neuroprotective effects (BDNF and H(1)-MRS). No statistically significant differences in UPDRS scores, plasma BDNF levels, or H(1)-MRS measures were indicated.

The groups treated with placebo and cannabidiol 300 mg/day had significantly different mean total scores in the PDQ-39 ($p = 0.05$). The findings point to a possible effect of cannabidiol in improving quality of life measures in Parkinson's disease patients with no psychiatric comorbidities.

Cannabis is widely used to self-medicate by older people and people with brain disorders such as amyotrophic lateral sclerosis, Multiple sclerosis, Alzheimer's disease, Parkinson's disease, bipolar disorder, and schizophrenia. A review was conducted to determine the benefits and adverse mental health effects of *Cannabis* use in people with amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's and Parkinson's diseases, bipolar disorder, and schizophrenia [56].

The studies indicate that *Cannabis* use diminishes some symptoms associated with the disorders. *Cannabis* use decreases pain and spasticity in people with Multiple sclerosis, decreases tremor, rigidity, and pain in people with Parkinson's disease, and improves the quality of life of amyotrophic lateral sclerosis patients by improving appetite and decreasing pain and spasticity. *Cannabis* is more common among people with schizophrenia than healthy controls. *Cannabis* use is a risk factor for schizophrenia which increases positive symptoms in schizophrenia patients and diminishes negative symptoms. *Cannabis* use worsens bipolar disorder, and there is no evidence that bipolar patients derive any benefit from *Cannabis*. In late-stage Alzheimer's patients, *Cannabis* products may improve food intake, sleep quality, and diminish agitation.

Parkinson's disease is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, leading to classical Parkinson's disease motor symptoms. Therapies are purely symptomatic and do not modify the progression of the disease. Cannabidiol exhibits a large spectrum of therapeutic properties,

including anti-inflammatory and antioxidant effects, suggesting its potential as a disease-modifying agent for Parkinson's disease.

A study was conducted to evaluate the effects of chronic treatment with cannabidiol (10 mg/kg, i.p.) on Parkinson's disease-associated neurodegenerative and neuroinflammatory processes and motor deficits in the 6-hydroxydopamine model [57]. The potential mechanisms by which cannabidiol exerted its effects in this model were examined. Cannabidiol-treated animals showed reduced nigrostriatal degeneration, damping of the neuroinflammatory response, and improved motor performance. In particular, cannabidiol exhibits a preferential action on astrocytes and activates the astrocytic transient receptor potential vanilloid 1, thus, enhancing the endogenous neuroprotective response of ciliary neurotrophic factor. The results support the potential therapeutic utility of cannabidiol in Parkinson's disease as both a neuroprotective and symptomatic agent.

Preclinical studies using different models of experimental Parkinson's disease have investigated the effects of both agonists and antagonists of the cannabinoid receptor, used alone or as adjuvants [59]. CB1 agonists inhibit basal ganglia dopamine release and are expected to be ineffective in alleviating Parkinson's disease motor symptoms. CB1 agonists exacerbated bradykinesia in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned primates. However, different CB1 agonists have also been reported to improve motor impairment, possibly through non-dopaminergic mechanisms, including interactions with adenosine A2A and 5-HT receptors. Studies of CB1 antagonists more consistently indicated improvement of motor symptoms.

Blockade of CB1R with rimonabant or other antagonists reduced akinesia and motor impairment in experimental models of Parkinson's disease, although a few other studies showed conflicting results. The effects involve non-dopaminergic mechanisms, including enhanced striatal glutamate release. Moreover, rimonabant was more effective when used at low doses and in very advanced phases of the disease characterized by extreme nigral damage.

Although the results are controversial, the endocannabinoid system might be involved in Levodopa-induced dyskinesia. Although this system is modulated in different experimental models of Parkinson's disease and response to chronic levodopa treatment, whether the changes are compensatory or causal is unknown. Preclinical studies showed that both CB1R agonists and antagonists represent potentially useful antidyskinetic agents [64].

The antidyskinetic effects of cannabinoid receptor agonists are mediated by the normalization of cAMP/PKA signaling and are associated with increased DARPP-32 phosphorylation. As higher doses of CB1 agonists may impair motor function, the effects on levodopa-induced dyskinesia may be related to global motor inhibition. In one study, FAAH inhibitors failed to reproduce the beneficial effects of cannabinoid agonists when given alone. As FAAH inhibitors showed antidyskinetic properties only when combined with a TRPV1 receptor antagonist, it is conceivable that CB1 and TRPV1 receptors operate in opposite directions to control Levodopa-induced dyskinesia.

Another study added more complexity by suggesting that certain cannabinoids (e.g., AEA) may reduce Levodopa-induced dyskinesia by activating PPAR- γ . Beneficial effects were also reported for the PPAR- α receptor endogenous lipid ligand oleoylethanolamide. However, the antidyskinetic effect was attributed to the blockade of TRPV1 receptors rather than the activation of PPAR- α receptors.

Observational studies suggest cannabinoids may improve some motor and non-motor symptoms associated with Parkinson's disease. Two published surveys of Parkinson's disease patients who smoked *Cannabis* were reported to experience some relief in motor and non-motor symptoms. However, the studies present several limitations that could have influenced the results [65]. A small case series indicated no benefit for tremors following a single administration of smoked *Cannabis*. In contrast, a small open-label study assessing motor exam 30 min after smoking *Cannabis* reported improvement in tremor, rigidity, bradykinesia, pain, and sleep [50]. Regarding non-motor symptoms, a small 4-week open-label study of cannabidiol for psychosis in Parkinson's disease found improvement on the Brief Psychiatric Rating Scale and Parkinson's Psychosis Questionnaire, and another small case series reported benefits for rapid eye movement sleep behavior disorder.

Cannabis has been proposed to have therapeutic potential for many neuropsychiatric disorders. In a review, some preclinical and clinical studies on the effects of cannabidiol in neuropsychiatric disorders have been examined. In preclinical studies, cannabidiol was demonstrated to potentially exhibit anti-epileptic, antioxidant, anti-inflammatory, antipsychotic, anxiolytic, and antidepressant properties [66].

Cannabidiol was shown to be safe, well-tolerated, and efficacious in mitigating the symptoms associated with several types of seizures and epilepsy and reducing the addictive effects of some drugs of abuse. Since treatment with cannabidiol alone was insufficient for managing choreic movements in patients with Huntington's disease, other *Cannabis*-derived treatments were investigated. Patients with Parkinson's disease reported improved sleep and quality of life with cannabidiol. The anxiolytic properties of cannabidiol were reported in Social Anxiety Disorder, antipsychotic effects in schizophrenia, and anti-addictive qualities in alcohol and drug addictions.

Delta-9-THC elicits psychoactive effects by modulating dopaminergic neurons, thereby altering dopamine levels in the brain. A case report highlights the complexity associated with medicinal *Cannabis* and the health risks associated with its use [67]. A 57-year-old male with Parkinson's disease was experiencing worsening tremors and vivid hallucinations despite therapy optimization attempts.

It was discovered that the patient took *Cannabis* for chronic back pain, and a pharmacogenomics test indicated the presence of variants for the catechol-O-methyltransferase (COMT) and *HTR2A* genes. The variants could increase dopamine levels and predispose patients to visual hallucinations. Once the *Cannabis* was discontinued, the patient's hallucinations slowly dissipated. This patient case suggests that *Cannabis* use in combination with dopamine-promoting drugs, especially in a patient with genetic variants, can increase the risk for vivid hallucinations. The condition supports the importance of considering herb/drug interactions and pharmacogenomics data when performing medication safety reviews.

To assess *Cannabis* use in Parkinson's disease and multiple sclerosis, a self-reported assessment of neurological disability between *Cannabis* users and non-users was conducted [68]. The anonymous survey collected demographic and *Cannabis* use information and used standardized questionnaires to assess neurological function, fatigue, balance, and physical activity participation. Analysis of variance and chi-square tests were used for the analysis. The survey was viewed 801 times, and 595 participants were involved in the final data set. Seventy-six percent and twenty-four percent of the respondents reported Parkinson's disease and multiple sclerosis, respectively. The users reported high efficacy of *Cannabis*, 6.4 (SD 1.8) on a scale from 0 to 7, and 59% reported reducing prescription medication since beginning *Cannabis* use. *Cannabis* users were younger and less likely to be classified as obese ($p < 0.035$). *Cannabis* users also had lower levels of disability, specifically in mood, memory, and fatigue ($p < 0.040$).

People with Parkinson's disease living in Norway and their caregivers participated in an anonymous online survey to investigate the frequency of use, attitudes toward, and experiences with *Cannabis* and *Cannabis*-related products. In the study, 530 people with Parkinson's disease completed a 24-item survey that included data on the participants' history of *Cannabis* use, perceived benefits and adverse effects of *Cannabis* use, and expectations toward health care professionals. One hundred and eight caregivers also completed a survey detailing their experience with *Cannabis* use.

The use of *Cannabis* is not often reported, and care professionals are not aware of the use. Fifty-nine (11.3%) of people with Parkinson's disease reported previous or current use of *Cannabis*, compared to seven (6.6%) of caregivers. *Cannabis* use was associated with increased disease duration but not age or gender. Improvement in motor function (69.5%), sleep (52.5%), and pain (37.3%) were the most frequently perceived benefits of *Cannabis* use, with benefits more frequently reported by current than previous users. While half (50.8%) of the *Cannabis* users had sought advice from a health care professional regarding *Cannabis* use, 55 (19.9%) of non-users with an interest in *Cannabis* use had discussed their interest with health care professionals [69]. Overall, one in 20 people with Parkinson's disease reports *Cannabis* use, and non-users report widespread interest in *Cannabis*.

Parkinson's disease is the most rapidly growing neurodegenerative disease; its global burden has doubled over the past generation. The onset of Parkinson's disease can arise due to environmental, sporadic, or genetic factors. Nevertheless, most Parkinson's disease cases have an unknown etiology. Chemicals, such as the anthropogenic pollutant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and amphetamine-type stimulants, have been associated with the onset of Parkinson's disease. Conversely, cannabinoids have been associated with the treatment of the symptoms. Parkinson's disease and *Cannabis* is currently under the spotlight, and research to find its benefits on Parkinson's disease is ongoing.

The described clinical applications and safety of pharmacotherapy with *Cannabis* products are still not fully supported by scientific evidence. Psychoactive substances are currently a popular alternative to classical drugs of abuse, representing an unknown health hazard for young adults who may develop Parkinson's disease later

in their lifetime [70]. A review addressed the neurotoxic and neuroprotective impact of illicit substance consumption in Parkinson's disease, presenting clinical evidence and molecular and cellular mechanisms of the association. The research area is utterly important for contemporary society since illicit drug legalization is under discussion, which may have consequences both for the onset of Parkinson's disease and for the treatment of its symptoms.

A study was conducted to determine the effects and tolerability of a range of doses in a Parkinson's disease population and explored the efficacy of a relatively high dose of ~20 mg/kg/day of purified cannabidiol. The study indicated that the relatively high dose of cannabidiol, ~1600 mg/day, with <0.15% delta-9-THC (Epidiolex), taken orally, is associated with mild adverse effects, especially somnolence, fatigue, and diarrhea, and perhaps hepatotoxicity in a population of Parkinson's disease. In the study, somnolence occurred early in dose titration but improved; diarrhea was common and increased with higher doses; the 25 mg/kg/day dose was poorly tolerated.

The adverse effects were similar to those reported in prior studies of pediatric epilepsy at this dose [71]. However, they were more frequent in this study, perhaps due to different characteristics of the Parkinson's disease population or the smaller study sample size. The dose-related adverse event was diarrhea, which could also be related to sesame oil used as a vehicle. In three controlled trials conducted by GW Pharmaceuticals, manufacturer of Epidiolex, diarrhea was reported in 9% of participants on placebo, 9% on 10 mg/kg/day, and 20% on 20 mg/kg/day, indicating that the diarrhea was related to the cannabidiol rather than sesame oil.

In the study, five participants (38%) had transiently elevated liver enzymes, one symptomatic and four asymptomatic, resolving after discontinuing the study drug [72]. The pattern of enzyme changes in the symptomatic patient was consistent with a cholestatic rather than hepatocellular process. The drug may have caused idiopathic or bland cholestasis and, much less likely, granulomatous hepatitis or vanishing bile duct syndrome since all liver tests normalized. None of the participants had significantly elevated bilirubin or internalized normalized ratio, indicating no change in liver function.

While liver enzyme elevations in a hepatocellular pattern have been reported with Epidiolex on similar doses, the cholestatic pattern has not [73]. Factors such as older age, having Parkinson's disease, concomitant medications, and relatively high cannabidiol dosage may have contributed. The results indicated cannabidiol benefits total and motor MDS-UPDRS scores, nighttime sleep, and emotional and behavioral dyscontrol. Interestingly, the *p*-values on the assessments were significantly improved 2 weeks after stopping cannabidiol, compared to baseline. It is possible that cannabidiol was still having some effect since it was still present in the participants' plasma.

The effect of *Cannabis* on motor symptoms and pain parameters in patients with 20 patients with Parkinson's disease, who were licensed to use *Cannabis*, underwent evaluation before and 30 min after *Cannabis* consumption and again after long-term use. Motor function was assessed with the Unified PD Rating scale (UPDRS) by two raters, one blinded. The pain was assessed with the Pain Rating

Index (PRI) and Visual Analog Scale (VAS) of the short-form McGill Pain Questionnaire. Thermal quantitative sensory testing (QST) was performed on 18 patients. The two consecutive QST measurements were validated in 12 *Cannabis*-naïve patients with Parkinson's disease.

There was a significant decrease from baseline to 30 min after *Cannabis* consumption in mean motor UPDRS score (38.1 ± 18 to 30.4 ± 15.6 , $p < 0.0001$), total PRI (27 ± 13.5 to 9.7 ± 11 , $p = 0.001$), and VAS score (6.4 ± 2.8 to 3.6 ± 3.1 , $p = 0.0005$). The mean cold pain threshold decreased significantly in the more affected limb, but only after excluding two patients who consumed *Cannabis* by vaporizer rather than smoking (19.5 ± 5.2 to 15.6 ± 8.7 °C, $p = 0.02$). After long-term (median 14 weeks) exposure, the mean heat pain threshold decreased significantly in the more affected limb in all of the treated patients (43.6 ± 3.5 to 40.9 ± 3.3 °C, $p = 0.05$) and *Cannabis* smokers (43.7 ± 3.6 to 40.3 ± 2.5 °C, $p = 0.008$).

Cannabis improved motor scores and pain symptoms in Parkinson's patients, with a dissociate effect on heat and cold pain thresholds. *Cannabis* modulates peripheral and central pathways. Quantitative sensory test results are significantly altered following *Cannabis* consumption in patients with Parkinson's disease [74]. *Cannabis* probably acts on pain in Parkinson's disease via peripheral and central pathways.

To determine the efficacy of medical *Cannabis* in several neurologic conditions, a systematic review of medical *Cannabis* was conducted to address the treatment of symptoms of multiple sclerosis, epilepsy, and movement disorders [75]. The studies were graded according to the American Academy of Neurology classification scheme for therapeutic articles. The following were studied in patients with multiple sclerosis, spasticity: oral *Cannabis* extract is effective, and nabiximols and delta-9-THC are probably effective for reducing patient-centered measures. Both oral *Cannabis* extract and delta-9-THC were likely effective for reducing both patient-centered and objective measures at 1 year. Central pain or painful spasms, including spasticity-related pain, excluding neuropathic pain: Oral *Cannabis* extract was effective; delta-9-THC and nabiximols are probably effective. Urinary dysfunction: nabiximols are probably effective for reducing bladder voids/day; delta-9-THC and oral *Cannabis* extract are probably ineffective for reducing bladder complaints. Tremor: delta-9-THC and oral *Cannabis* extract are probably ineffective; nabiximols are possibly ineffective. Other neurologic conditions: oral *Cannabis* extract is ineffective for treating levodopa-induced dyskinesia in patients with Parkinson's disease. Oral cannabinoids are of unknown efficacy in non-chorea-related symptoms of Huntington's disease, Tourette's syndrome, cervical dystonia, and epilepsy. The risks and benefits of medical marijuana should be weighed carefully. The risk of serious adverse psychopathologic effects was approximately 1%.

The protective effects of cannabidiol were evaluated in retinoic acid-differentiated SH-SY5Y cells exposed to 1-methyl-4-phenylpyridinium (MPP+) in an in vitro Parkinson's disease model [76]. To evaluate which receptor is involved in cannabidiol actions CB1, CB2, and TRPV1 receptor antagonists were used. Cannabidiol counteracted the loss of cell viability caused by MPP+, reducing apoptosis as

demonstrated by the reduction of Bax and caspase-3. Cannabidiol reduced the nuclear levels of PARP-1. The protective effects of cannabidiol seem to be mediated by the activation of ERK and AKT/mTOR pathways. The treatment with AKT1/2 inhibitor and the mTOR inhibitor rapamycin abolished cannabidiol protective effects. The cannabidiol-induced ERK activation may be mediated by cannabidiol interaction with CB2 and TRPV1. The protein levels of the autophagic proteins LC3 and beclin 1 were also investigated. Cannabidiol reduced the MPP+-induced increase of LC3 by CB2 and TRPV1 receptors. The data indicated the involvement of ERK in the modulation of autophagy. However, beclin 1 level was not modified by MPP+ and by cannabidiol.

Acute and chronic *Cannabis* exposure has been associated with subjective feelings of pleasure and relaxation but also with the onset of psychiatric syndromes, a decrease in the efficacy of neuroleptics, and alterations in the extrapyramidal system regulation of motor activity. The actions point to the association of the cannabinoid system with the brain dopaminergic circuits involved in addiction, and the clinical manifestation of positive symptoms of schizophrenia and Parkinson's disease [76].

Cannabinoid CB1 receptors are present in dopamine-projecting brain areas. In primates and certain rat strains, it is also located in dopamine cells of the A8, A9, and A10 mesencephalic cell groups and hypothalamic dopaminergic neurons controlling prolactin secretion. CB1 receptors co-localize with dopamine D1/D2 receptors in dopamine-projecting fields. Manipulation of dopaminergic transmission can alter the synthesis and release of anandamide and the expression of CB1 receptors. Additionally, CB1 receptors can switch their transduction mechanism to oppose the ongoing dopamine signaling. Acute blockade of the CB1 receptor potentiates dopamine D2 receptor agonists' facilitatory role in the movement [77]. CB1 stimulation results in sensitization to the motor effects of indirect dopaminergic agonists. The dynamics of the changes indicate that the cannabinoid system is an activity-dependent modulator of dopaminergic transmission, a hypothesis relevant for designing new therapeutic strategies for dopamine-related diseases such as psychosis and Parkinson's disease.

The development of levodopa-induced dyskinesia may complicate the long-term treatment of Parkinson's disease. Clinical and animal model data support the view that modulation of cannabinoid function may exert an antidyskinetic effect. A randomized, double-blind, placebo-controlled crossover trial was conducted to examine the effects of *Cannabis* on dyskinesia in Parkinson's disease [78]. A 4-week dose escalation study was performed to assess the safety and tolerability of *Cannabis* in six Parkinson's disease patients with levodopa-induced dyskinesia. Then a randomized placebo-controlled crossover study was performed, in which 19 Parkinson's disease patients were randomized to receive oral *Cannabis* extract followed by placebo or vice versa. Each treatment phase lasted 4 weeks, with an intervening 2-week washout phase.

The primary outcome measure was a change in Unified Parkinson's Disease Rating Scale (UPDRS) (items 32–34) dyskinesia score. Secondary outcome measures included the Rush scale, Bain scale, tablet arm drawing task, and total UPDRS

score following a levodopa challenge, and patient-completed measures of dyskinesia activities of daily living scale, the PDQ-39, on-off diaries, and a range of category rating scales. Seventeen patients completed the randomized, double-blind, placebo-controlled crossover trial. *Cannabis* was well tolerated and had no pro- or anti-Parkinsonian action. There was no evidence of a treatment effect on levodopa-induced dyskinesia as assessed by the UPDRS or any of the secondary outcome measures.

The involvement of neuritogenesis, NGF receptors (trkA), NGF, and neuronal proteins in the mechanism of neuroprotection by cannabidiol against MPP(+) toxicity in PC12 cells were investigated. Cannabidiol increased cell viability, differentiation, and the expression of axonal (GAP-43) and synaptic (synaptophysin and synapsin I) proteins [79]. Its neuritogenic effect was not dependent or additive to NGF, but it was inhibited by K252a (trkA inhibitor). Cannabidiol did not increase the expression of NGF but protected against its decrease induced by MPP(+), probably by an indirect mechanism. The neuritogenesis in SH-SY5Y cells did not express trkA receptors. The study reports the involvement of neuronal proteins and trkA in the neuroprotection of cannabidiol. The findings suggest that cannabidiol has a neurorestorative potential independent of NGF that might contribute to its neuroprotection against MPP(+), a neurotoxin relevant to Parkinson's disease.

Cannabinoid type 1 receptor is among the most abundant receptors expressed in the central nervous system. It is mainly located on the axon terminals of presynaptic neurons. It participates in the modulation of neuronal excitability and synaptic plasticity, playing an important role in the pathogenesis of various neuropsychiatric diseases [80]. In recent years, the consistent development of CB1R radioligands and the maturity of molecular imaging techniques, particularly positron emission tomography (PET), may help to visualize the expression and distribution of cannabinoid type 1 receptors in the central nervous system. Cannabinoid type 1 receptor PET imaging can effectively evaluate the changes of cannabinoid type 1 receptor levels in neuropsychiatric diseases such as Huntington's disease and schizophrenia, and its correlation with the disease severity, therefore providing new insights for the diagnosis and treatment of neuropsychiatric diseases such as Alzheimer's, Parkinson's, and Huntington's diseases, schizophrenia, post-traumatic stress disorder, *Cannabis* use disorder, and depression.

Parkinson's disease affects the basal ganglia and is characterized by tremors, rigidity, bradykinesia, and muscle stiffness. The piecemeal loss of melanin-containing nigral neurons leads to aberrant dopaminergic transmission, with tremors emerging due to subthalamic overactivity [81]. The increase in the levels of endocannabinoids and alteration in both CB1R and CB2R expression in different stages of Parkinson's disease opens the possibility of manipulation of receptor function in managing the disease [65].

Studies have demonstrated that, in the early stages of Parkinson's disease, CB1R is downregulated, while in advanced and symptomatic phases, there is a switch to CB1R upregulation [82]. The findings may suggest a basis for the use of cannabinoid antagonists in order to alleviate the motor deficits associated with advanced Parkinson's disease. Despite findings supporting this suggestion, it failed to show

the proficiency of the CB1R antagonist SR141716A (rimonabant) in mitigating symptoms in a non-human primate model of Parkinson's disease. In a study using tacrin, an acetylcholinesterase inhibitor, to induce Parkinsonian tremor, pretreatment with rimonabant was without significant effect on tremor bursts, whereas in combination with the adenosine A2A receptor antagonist SCH58261, a marked reduction in tremor bursts was seen; however, SCH58261 produced a pronounced decrease in tremor bursts [83].

A clinical report suggests no advantageous effects of *Cannabis* on tremors among five unresponsive patients to anticholinergics and beta blockers [84]. By contrast, a questionnaire-based study revealed a 30.6% improvement in rest tremors in tremulous patients with Parkinson's disease. A plausible argument for using cannabinoid agonists as therapeutic agents has arisen from studies describing the inhibitory effect of CB1R activation on glutamate release and its capability to oppose subthalamonigral neuronal overactivity and subsequently reduce tremors. It has also been suggested that cannabinoid agonists and endocannabinoids can block dopamine transporters (DAT1), inhibiting dopamine reuptake from ameliorating dyskinesia [85]. However, other studies assert no beneficial effects of cannabinoid agonists in alleviating motor symptoms.

In an open-labeled observational study, the efficacy of *Cannabis* treatment on patients who had experienced severe Parkinson's disease-related pain and tremor and whose symptoms were insufficiently controlled with current anti-Parkinson's medications was assessed; the results indicated a significant improvement in tremor and rigidity, with a lesser beneficial effect on bradykinesia, among *Cannabis* smokers. Alterations to the endocannabinoid system may play a role in Parkinsonian tremor pathogenesis. A range of individual cannabinoids should be examined as potential therapeutic agents for managing signs and symptoms of Parkinson's disease, in particular, tremors.

A study examined the prevalence and characteristics of pain and response to pain medications in patients with Parkinson's disease and Parkinson's plus syndromes. The cohort consisted of 371 patients: 300 (81%) with Parkinson's disease and 71 (19%) with Parkinson's plus syndromes [86]. Data on clinical parameters and pain were collected by questionnaire. Disease severity was measured with the Unified PD Rating Scale for patients with Parkinson's disease. Two hundred seventy-seven patients reported pain (74%): 241 with Parkinson's disease and 36 with Parkinson's plus syndromes.

The prevalence of pain was significantly higher in the patients with Parkinson's disease than in the patients with Parkinson's plus syndromes (80% vs. 50%, $p < 0.001$) and higher in patients with synucleinopathies than in patients with tauopathies (70% vs. 40%, $p < 0.001$). In the synucleinopathies, the most common pain was central pain (32%), whereas in the tauopathies, only 4% of patients had central pain. Anti-Parkinson's treatment relieved the pain in 21% of the patients with Parkinson's disease. Only 114 patients (48%) experienced pain and were treated with pain medications. The most beneficial analgesics were nonsteroidal anti-inflammatory drugs and medical *Cannabis*.

Musculoskeletal orofacial pain is a complex symptom of Parkinson's disease resulting in stomatognathic system dysfunctions aggravated by the disease's rigidity and postural instability. The effect of cannabidiol was tested in Parkinson's disease-related myofascial pain [87]. Adult Wistar adult female and male rats' orofacial allodynic and hyperalgesic responses were tested by Von Frey and formalin tests before and 21 days after the 6-OHDA lesion. The algesic response was tested after masseter muscle injection of cannabidiol at 10, 50, and 100 μg in 10 μL and vehicle. Compared to females in all estrous cycle phases, males presented reduced orofacial allodynia and hyperalgesia. In the estrous cycle's phases, females presented distinct orofacial nociceptive responses for nociceptive analysis after the 6-OHDA lesion.

Dopaminergic neuron lesions decreased mechanical and inflammatory nociceptive thresholds in females and males in a higher proportion in females. Cannabidiol local treatment reduced the increased orofacial allodynia and hyperalgesia in males and females. The female rats were more sensitive to the cannabidiol effect considering allodynia, responding to the lowest dose. Although females and males respond to the effect of three doses of cannabidiol in the formalin test, males showed a superior reduction in the hyperalgesic response. The results indicate that hemi-Parkinsonian females in the estrus phase and males respond differently to the different doses of cannabidiol therapy and nociceptive tests.

The discovery of specific cannabinoid receptors and a family of endogenous ligands of those receptors has attracted much attention to cannabinoids. One of the most exciting and promising areas of current cannabinoid research is the ability of the compounds to control the cell survival/death decision. Cannabinoids may induce proliferation, growth arrest, or apoptosis in some cells, including neurons, lymphocytes, and transformed neural and non-neural cells. The variation in drug effects may depend on experimental factors such as drug concentration, the timing of drug delivery, and the type of cell examined.

Regarding the central nervous system, most experimental evidence indicates that cannabinoids may protect neurons from toxic insults such as glutamatergic overstimulation, ischemia, and oxidative damage. In contrast, cannabinoids induce apoptosis of glioma cells in culture and regression of malignant gliomas in vivo. Breast and prostate cancer cells are also sensitive to cannabinoid-induced antiproliferation. Regarding the immune system, low doses of cannabinoids may enhance cell proliferation, whereas high doses of cannabinoids usually induce growth arrest or apoptosis [88]. The neuroprotective effect of cannabinoids has potential clinical relevance for treating neurodegenerative disorders such as multiple sclerosis, Parkinson's disease, and ischemia/stroke. In contrast, their growth-inhibiting action on transformed cells might be useful for managing malignant brain tumors.

Regular *Cannabis* use induces a decreased capacity for dopamine synthesis in the brain. The retina is considered an easy way to investigate synaptic transmission dysfunctions in the brain. The impact of regular *Cannabis* use on retinal function has been studied [89]. Using the N95 wave of the pattern electroretinogram, a 6 ms-delayed ganglion cells response was revealed. Using the b-wave of the photopic flash electroretinogram, a 1 ms-delayed bipolar cells response was found. Amacrine cells' function was investigated because the cells are located between the bipolar

cells and the ganglion cells and contribute to amplifying the signal between the two retina layers. The effect of regular *Cannabis* use on the retinal dopaminergic cells was tested, and the role of the cells in amplifying the delay observed previously was assessed.

Oscillatory potentials could be used as markers of central dopaminergic modulation. Dark-adapted 3.0 Flash ERG oscillatory potentials were recorded in 56 regular *Cannabis* users and 29 healthy controls, and the amplitude and implicit time of OP1, OP2, OP3, and OP4 were evaluated. *Cannabis* users showed a significant decrease in OP2 amplitude ($p = 0.029$, Mann-Whitney test) and OP3 amplitude ($p = 0.024$, Mann-Whitney test). No significant difference was found between the groups for OP1 and OP4 amplitude or for the implicit time of oscillatory potentials. The results reflect the impact of regular *Cannabis* use on amacrine cell function. It highlights abnormalities in the dopaminergic transmission that are similar to those found in Parkinson's disease.

In Parkinson's disease patients, cannabidiol can attenuate the psychotic symptoms induced by L-DOPA and improve quality of life. Repeated administration of reserpine in rodents induces motor impairments accompanied by cognitive deficits and has been applied to model both tardive dyskinesia and Parkinson's disease.

A study investigated whether cannabidiol administration would attenuate reserpine-induced motor and cognitive impairments in male Wistar rats receiving four injections of cannabidiol (0.5 or 5 mg/kg) or vehicle (days 2–5) [97]. On days three and five, the animals received one injection of 1 mg/kg reserpine or vehicle. Locomotor activity, vacuous chewing movements, and catalepsy were assessed from day one to day seven. On days eight and nine, the animals' performance on the plus-maze discriminative avoidance task was evaluated for learning/memory assessment.

The data indicated that cannabidiol could attenuate motor and cognitive impairments induced by reserpine, a compound in the pharmacotherapy of Parkinson's disease and tardive dyskinesia. Cannabidiol attenuated the increase in catalepsy behavior and oral movements but not the decrease in locomotion induced by reserpine. Cannabidiol (0.5 mg/kg) also ameliorated the reserpine-induced memory deficit in the discriminative avoidance task.

Preclinical and clinical data have suggested that cannabidiol shares more similarities with atypical antipsychotics than typical, which are customarily used to manage schizophrenia symptoms. The molecular pathways modulated by cannabidiol were compared with the antipsychotics clozapine (atypical) and haloperidol (typical), and the effects of benztropine, a muscarinic receptor antagonist that displays a protective effect in oligodendrocytes and myelination. Nano-chromatography coupled with mass spectrometry was used to investigate the proteomic response to the drugs in healthy oligodendrocytic cells and a cuprizone-based toxicity model using the human oligodendrocyte precursor cell line MO3.13.

Cannabidiol shares similarities in biochemical pathways with clozapine and benztropine, in agreement with other studies that indicated an atypical antipsychotic profile. All drugs tested affected metabolic and gene expression pathways, and cannabidiol, benztropine, and clozapine modulated cell proliferation and apoptosis

when administered after cuprizone-induced toxicity. The general pathways are associated with cuprizone-induced cytotoxicity in MO3.13 cells, indicating a possible proteomic approach when acting against the toxic effects of cuprizone [98]. However, modeling oligodendrocytic cytotoxicity with cuprizone does not represent the entirety of the pathophysiology of oligodendrocyte impairments. The results provide insight into the mechanisms associated with the effects of cannabidiol and antipsychotics against cuprizone toxicity, offering new study directions for myelin-related processes and deficits.

Cannabis has been an adjunctive treatment for Parkinson's disease. Haloperidol is a classic antipsychotic drug known for its propensity to cause extrapyramidal symptoms due to the blockade of dopamine D2 receptors in the striatum. The effect of repeated administration of delta-9-tetrahydrocannabinol on catalepsy and brain oxidative stress induced by haloperidol administration in mice was investigated.

Delta-9-tetrahydrocannabinol was given subcutaneously at 5, 10, or 20 mg/kg daily for 18 days, and the effect on 1 mg/kg haloperidol, administered intraperitoneally, was examined for haloperidol-induced catalepsy at selected time intervals using the bar test. Malondialdehyde (MDA), reduced glutathione (GSH), and nitric oxide were determined in the brain and liver [99]. The mice were euthanized 18 days after starting the *Cannabis* injection.

In saline-treated mice, no catalepsy was observed at doses of *Cannabis* up to 20 mg/kg. The mice treated with haloperidol at 1 mg/kg exhibited a significant cataleptic response. Mice treated with *Cannabis* and haloperidol showed a significant decrease in catalepsy duration compared to those treated with haloperidol. The decrease in catalepsy duration was evident on days one to 12 after starting the *Cannabis* treatment. Later, the effect of *Cannabis* was not apparent.

The administration of only *Cannabis*, at doses of 10 and 20 mg/kg, decreased brain MDA by 17.5% and 21.8%, respectively. The level of nitric oxide was decreased by 18% after the administration of *Cannabis* at the dose of 20 mg/kg. Brain glucose decreased by 20.1% after 20 mg/kg of *Cannabis*. The administration of haloperidol increased MDA by 22.2%, decreased GSH by 25.7%, and increased brain nitric oxide by 44.1%. The administration of *Cannabis*, 10 and 20 mg/kg, to haloperidol-treated mice resulted in a significant decrease in brain MDA and nitric oxide and a significant increase in GSH and glucose compared to the haloperidol-control group. *Cannabis* had no significant effects on liver MDA, GSH, and nitric oxide in the saline- or haloperidol-treated mice. *Cannabis* improves catalepsy induced by haloperidol, but the effect is not maintained on repeated *Cannabis* administration. *Cannabis* alters the oxidative status of the brain in favor of reducing lipid peroxidation but reduces brain glucose.

The use of illicit stimulants such as methamphetamine, cocaine, and ecstasy is an increasing health problem. Chronic use can cause neurotoxicity in animals and humans, but the long-term consequences are still not obvious. A study was conducted to investigate the long-term effect of stimulant use on the morphology of the human substantia nigra [100]. The morphology of the substantia nigra was assessed in abstinent stimulant users ($n = 36$; 31 ± 9 years) and two groups of control subjects: non-drug users ($n = 29$; 24 ± 5 years) and *Cannabis* users ($n = 12$; 25 ± 7 years).

Substantia nigra morphology was viewed with transcranial sonography, and the area of echogenicity at the anatomical site of the substantia nigra was measured to its greatest extent. The area of substantia nigra echogenicity was significantly larger in the stimulant group, $0.273 \pm 0.078 \text{ cm}^2$, than in the control $0.201 \pm 0.054 \text{ cm}^2$; $p < 0.001$, and *Cannabis* groups, $0.202 \pm 0.045 \text{ cm}^2$; $p < 0.007$.

The study results indicated that individuals with a history of illicit stimulant use exhibit abnormal substantia nigra morphology. Fifty-three percent of stimulant users exhibited echogenicity that exceeded the ninetieth percentile for the control group. Substantia nigra hyperechogenicity is a strong risk factor for developing Parkinson's disease later in life.

Parkinson's disease is associated with dopaminergic neuronal loss in the basal ganglia. Several other neurotransmitters, such as serotonin, gamma-aminobutyric acid, and glutamate, are also related to the symptoms of Parkinson's disease patients and their response to levodopa treatment. The co-expression of cannabinoid and dopamine receptors in the basal ganglia indicates a potential role for endocannabinoids in the control of voluntary movement in Parkinson's disease. In one study, unilaterally 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine)-lesioned rats were treated with the enantiomers of the synthetic cannabinoid, 7-hydroxy-delta6-tetrahydrocannabinol 1,1-dimethylheptyl [101].

The treatment with (–)- (3R, 4R), the enantiomer (code-name HU-210), a potent cannabinoid receptor type 1 agonist, reduced the rotations induced by levodopa/carbidopa or apomorphine by 34% and 44%, respectively. In contrast, treatment with the (+)- (3S, 4S) enantiomer (code-name HU-211), an N-methyl-D-aspartate antagonist, as well as the psychotropically inactive *Cannabis* constituent, cannabidiol and its primary metabolite, 7-hydroxy-cannabinol, produced no reduction of rotational behavior. The results indicate that activation of the CB1 stimulates the dopaminergic system ipsilaterally to the lesion and may have implications in treating Parkinson's disease.

The molecular mechanisms underlying the antidepressant-like effects of cannabidiol are not clearly understood. Studies in differentiated skin cells demonstrated that cannabidiol regulates DNA methylation, an overall repressive epigenetic mechanism. Stress exposure and antidepressant treatment can modulate DNA methylation in the brain and lead to gene expression changes associated with depression neurobiology. The antidepressant effect of cannabidiol association with changes in DNA methylation was investigated in the prefrontal cortex and hippocampus of mice submitted to the forced swimming test [102].

The behavioral effects induced by cannabidiol and DNA methylation inhibitors (DNMTi: 5-AzaD and RG108), alone or in association, and the effects induced by cannabidiol and DNMTi in global DNA methylation and DNMT activity in the prefrontal cortex and hippocampus were examined. The results indicated that the treatment with cannabidiol, at a dose of 10 mg/kg and 5-AzaD and RG108 at a dose of 0.2 mg/kg, induced an antidepressant-like effect in the prefrontal cortex. Similar effects were observed after combining sub-effective doses of cannabidiol, 7 mg/kg, and 5-AzaD or cannabidiol at 7 mg/kg and RG108 at 0.1 mg/kg. Also, the stress reduced DNA methylation and DNMT activity in the hippocampus and increased it

in the prefrontal cortex. Cannabidiol and DNMTi treatment prevented the changes in both brain structures. The results indicate that cannabidiol regulates DNA methylation in brain regions relevant to depression neurobiology, indicating that the mechanism could be related to cannabidiol-induced antidepressant effects.

Sonographic appearance of the substantia nigra is abnormally bright and enlarged (hyperechogenic) in young adults with a history of illicit stimulant use. The abnormality is a risk factor for Parkinson's disease. A study was conducted to identify the type of illicit stimulant drug associated with substantia nigra hyperechogenicity and to determine if individuals with a history of illicit stimulant use exhibit clinical signs of Parkinsonism [103]. The area of echogenic signal in the substantia nigra was measured in abstinent human amphetamine users ($n = 27$; 33 ± 8 years) and in three control groups comprising 'ecstasy' users ($n = 19$; 23 ± 3 years), (b) *Cannabis* users ($n = 30$; 26 ± 8 years), and non-drug users ($n = 37$; 25 ± 7 years). A subset of subjects ($n = 55$) also underwent a neurological examination comprising the third and fifth part of the Unified Parkinson's Disease Rating Scale.

The area of substantia nigra echogenicity was significantly larger in the amphetamine group (0.276 ± 0.080 cm²) than in the control groups (0.200 ± 0.075 , 0.190 ± 0.049 , 0.191 ± 0.055 cm²), respectively ($p < 0.002$). The score on the clinical rating scale was also significantly higher in the amphetamine group (8.4 ± 8.1) than in pooled controls (3.3 ± 2.8 ; $p = 0.002$). The Illicit use of amphetamines is associated with abnormal substantia nigra morphology and subtle clinical signs of Parkinsonism. The results support epidemiological findings linking the use of amphetamines, particularly methamphetamine, with an increased risk of developing Parkinson's disease later in life.

Cannabidiol exhibits therapeutic potential for chronic neurodegenerative diseases, such as Alzheimer's and Parkinson's, ischemic stroke, epilepsy, and other convulsive syndromes, neuropsychiatric disorders, neuropathic allodynia, and certain types of cancer. Cannabidiol does not bind directly to endocannabinoid receptors 1 and 2. The sigma 1 receptor ($\sigma 1R$) antagonists inhibit glutamate N-methyl-D-aspartate acid receptor (NMDAR) activity and positively affect most diseases. Thus, the effects of cannabidiol were investigated on three animal models in which NMDAR overactivity plays a critical role: opioid analgesia attenuation, NMDA-induced convulsive syndrome, and ischemic stroke [104].

In an in vitro assay, cannabidiol disrupted the regulatory association of $\sigma 1R$ with the NR1 subunit of NMDAR, an effect shared by $\sigma 1R$ antagonists, such as BD1063 and progesterone, and prevented by $\sigma 1R$ agonists, such as 4-IBP, PPCC, and PRE084. The in vivo administration of cannabidiol or BD1063 enhanced morphine-evoked supraspinal antinociception, alleviated NMDA-induced convulsive syndrome, and reduced the infarct size caused by permanent unilateral middle cerebral artery occlusion. The positive effects of cannabidiol were reduced by the $\sigma 1R$ agonists PRE084 and PPCC and were absent in $\sigma 1R$ -/- mice. Thus, cannabidiol displays antagonist-like activity toward $\sigma 1R$ to reduce the negative effects of NMDAR overactivity.

Studies have revealed that cannabidiol could interconvert into delta-8- and delta-9- tetrahydrocannabinol. The plasma samples of 120 healthy human subjects,

60 male and 60 female, 60 in fasting and 60 under normal feeding conditions after the acute administration of an oral solution containing 300 mg of cannabidiol [105]. Delta-9-THC was not detected in the plasma after the administration of cannabidiol, and the study participants did not present psychotomimetic effects. The findings are consistent with previous evidence that the oral administration of cannabidiol in a corn oil formulation is a safe route for administering the active substance without bioconversion to delta-9-THC in humans.

Cannabinoids present neurotoxic and neuroprotective properties in in vitro studies, inconsistent alterations in human neuroimaging studies, neuropsychological deficits, and an increased risk for psychotic episodes. Proton magnetic resonance spectroscopy ((1)H-MRS), neuropsychological testing, and hair analysis for cannabinoids were performed on 13 male non-treatment-seeking recreational *Cannabis* users and 13 male control subjects [106]. A significantly diminished N-acetylaspartate/total creatine (NAA/tCr) ratio in the dorsolateral prefrontal cortex was observed in the *Cannabis* users ($p = .0003$). The NAA/tCr in the putamen/globus pallidum region correlated significantly with cannabidiol ($R(2) = .66, p = .004$).

The results of the Wisconsin Card Sorting test, Trail Making Test, and D2 test for attention were influenced by cannabinoids. Chronic recreational *Cannabis* use is associated with an indication of diminished neuronal and axonal integrity in the DLPFC. As chronic *Cannabis* use is a risk factor for psychosis, the results are interesting because diminished NAA/tCr ratios in the dorsolateral prefrontal cortex and neuropsychological deficits were also reported in schizophrenia. The strong positive correlation of NAA/tCr and cannabidiol in the putamen/globus pallidum is in line with the neuroprotective properties of cannabidiol, which were also observed in in vitro model studies of Parkinson's disease.

Hempseed is rich in polyunsaturated fatty acids, which have the potential as therapeutic compounds for the treatment of neurodegenerative and cardiovascular diseases. The effects of the intake of hempseed meal and polyunsaturated fatty acids were assessed on oxidative stress, cytotoxicity, neurological phenotypes, and cholesterol uptake using *Drosophila* models [107].

The intake of hempseed meal, and linoleic or linolenic acids, the major polyunsaturated fatty acids components of hempseed meal, was shown to ameliorate A β 42-induced eye degeneration, thus revealing that the compounds exert a protective effect against A β 42 cytotoxicity. Hempseed meal intake was shown to reduce H $_2$ O $_2$ toxicity markedly, indicating that hempseed meal exerts a profound antioxidant effect. Locomotion and longevity in the Parkinson's disease model and eye degeneration in the Huntington's disease model were unaffected by hempseed meal feeding.

The results indicate that hempseed meal and linoleic acid exert inhibitory effects on both A β 42 cytotoxicity and cholesterol uptake and are potential candidates for treating Alzheimer's and cardiovascular diseases. The hempseed meal or linoleic acid intake was also shown to reduce cholesterol uptake significantly. Linoleic acid intake has been shown to delay pupariation, and cholesterol feeding rescued the linoleic acid-induced larval growth delay, indicating that linoleic acid acts antagonistically with cholesterol during larval growth.

Progressive supranuclear palsy (PSP) is a severe, debilitating, and often fatal disease resembling other neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, which have been successfully treated with cannabinoids. The case of a 71-year-old woman diagnosed with progressive supranuclear palsy, displaying severe impairment of motor and language functions. The patient had a history of 6 years of progressive gait disturbance, Parkinsonism, and severe language disturbance. She had lost the capacity to produce intelligible speech except for moans and unintelligible noises (dysarthria) and displayed ophthalmoplegia. She was also unable to walk and had rigidity in her limbs. Serum levels of folic acid, total calcium, creatinine, iron, ferritin, magnesium, phosphorus, and vitamin B12 were within normal ranges.

A brain MRI was performed and indicated (a) mesencephalon-pontine disproportion characterized by marked reduction of the midbrain, with relative preservation of the pons associated with loss of the usual morphology of the midbrain, with dorsolateral concavity and reduction of anteroposterior diameter; (b) reduction of thalamic volume and narrowing of superior cerebellar peduncles, in addition to hypersignal of periaqueductal gray matter on FLAIR and T2WI; (c) signs of neurodegeneration with T2SE-hyposignal mainly of the midbrain and posterolateral aspect of putamens, coinciding with volumetric reduction; (d) large cerebellar fissures, inferring some degree of cerebellar volumetric reduction; (e) enlargement of cortical and cerebellar sulci and brain fissures, predominantly in frontal and insular regions; (f) rare sparse foci with T2/FLAIR hyperintense signal without diffusion restriction on the white matter of the cerebral hemispheres, around the anterior horns and atria of the lateral ventricles, in the semioval nuclei/radiated crown and in the subcortical frontoparietal regions.

A full extract of *Cannabis* oil with the approximate ratios of the main cannabinoids of 0.5% cannabidiol, 2% delta-9-THC, 10% CBDA (cannabidiolic acid), and 7% THCA (tetrahydrocannabinolic acid) (approximately 11 mg/mL of cannabidiol + CBDA and 9 mg/mL of delta-9-THC + THCA) was prescribed, beginning with two sublingual drops at night and doubling the dose weekly [108]. The oil was acquired by the patient's family from ABRACE (a non-profit organization founded by relatives of children with refractory epilepsy that benefited from *Cannabis* oil and was conferred the right to grow, extract, and commercialize *Cannabis* products in Brazil). There were no side effects attributable to the oil.

After 3 weeks, an improvement in dysarthria was perceived. The patient started to articulate words, although still in an unintelligible manner, attempting to communicate. One week later, she improved word articulation and presented a slight return of gaze function. After 5 weeks of *Cannabis* oil administration, the patient's oral output was further improved, and the family and clinical staff could understand her speech's words and meaning. The patient also showed improved hand tremors and regained the ability to move her limbs. Finally, the patient could also stand on her feet and walk with assistance.

Homeodomain-interacting protein kinase 2 is a conserved serine/threonine kinase that regulates transcription, cell differentiation, proliferation, and apoptosis. Homeodomain-interacting protein kinase 2 could be involved in the pathogenesis of

neurodegenerative diseases as a target for Parkinson's disease therapeutic development. Gene microarray analysis was performed to verify the key regulatory function of Homeodomain-interacting protein kinase 2 in Parkinson's disease. (Z)-methylp-hydroxycinnamate with 18 other compounds were isolated from *Cannabis sativa* subsp. *Sativa*, growing in Bama Yao Autonomous County [109].

(Z)-Methylp-hydroxycinnamate was identified to bind Homeodomain-interacting protein kinase 2 with high affinity through molecular modeling and molecular dynamics simulations. Cell morphology, flow cytometry, and western blot assay suggested that (Z)-methylp-hydroxycinnamate inhibited homeodomain-interacting protein kinase 2, which attenuated MPP⁺-induced apoptosis in SH-SY5Y cells. The findings revealed a natural product that inhibits homeodomain-interacting protein kinase 2 and highlighted that (Z)-methylp-hydroxycinnamate could be a potential precursor agent for Parkinson's disease therapy.

Iron accumulation has been described in several brain regions and cell types in normal aging. However, in neurological disorders such as Alzheimer's, Parkinson's, and Huntington's, iron accumulates in selective brain areas such as the hippocampus, substantia nigra, cortex, and basal ganglia, regions relevant to disease-associated neurodegenerative processes [110]. The mechanisms that underlie neurotoxicity induced by iron and other metals still need to be completely understood. It has been described that iron overload induces severe and persistent long-term impairments in spatial, recognition, and emotional memories [111].

In molecular analyses, it was found that lipid peroxidation and oxidative damage were associated with iron excess, increased apoptotic markers, Par4 and caspase-3, accumulation of ubiquitinated proteins, and reactive gliosis. Iron treatment in the neonatal period decreased acetylcholinesterase activity in the striatum and affected the regulation of iron homeostasis proteins in the hippocampus, cortex, and striatum of aged rats [112]. Also, there was a decrease in synaptophysin levels, a marker of synaptic viability, and changes in DNAIL levels, a protein critically involved in mitochondrial dynamics in the hippocampus of iron-treated rats. Iron chelation has been demonstrated to prevent memory impairments and oxidative stress in aged rats, supporting that cognitive deficits associated with aging might be related to iron accumulation in the brain.

Apoptosis is a major form of programmed cell death implicated in neurodegenerative disorders. Studies have consistently reported deregulations in the expression of apoptotic proteins in the brains of both Parkinson's disease and Alzheimer's disease patients and experimental models of neurodegenerative disorders [114]. Among the stimuli known to trigger apoptosis are alterations of the redox balance and oxidative damage [113]. Cannabidiol was investigated as a potential therapeutic option for neurodegenerative disorders. Studies indicate that cannabidiol has antioxidant, antiapoptotic, and neuroprotective properties. Studies have shown that cannabidiol improves iron-induced memory deficits and regulates markers of synaptic viability and mitochondrial dynamics in the hippocampus of iron-overloaded rats.

Iron accumulation in the brain has been recognized as a common feature of normal aging and neurodegenerative diseases. Cognitive dysfunction has been associated with iron excess in brain regions in humans. There is evidence that iron overload

leads to severe memory deficits, including spatial, recognition, and emotional memory impairments in adult rats. In an attempt to establish a causative role of excessive iron on cell death in the nervous system leading to memory dysfunction, the effects of neonatal iron overload were investigated on proteins involved in apoptotic pathways, such as Caspase-8, Caspase-9, Caspase-3, Cytochrome C, APAF1, and PARP in the hippocampus of adult rats. Cannabidiol was examined as a potential drug to reverse iron-induced effects on the parameters analyzed. Male rats received vehicle or iron carbonyl, at a dose of 30 mg/kg, from the twelfth to the fourteenth postnatal days and were treated with vehicle or cannabidiol at a dose of 10 mg/kg for 14 days in adulthood.

The results indicated that iron could trigger cell death pathways by inducing intrinsic apoptotic proteins. Iron increased caspase-9, cytochrome C, APAF1, caspase-3, and cleaved PARP without affecting cleaved caspase-8 levels. Cannabidiol reversed iron-induced effects, recovering apoptotic proteins caspase-9, APAF1, and caspase-3 and cleaved PARP to the levels found in controls. The reversal of iron-induced effects by cannabidiol indicates the neuroprotective potential through its antiapoptotic action [110]. The results revealed that neonatal iron treatment led to significant changes in the concentration of apoptotic proteins, increasing the intrinsic apoptotic pathway proteins.

Cannabis Use and Dopaminergic Gene Polymorphism

Twenty-three million individuals regularly use *Cannabis*. Regular *Cannabis* use may be detrimental to the brain and associated with neuropsychological, emotional, and motivational impairments. The hippocampus, in particular, may be especially sensitive to the impact of heavy *Cannabis* use, given its high concentration of cannabinoid receptors type 1, evidenced by neuroimaging results of dose-dependent hippocampal volume reductions in regular *Cannabis* users [90]. Hippocampal alteration has not been consistently observed across studies, although it remains unclear why hippocampus volumetric reduction has not been consistently observed in *Cannabis* users. Evidence suggests that non-*Cannabis*-specific vulnerability factors, including dopamine gene polymorphism, may have played a role in this heterogeneity [91].

A dopamine gene polymorphism may also contribute to adverse mental health outcomes in *Cannabis* users. Given the rising prevalence of *Cannabis* use, treatment seekers, and the global trends toward decriminalization of *Cannabis* products, it is vital to address the heterogeneity in *Cannabis* use-related effects across users by examining the contribution of dopamine gene polymorphism and *Cannabis* use on hippocampal morphology [92].

The neuroanatomy of the hippocampus and hippocampal subregions – subiculum, presubiculum, cornu ammonis (CA) subfields CA1-4, dentate gyrus (DG), and fimbria are affected by variation in the expression of genes implicated in dopamine regulation. The genes include the catechol-O-methyltransferase (COMT) gene, the

dopamine transporter (DAT1) gene, and the brain-derived neurotrophic factor (BDNF) gene, all of which are highly expressed within the hippocampus. The COMT gene codes for the enzyme that metabolizes dopamine and may present as one of three variants (i.e., a single nucleotide polymorphism (SNP) at *Val158Met*, rs4680): *val/val*, *val/met*, and *met/met*, which are associated with the low, medium, and high dopamine availability, respectively.

The DAT1 gene, which is involved in dopamine transport and reuptake, consists of a polymorphic 40-base pair variable number of tandem repeats (VNTR) in an untranslated region and has common variants, including the 9-repeat (9/9R) and the 10-repeat allele (10/10R) associated with high and low dopamine availability, respectively [93]. The BDNF gene is involved in neuronal plasticity and dopamine receptor expression. It may present with an SNP (*Val66Met*) (rs6265), which can lead to a significant reduction in BDNF trafficking to secretory granules and, as a result, to reduced BDNF production in *met* carriers [94].

The effect of hippocampal volumes by *Cannabis* use $\times$ COMT–DAT1 cross-products revealed that COMT, DAT1, and BDNF polymorphisms affect the neuroanatomy of the hippocampus and its subregions. The gene variants may further interact with *Cannabis* use to affect cognitive functions ascribed to the hippocampus (e.g., verbal and visuospatial learning). It remains unclear whether polymorphisms in genes other than COMT, linked to dopamine regulation, such as DAT1 and BDNF, affect hippocampus volume and its subregions in *Cannabis* users. The combined effects of *Cannabis* and genetic polymorphism (i.e., DAT1, COMT, and BDNF) on the hippocampus volume and its subregions were quantified using a validated, highly reliable hand-tracing method. Reduction in hippocampal (total and subregional) volumes was explored in *Cannabis* users versus controls and *Cannabis* users carrying variants linked to high relative to low dopamine level (i.e., COMT *met* vs. *val/val*, DAT1 9/9R vs. 10/10R, and BDNF *val/val* vs. *met* allele carriers), as elevated extracellular dopamine levels may mediate neurotoxic effects.

Fifty-nine male participants, including 30 *Cannabis* users and 29 controls, were matched by age, intelligence quotient, and education, symptoms of depression, anxiety, and psychosis. The participants underwent a detailed medical history check, physical/neurological examinations, and urine and hair toxicology tests through immunometric assay kits (Instant-View, ASD, Inc., Poway, CA). Exclusion criteria for all participants were (i) lifetime Axis I, DSM-IV disorder apart from nicotine use disorder, or *Cannabis* use disorder in *Cannabis* users; (ii) positive urine toxicology return for opiates, cocaine, amphetamines, and benzodiazepines; (iii) current psychoactive medications; (iv) history of chronic medical illness or neurological conditions; (v) head trauma with loss of consciousness for more than 2 min; (vi) left-handedness; and (vii) uncorrected visual impairment, color blindness, and hearing impairment.

The COMT *val108/158met* (rs4680) and BDNF *val66met* (rs6265) SNP allelic variants were determined using the 59 exonucleases TaqMan assay with ABI 7900HT Sequence Detection System (real-time PCR) supplied by Applied Biosystems. After genotype determination, the sample was divided into subgroups based on (i) COMT polymorphism (i.e., *val/val* homozygote and *met*-allele carriers

including *val/met* and *met* homozygous); (ii) DAT1 polymorphism (i.e., *9/9R* and *10/10R* allele carriers); (iii) BDNF polymorphism (i.e., *val/val* homozygote and *met*-allele carriers including *val/met* and *met* homozygous); and (iv) combinations of DAT1–COMT variants associated with different dopamine availability levels. The distribution of COMT, DAT1, BDNF, and COMT–DAT1 genetic polymorphism was in Hardy–Weinberg equilibrium. MRI images were acquired with a 1.5T Signa Excite system equipped with an eight-channel phased-array head coil.

A protocol was developed to separate the hippocampus from the amygdala. High-resolution T1-weighted anatomical images were obtained by using a three-dimensional fast spoiled gradient inversion-recovery prepared sequence with 130 contiguous slices (TR, 11.8 msec; TE, 4.2 msec; flip angle, 15°; field of view, 30 cm; pixel matrix, 256 × 256; and slice thickness, 1.2 mm). Tracing was performed on coronally displayed MRI slices from the caudal to the rostral portion of the brain. Hippocampal boundaries were set *medially* by the cerebral spinal fluid of the uncus cistern, *laterally* by the cerebral spinal fluid in the temporal horn of the lateral ventricle, and *inferiorly* by the parahippocampal white matter running medially from the temporal horn of the lateral ventricle.

Intracranial volumes (ICVs) were obtained through the Voxel Brain Morphometry module of the Statistical Parametric Mapping software. To assess the hippocampal subregional effect, the hippocampus was also segmented using the FreeSurfer image analysis environment version 5.3.0 into several subregions (i.e., fimbria, presubiculum, subiculum, CA1, CA2/3, CA4/DG fields, and hippocampus fissure). Intraclass correlation coefficients (absolute agreement) were computed to assess tracing reliability based on ten randomly selected images. Intrarater reliability was 0.96 and 0.95, whereas inter-rater reliability against an experienced hippocampus tracer (VL) was 0.94 and 0.90 for the right and left hemispheres, respectively.

A series of four repeated-measures analyses of covariances, with total and subregional hippocampal volumes as dependent variables, hemisphere (i.e., left and right) as a repeated measure, and ICV as a covariate was done. Factors in the first three models included COMT genetic polymorphisms (i.e., *val/val* vs. *met*), DAT1 genetic polymorphisms (*9/9R* vs. *10/10R*), and BDNF genetic polymorphisms (*val/val* vs. *met* carriers including mostly *val/met* carriers and three *met/met* carriers), respectively, as well as *Cannabis* use (i.e., user vs. non-user).

Factors in the exploratory model were *Cannabis* use × cross-product of COMT–DAT1 genotypes associated with varying levels of dopamine availability: (i) low dopamine, including carriers of alleles *val/val* and *10/10R*; (ii) high dopamine, including carriers of *met* and *9/9R*; (iii) medium dopamine, including carriers of alleles (a) *val/val* and *9/9R* and (b) *met* and *10/10R* and one *Cannabis* using a carrier of the *val/val* and *9/9R*. In addition, the impact of *Cannabis* use as the only factor (without genetic polymorphism as a factor) on hippocampal volumes, using the same model already outlined was explored.

Nicotine use (monthly cigarettes) and alcohol use were not included as covariates in the analyses. Monthly cigarettes were not associated with hippocampal volumes in *Cannabis* users. Nine controls were tobacco smokers, of which only two used more than ten cigarettes per day. Alcohol use did not affect any analyses, at

either a significant or trend level, when used as a covariate. Partial correlations to explore the association between hippocampal total and subregional volumes and *Cannabis* use levels (lifetime, past year, and past month a cumulative number of joints) and monthly cigarettes, controlling for ICV, were run. No outlier was observed in hippocampal volume distribution. All analyses were performed using SPSS version 19.0. For all the analyses, the significance threshold was $p < 0.05$.

Cannabis use and dopaminergic gene polymorphism had distinct and interactive effects on total and subregional hippocampal volumes. The trend emerged when *Cannabis* use was the only factor in the model and dissipated when genetic polymorphism was added as an additional factor (COMT, DAT1, BDNF, and COMT×DAT1) cross-products. The left hippocampus volume was not significantly reduced in *Cannabis* users relative to controls ($F_{52} = 3.70$, $p = 0.06$). *Cannabis*-using samples from past studies, with consumption levels similar to those of this study (i.e., mean 15 years age of onset, smoking 22 days/month, and 3 years duration), also show no significant hippocampal differences relative to non-using controls. In contrast, samples of heavier users with longer periods of use (i.e., daily use for more than 10 years) show hippocampal reductions relative to non-users.

Duration of heavy use may have played a role in the discrepancy of the findings, with longer duration of heavy use driving reduced hippocampi. That was not surprising, given the direct association between cannabinoid exposure and receptor organization in the hippocampus. A longer duration of heavy *Cannabis* use may be required before significant hippocampal reduction is observed. Accordingly, in the sample, an association between smaller total hippocampal volumes and higher cumulative *Cannabis* joints smoked during users' lifetime ($R = -0.41$, $p = 0.046$), past year ($R = -0.35$, $p = 0.08$). The past month was found ($R = 0.34$, $p = 0.08$), suggesting a dose-dependent relationship between *Cannabis* use and hippocampal volume.

Significant effects of *Cannabis* on specific subregions were also found, with a larger volume in CA1 (i.e., in two of the four analyses, which used COMT and DAT1 as factors), CA2/3, and CA4 (i.e., in one analysis using COMT and *Cannabis* as factors) in *Cannabis* users relative to non-users, when controlling for COMT gene polymorphism and ICV; this is alongside dose-dependent relationships between the volume of various subregions and *Cannabis* use levels – including larger right CA1 and greater monthly/yearly joints ($R_{53} = 0.29$, $p < 0.05$ and $R_{53} = 0.25$, $p = 0.07$), larger right CA2/3 and greater monthly/yearly/lifetime joints ($R_{53} = 0.40$, $p < 0.005$, $R_{53} = 0.36$, $p < 0.01$ and $R_{53} = 0.33$, $p < 0.05$), and larger right CA4 and greater monthly, yearly, and lifetime joints ($R_{53} = 0.30$, $p < 0.05$, $R_{53} = 0.26$, $p = 0.06$ and $R_{53} = 0.23$, $p = 0.09$). Total hippocampal volume and the fissure subregion were affected by *Cannabis*×DAT1 polymorphism (i.e., 9/9R and in 10/10R alleles), reflecting high and low levels of dopamine availability ($F_{152} = 4.93$, $p = 0.031$).

The average hippocampal volume was largest in control carriers of the 10/10R allele, followed by *Cannabis*-using carriers of the 9/9R and the 10/10R allele, and finally by control carriers of the 9/9R allele. 10/10R-Carrying *Cannabis* users had significantly smaller hippocampus than 10/10R-carrying controls in the left

hemisphere ($T27 = 7.81$, $p < 0.001$, Cohen's $d R = -0.8$, large effect size) and right hemisphere ($T26 = 4.07$, $p < 0.0001$; Cohen's $d R = -0.7$, large effect size). The inverse pattern was observed in *9/9R* carriers, with *9/9R*-carrying *Cannabis* users having larger left hippocampal volumes than *9/9R*-carrying controls ($T26 = 4.07$, $p < 0.0001$, Cohen's $d R = -0.6$, large effect size); this may be related to the *DAT1* genotype affecting total hippocampal volumes in controls (i.e., *9/9R* and *10/10R* carriers showed the smallest and largest volumes, respectively – left: $T27 = 2.69$, $p = 0.012$ and – right $T27 = 1.28$, $p = 0.21$), but not in *Cannabis* users (i.e., *9/9R* and *10/10R* carriers had similar hippocampal volumes – left, $T26 = -0.68$, $p = 0.50$ and right, $T26 = -0.26$, $p = 0.79$). Notably, the *Cannabis*×*DAT1* effect on total hippocampal volumes may be driven by a specific subregion (i.e., hippocampal fissure), where the effect was also observed. The interaction between dopamine genetic polymorphism (*DAT1*) and *Cannabis* may partly explain the mixed evidence on smaller hippocampal volumes in *Cannabis* users than in controls. This notion remains unexplored, as genetic polymorphism remains underinvestigated in neuroimaging studies of *Cannabis* users.

The findings indicated that carrying the *9/9R* allele of *DAT1* predates smaller hippocampus, and *Cannabis* use may reverse the process. Although the underlying mechanism remains unclear, it is speculated that *Cannabis* exposure may selectively interact with *DAT1* gene variants to alter the normal effect of dopaminergic transmission on neuroanatomy. Chronic *Cannabis* use may alter “optimal” extracellular dopamine levels (e.g., lower synthesis/release and contributing to hypersensitivity of postsynaptic receptors) and compromise neuronal growth. The *DAT1* gene influences neuronal growth, survival, and differentiation and is highly expressed within the hippocampus [95]. Importantly, emerging evidence shows that dopaminergic genes (e.g., *DAT1*) mediate the effects of specific cannabinoid compounds on the brain [96]. It remains unclear whether the effects change for distinct cannabinoid compounds that have very different effects on neuroanatomy, neural activity, and emotion processing, such as the neuroprotective cannabidiol and the psychotogenic tetrahydrocannabinol.

Parkinson's Disease and Complementary Therapies

Parkinson's disease is characterized by bradykinesia, rigidity, resting tremors, and postural instability. Non-motor symptoms, including pain, fatigue, insomnia, anxiety, and depression, are increasingly recognized and often just as disabling as motor symptoms. The mainstay of treatment is dopamine replacement; however, the beneficial effects tend to wane with disease progression, and patients often experience motor fluctuations and medication side effects.

The lack of a disease-modifying intervention and the shortcomings of traditional symptomatic medications have led many patients to pursue complementary therapies to alleviate motor and non-motor symptoms associated with Parkinson's

disease. The term complementary implies that the therapy is used with conventional medicine and may include supplements, manipulative treatments such as chiropractic massage, exercise-based programs, and mind-body practices. As the practices become more widespread in medical care, there is a growing interest in evaluating their effects on medical conditions, including Parkinson's disease.

Parkinson's disease affects more than 1% of individuals over 60 years, and the prevalence is rising dramatically. The symptoms are often quite responsive to dopaminergic therapy early in the disease course, and levodopa remains the mainstay of symptomatic treatment. Despite a robust initial response to levodopa, patients tend to experience less consistent medication effects over time because of the ongoing disease progression and increasing medication requirements, which can manifest as motor fluctuations (dyskinesia, wearing off, and sudden off periods) or medication side effects (hallucinations, orthostasis, and fatigue). Parkinson's disease-related non-motor features, including cognitive, behavioral, sleep, and autonomic dysfunctions, are often progressive and may not respond to dopaminergic therapy, thus profoundly impacting the quality of life.

The lack of a disease-modifying treatment and the shortcomings of the traditional symptomatic medications have led many patients with Parkinson's disease to pursue complementary therapies in addition to established, FDA-approved medications. According to the National Center for Complementary and Integrative Health, the term complementary refers to non-mainstream practices that are used together with conventional medicine versus alternative, which indicates that the non-mainstream practice is used in place of conventional medicine. Typically, the terms are used together as complementary and alternative medicine or interchangeably. Complementary medicine encompasses various interventions, including supplements, manipulative therapies such as massage, chiropractic physical exercise, and mind-body practices. Patients have reported using the therapies for motor and non-motor symptoms of Parkinson's disease and general health.

In a survey prior to 2013, the use of complementary treatments to address Parkinson's disease symptoms ranged from 26 to 76 in various countries. The numbers are higher in Asian populations, where many therapies viewed as complementary in Western medicine have been traditional practices for centuries. More recent surveys across the United States have reported 50–75% of users. In 2006, the American Academy of Neurology released an evidence-based review of disease-modifying and complementary therapies for Parkinson's disease. The report provides insufficient evidence to support or refute acupuncture, manual therapy, or biofeedback. However, since the report was published, the number of trials of various complementary therapies has vastly increased.

Synthetic Cannabinoids and Parkinson's Disease

Synthetic cannabinoids are representative of scientific research. Cannabinoid receptor agonists have been developed for therapeutic purposes. However, most of the substances were not approved by the government regulating medicines. The substances were misused for recreational purposes. Synthetic cannabinoids are a group of novel psychoactive substances with similar properties as delta-9-THC that appeared in the drug market in 2004 as an herbal blend. Since then, the composition of cannabinoid-containing herbal products has substantially changed to include more potent new psychoactive compounds that circumvent the law. The illegal market turned into potent, dangerous recreational drugs that strongly activate the brain reward pathway and expose the user to a higher risk of developing addiction and other severe illnesses, including psychosis.

In vivo studies showed that synthetic cannabinoids 2-(2-methoxyphenyl)-1-(1-pentyl-1H-indol-3-yl)ethenone (JWH-250) and 1-butyl-3-(1-naphthoyl)indole (JWH-073), are major agonists of CB1. They induced several effects when administered separately, such as impaired sensorimotor responses (visual, acoustic, and tactile), causing seizures, myoclonia, and hyper-reflexia, and promoting aggressiveness in mice [11]. The co-administration of ineffective doses of JWH-250 and JWH-073 impaired visually stimulated mesolimbic dopamine transmission in mice. The experiment has shown the potential synergistic action of synthetic cannabinoids suggesting that co-administration of the novel psychoactive substances may potentiate the harmful effects of individual compounds, increasing their danger.

The impact of 1-pentyl-3-(1-naphthoyl)indole (JWH-018), a more potent agonist of CB1 than delta-9-THC, was described at the molecular and cellular level, using yeast as a eukaryotic model. In the presence of JWH-018, the cells' growth rate is higher due to an enhanced glycolytic flux at the expense of a decrease in the pentose phosphate pathway. The pentose phosphate pathway generates NADPH, which is critically important since its production provides reducing power to deal with oxidative stress. In the brain, reactive oxygen species come mainly from dopamine metabolism, mitochondrial dysfunction, and neuroinflammation, and its oxidative stress contributes to Parkinson's disease [12]. Consequently, reducing NADPH production during a long period might allow the proliferation of reactive oxygen species, thus increasing the probability of developing Parkinson's disease in JWH-018 consumers.

Despite the possible neurotoxic effects of synthetic cannabinoids, (6aR,10aR)-6,6,9-trimethyl-3-(2-methylpentan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromene (JWH-133), a potent agonist of CB2, indicated to be neuroprotective in an MPTP model of Parkinson's disease. The response was associated with the suppression of the blood-brain barrier damage, astroglial myeloperoxidase expression, infiltration of peripheral immune cells, and production of inducible nitric oxide synthase, proinflammatory cytokines, and chemokines by activated microglia [13]. In synthetic

cannabinoids, the affinity to CB1 or CB2 seems essential in understanding if a compound might be neurotoxic or protective against Parkinson's disease, respectively.

Patients use phytocannabinoids legally or illegally, although the EMA and FDA have not approved their use for Parkinson's disease. The effects of the phytocannabinoids on Parkinson's disease appear protective either by binding to the CB1 receptor or CB2. For example, the binding of delta-9-THC to CB1 restores membrane potential, decreases reactive oxygen species, and increases CB1 protein level, increasing the amount of synaptic vesicle inhibition. Moreover, after a decrease of the mitochondrial membrane potential by MPP⁺ exposition, the activation of CB2 might be neuroprotective by inhibiting the apoptosis by target-derived neurotrophin nerve growth factor.

Opiates and Parkinson's Disease

Opiates comprise the naturally occurring alkaloids in the opium poppy from the plant *Papaver somniferum*, such as morphine, codeine, and their semi-synthetic derivatives: heroin, hydrocodone, oxycodone, and buprenorphine, among others. Most pharmaceutical opioids are controlled under the Single Convention on Narcotic Drugs of 1961, with some exceptions, such as buprenorphine, controlled under the Convention on Psychotropic Substances of 1971. The prevalence of opiate consumption in Europe in 2017 was estimated at 0.7% of the adult population, representing nearly 3.8 million opioid users. In Western and Central Europe, there are an estimated two million opioid users, 0.6% of the adult population. The use of opioids is dominated by heroin. In addition to heroin, the most common opioids are opium, morphine, methadone, buprenorphine, tramadol, and fentanyl analogs.

Opioid binds to G protein-coupled (Gi and Go) receptors. The opioid receptors in CNS are classified into four types: μ , κ , δ , and nociceptin. μ -Receptors mediate natural rewards initiating addictive behaviors, whereas δ and κ -receptor activity appear to play a role in improving mood states [14]. The receptors bind to endogenous and exogenous opioids structurally related to the natural plant alkaloids found in opium but also to small opioid peptides. Nociceptin receptor binds to medium size endogenous opioid peptides such as orphanin. The receptors exhibit seven transmembrane helices, typical of G protein-coupled receptors structures.

The atomic structures of opioid receptors revealed common features for opioid recognition, as predicted previously. Opioid receptors binding sites contain an anionic aspartic acid residue that forms an ionic bond with the amino group of opioid ligands. The hydrophobic binding pocket accommodates the aliphatic substituents on the amino group, and the phenolic group of morphine engages an extended hydrogen-bonding network between two water molecules and a conserved histidine residue in the transmembrane helix 6 (TM6). The activation of the receptor is due to a conformational change displacing TM6 10Å and, to a lesser extent, TM5 and TM7. The movements open a large pocket in the intracellular side of the receptor allowing coupling with heterotrimeric G proteins [15].

Since opioid receptors are expressed on excitatory and inhibitory neurons, they can activate or inhibit the neural circuits. Opioids inhibit neurons by decreasing either neuronal firing on the postsynaptic localization or neurotransmitter release on the presynaptic localization of the receptors. After binding to the receptors, opioids inhibit voltage-dependent Ca^{2+} channels or activate inwardly rectifying potassium channels, thereby diminishing neuronal excitability. Opioids also inhibit the cyclic adenosine monophosphate pathway and activate mitogen-activated protein kinase cascades, affecting the cell's cytoplasmic events and transcriptional activity [16]. In addition to opioids, opioid peptides share a common N-terminal Tyr-Gly-Gly-Phe signature sequence that also interacts with opioid receptors, namely, β -endorphin, enkephalins, and dynorphins which bind to μ , δ , and κ , respectively.

In the rat model of Parkinson's disease, studies revealed the involvement of opioid pathways in the mechanisms modulating nociceptive thresholds. One study indicated an increase in the survival rate of dopaminergic neurons treated with δ -opioid peptide when exposed to the neurotoxin 6-OHDA in vitro and in vivo. The results suggest that δ -opioid receptors may be protective in Parkinson's disease [16]. Studies performed in rat and primate models of Parkinson's disease indicate that δ -opioid receptors reduce dyskinesia induced by levodopa [17].

Regarding endogenous opioids, it is evident that endogenous morphine, structurally similar to vegetal morphine-alkaloid, is synthesized by mammalian cells from dopamine. It binds to the μ -opioid receptor and induces antinociceptive effects. Since the striatum is rich in both μ - and δ -opioid receptors, the substances can act as modulators of dopamine, gamma-aminobutyric acid (GABA), and glutamate neurotransmission. In Parkinson's disease patients, the levels of endogenous morphine and its metabolites were increased. The increment may be associated with fatigue, depression, and pain symptoms experienced by Parkinson's disease patients [18]. Opioids also affect locomotion and reward behavior mediated by the basal ganglia.

An increase in opioid transmission in the two main striatal outputs has been observed in monkeys and humans with dyskinesia induced by levodopa, indicating the involvement of the endogenous opioid system in mitigating the effect of abnormal dopaminergic stimuli [19]. Prolonged treatment with oxycodone/naloxone seems to affect only specific subgroups of Parkinson's disease patients with pain, which suggests that successful clinical improvements require a careful identification and characterization of Parkinson's disease patients.

Cellular studies indicated that δ -receptor activation attenuates α -synuclein expression and aggregation and reduces cytotoxicity in Parkinson's disease in vitro models exposed to MPP(+) stress. δ -Receptor activation can largely attenuate α -synuclein expression via DJ-1 upregulation in both genetic (α -synuclein wild-type or A53T-mutant α -synuclein) and environmental (hypoxic) conditions. The δ -receptor action involves the transducer of the regulated CREB1 (TORC1)/salt-inducible kinase 1 (SIK1) downregulation in the former condition, and cAMP response element-binding protein (CREB) phosphorylation in the latter condition.

The activation of the δ -receptor appears to be cytoprotective against both hypoxia and MPP+ through the regulation of PTEN-induced kinase 1 (PINK1) and caspase-3 pathways [20]. Although activation of δ -receptors has an anti-Parkinsonian effect,

adverse effects of opioids were also observed. Long-term exposure to tramadol is known to induce tremors, muscular rigidity, and tardive dyskinesia. The symptoms are possibly related to serotonin's inhibitory effect on dopamine neurotransmission within the basal ganglion system, which may result in the altered function in the striatum and the inhibition of serotonin reuptake inhibitors.

Morphine and Parkinson's Disease

It has been indicated that morphine raises dopamine levels in the brain by stimulating μ -opioid receptors, which inhibit GABA release and consequently enhance dopamine release. Morphine is a partial agonist for μ -opioid receptors and is a weak agonist for δ -opioid receptors. Morphine does not seem to act through κ -opioid receptors [21]. Therefore, μ -opioid receptors could be a potential therapeutic target for relieving Parkinson's disease symptoms.

Clinical observations indicated that morphine alleviates tremors significantly. However, the levels of α -synuclein protein in mice withdrawn from morphine for 48 h were significantly increased in the ventral striatum, namely the nucleus accumbens [22]. Two weeks after treatment cessation, the protein levels were still high. Morphine also increases the cell viability in PC12 cells after MPP+ exposure. MPP+ reduces cell viability and tyrosine hydroxylase expression, but the effect was reversed in the presence of morphine which acts on the PI3K/Akt pathway [23].

It was indicated that morphine has neuroprotective effects against 6-OHDA-induced SH-SY5Y dopaminergic cell damage and neurodegeneration [24]. Morphine essentially changes the expression of Parkinson's disease-associated genes. It has been reported that PARK2 was upregulated and PINK1 was down-regulated. The two genes are associated with juvenile Parkinson's disease. Mutations in PARK2 cause nearly 50% of autosomal recessive juvenile Parkinsonism [25]. PINK 1 overexpression activates Parkin's E3 ubiquitin ligase and recruits Parkin triggering selective autophagy [26].

Cocaine and Parkinson's Disease

Cocaine is extracted from leaves of two distinct species of the genus *Erythroxylum* (family Erythroxylaceae): *Erythroxylum coca* Lam. and *Erythroxylum novogranatense* (Morris) Hieron. Coca leaves chewing has been part of the Andean life-style for thousands of years. At the end of the nineteenth century, pharmaceutical and food products with coca leaf extracts were introduced into the market achieving high popularity. Later, the active principle in coca leaves was purified and used in medicine as a stimulant for psychoanalysis and as an anesthetic. Simultaneously, the use of pure cocaine for recreational purposes also started. The cocaine market is the second-largest illicit drug market after *Cannabis*.

Cocaine acts on presynaptic monoamine reuptake transporters inhibiting monoamine neurotransmitters reuptake, increasing its levels in the synaptic cleft [27]. The atomic structure of the dopamine transporter of *Drosophila melanogaster* bound to cocaine was obtained in 2015. The structure was used as a template, in combination with computational tools, to study the binding and modulation of human dopamine transporter function in dopamine and cocaine. The study revealed that cocaine competitively binds dopamine transporter. The binding affinity is dependent on the conformational state of the dopamine transporter; notwithstanding, cocaine binds competitively to the dopamine transporter inhibiting dopamine reuptake.

Cocaine exposure may have neurotoxic effects on dopaminergic neurons since the total number of melanized dopamine cells in the anterior midbrain is reduced in cocaine users. Additionally, chronic cocaine use leads to the downregulation of postsynaptic dopamine receptors, which results in putamen hypertrophy as a compensatory process to produce more dopamine to maintain dopaminergic transmission. However, in Parkinson's disease brains, caudate and putamen volumes were smaller compared to controls [28]. On the other hand, there is a published case report of a young adult that developed early Parkinsonism after chronic cocaine use.

Despite the scarcity of research data on cocaine and Parkinson's disease, studies have shown that cocaine is not a risk factor for Parkinson's disease onset. There is a consensus that high levels of cytosolic dopamine are neurotoxic. It was observed that after cocaine administration, the cytosol levels of dopamine remained unchanged, suggesting that cocaine administration may not be considered a risk factor in dopamine-induced neurodegeneration. This is reinforced by the observations that cocaine enhances dopamine levels in the dorsal, but not ventral, striatum [29]. A 3-day administration, via implanted pumps, of cocaine hydrochloride did not produce axonal degeneration in the frontal agranular cortex or neostriatum [30]. Interestingly, cocaine has been shown to alleviate the symptoms of Parkinson's disease in monkeys.

Like amphetamine, cocaine binds tightly to the N-terminus of intrinsically unstructured α -synuclein, inducing a folded conformation, which increases the likelihood of misfolding and possibly leads to an increased incidence of Parkinson's disease amongst drug users [31]. Cocaine has been shown to increase the levels of α -synuclein. A recent genetic study links cocaine abuse to secondary Parkinsonism due to a potential gene-environmental interaction, namely, a detected leucine-rich repeat kinase 2 (*LRRK2*) risk variant [32].

Amphetamines and Parkinson's Disease

According to the World Health Organization, amphetamine-type stimulants are a group of drugs of abuse with the principal members including amphetamine and methamphetamine. Amphetamine was first synthesized in 1887 in Germany as phenylisopropylamine by Romanian chemist Lazăr Edeleanu. Methamphetamine was

synthesized in 1893 by Nagayoshi from ephedrine, an alkaloid present in the plant *Ephedra sinica*, isolated for the first time in 1885 by G. Yamanashi and named by Nagai in 1887. Amphetamine-type stimulants have been used for recreational purposes to improve physical and mental performance in fatigued subjects. During World War II, amphetamine and methamphetamine were used extensively by allied and axis forces for their stimulant and performance-enhancing effects.

As a result of the United Nations 1971 Convention on Psychotropic Substances, amphetamine became a schedule II-controlled substance, as defined in the treaty, ratified by all 183 members. As the addictive properties of the drugs became known, governments began to place strict controls on their sale. Despite strict government controls, amphetamine and methamphetamine are used for recreational purposes, and according to the European Monitoring Centre for Drugs and Drug Addiction, amphetamines are associated with a large number of health emergencies in the north and east of Europe. The monitoring center estimates that 1.2 million young people in Europe, between 15 and 34 years of age, have consumed amphetamine-type stimulants in the last year, and 12.4 million Europeans have consumed them during their lifetime.

Amphetamine-type stimulants share structural features with the catecholamine neurotransmitters, such as norepinephrine and dopamine. Amphetamine-type stimulants have an aromatic ring and nitrogen on the aryl side-chain, which is a prerequisite for competitive binding to the monoamine reuptake transporters, norepinephrine transporter, dopamine transporter, and 5-HT transporter (SERT) [33]. All three transporters are membrane-embedded proteins expressed in the presynaptic neuronal terminals. Monoamine reuptake transporters mediate the uptake of neurotransmitters from the synaptic cleft into the presynaptic neuronal terminals using the energy gradient produced by Na^+/K^+ ATPase. Dopamine and norepinephrine transporters translocation of dopamine and norepinephrine are involved in the co-transport of two Na^+ and one Cl^- ion along with one substrate molecule. SERT co-transporters one 5-HT molecule with one Na^+ and one Cl^- along with one K^+ ion in the opposite direction.

Since amphetamine competes with endogenous monoamines for transport into the nerve terminals via the transporters, the higher the concentration of amphetamine present in the synapse, the less molecules of endogenous catecholamines are uptake due to competitive inhibition of dopamine transporter by amphetamine. Consequently, there is a greater stimulation effect on postsynaptic receptors by dopamine. Amphetamine also has an affinity for vesicular monoamine transporter 2, preventing the translocation of monoamines into the intraneuronal storage vesicles and reversing the direction of the reuptake transporter. Therefore, it pumps neurotransmitters out of neurons into the synapse [34]. In addition, amphetamine also increases synaptic monoamine concentrations inhibiting monoamine oxidase, which catalyzes the breakdown of monoamine neurotransmitters in the CNS.

The abuse of amphetamines-type stimulants has been largely described as affecting dopaminergic transmission and function, inducing dopamine depletion, raising extracellular dopamine levels, and prolonging dopamine receptor signaling in the striatum. The consequences of amphetamines-type stimulant intake indicate a

relationship between its consumption and the onset of Parkinson's disease. The studies performed with amphetamine and methamphetamine revealed that the two substances have similar pharmacokinetic profiles and their dopamine responses in the striatum are equivalent [35]. Some studies attempt to verify the amphetamine-like stimulants' effects in Parkinson's disease symptoms treatment; however, no significant improvement has been found [36].

Protein misfolding and aggregation processes are involved in several neurodegenerative diseases resulting from conformational changes in the amyloid protein precursors. In Parkinson's disease, α -synuclein aggregates form amyloid deposits in the brain called Lewy bodies, which are associated with the loss of dopaminergic neurons in the substantia nigra.

Researchers have studied the relationship between α -synuclein and amphetamine-type stimulants, such as conformational changes, post-translational modification, and increased protein expression. Amphetamine and methamphetamine bind tightly to the N-terminus of intrinsically unstructured α -synuclein, inducing a folded conformation. A putative fold conformation increases the likelihood of misfolding and aggregation. The researchers suggest that this mechanism may increase the incidence of Parkinson's disease among amphetamine and methamphetamine users [31]. Additionally, the temperature in the mid-brain region can exceed 41 °C upon ingestion of the stimulants.

Repeated bouts of excessive heat increase α -synuclein expression to prevent cells from heat damage by inhibiting stress signaling [37]. Consequently, this causes an accumulation of α -synuclein promoting its aggregation, damaging neurons [38]. Post-translational modifications of α -synuclein, such as phosphorylation, nitration, acetylation, and ubiquitination, have also been pointed as a risk or beneficial factor for Parkinson's disease; however, only protein nitration has been linked to the use of methamphetamine, which is pointed out as a risk due to the increased post-translational modifications of α -synuclein which seems to mediate neurotoxicity, as indicated by studies in human neuronal lines and mice brain cells [39].

Methamphetamine influences gene expression of normal dopaminergic innervations in the striatum via stimulation of dopamine and glutamate receptors. Low doses of methamphetamine were found to induce the expression of a different set of genes in lesioned denervated striatum, completely lacking dopamine. The observations implicate alternative gene expression activation independent of dopamine in the presence of methamphetamine. Additionally, it was suggested that the absence of dopamine might cause plastic changes that render the striatum differentially responsive to the effects of methamphetamine [40]. In another study, the neurotoxic effects of methamphetamine in rodent models, using epigenetic assays, revealed that the consumption of methamphetamine decreased cytosine methylation in the SNCA promoter region and consequently upregulates α -synuclein in substantia nigra, contributing to the Parkinson's-like behavior [41].

Regarding the cellular mechanisms underlying the neurotoxic effects of amphetamine-type stimulants, the substances activate nicotinic α -7 receptors, which increase intrasynaptosomal calcium, nitric oxide synthase, and protein kinase C, leading to the production of high levels of nitric oxide and to dopamine

oxidation, which promotes neurodegeneration [42]. The increase of nitric oxide synthase may modulate fundamental functions since nitric oxide is involved in almost all vital functions, from platelet aggregation to neurotransmission. Cells treated for 24 h with methamphetamines significantly increased nitric oxide synthase, causing a rise in nitric oxide and α -synuclein levels that consequently promoted the aggregation of α -synuclein.

Another study indicated that tyrosine hydroxylase, dopamine transporter, vesicular monoamine transporter 2, nitric oxide synthase, and reactive oxygen species might be involved in α -synuclein mediated methamphetamine-induced neuronal toxicity [43].

Oxidative stress induced by amphetamine-type stimulants is also linked to Parkinson's disease since it increases dopamine neurons' vulnerability. A study in pregnant primates exposed to methamphetamine revealed that high levels of oxidative stress in pregnancy could compromise the population of nigrostriatal dopamine neurons and potentially elevate the risk of Parkinson's disease in children born later in life.

The activation of a small stress response can be used to protect neurons against neurodegeneration and might be used pharmacologically. It was proposed that the higher levels of oxidative stress induced by amphetamine-like stimulants result from dopamine autooxidation and increasing excitotoxicity. There is also evidence that exposure to low levels of methamphetamine induces a certain degree of cellular stress that can reduce the vulnerability of dopamine neurons to insults [40]. The cellular mechanisms underlying stress-induced protection are associated with decrease in basal ERK 1/2 and kinase b levels (involve in multiple cellular processes such as apoptosis), reduced activity of protein phosphatase 2 (a protein phosphatase implicated in ERK1/2 dephosphorylation inhibition), and upregulation of the pro-survival protein BCL-2 which plays an anti-apoptotic role.

Clinical observations point out amphetamine-type stimulants as neurotoxic. The substances damage dopaminergic neurons involved in motor function and limbic-motor integration, increasing the predisposition to Parkinson's disease. The molecular studies indicated that amphetamine up regulates α -synuclein in substantia nigra, which accumulates, leading to aggregation, damaging neurons, and contributing to Parkinson's-like behavior. Conversely, there is evidence that exposure to low levels of methamphetamine may reduce dopamine neurons' vulnerability to insults.

Epidemiological studies indicate an increased risk of Parkinson's disease for amphetamine-type stimulant users independently of the lifestyle. A nearly threefold increased risk of Parkinson's disease in amphetamine-type stimulants users vs. non-consumers was revealed. A retrospective case-control study revealed that prolonged use of amphetamines is associated with an eightfold increased risk of Parkinson's disease, with an average of 27 years between amphetamine exposure and the onset of signs of the disease.

Innovative Therapies in Parkinson's Disease

Parkinson's disease is the most prevalent motor and second most prevalent neurodegenerative disorder, characterized by progressive death of dopaminergic neurons, predominantly in substantia nigra pars compacta and neostriatum, and subthalamic nucleus of basal ganglia at low amounts. The intracellular accumulation of Lewy bodies enriched in α -synuclein protein, and thread-like proteinaceous inclusions called Lewy neuritis, is known to provoke the motor symptoms in Parkinson's disease.

The genetic studies indicated the mutations in α -synuclein, *PINK1*, *DJ-1* (*PARK7*), ubiquitin genes such as *ubiqA*, *ubiqC*-hydrolase, and population-specific gene mutations such as glucocerebrosidase gene in Ashkenazi Jews directly relate to the onset of Parkinson's disease. Evidence suggests that mutations in molecular signaling, such as leucine-rich repeat kinase 2 and Miro GTPases, play a vital role in the onset and progression of Parkinson's disease.

In addition to the L-DOPA, other physiological methods, such as deep brain stimulation with the basis of Network Centric Therapy, are used to control the motor symptoms of Parkinson's disease, such as tremors and stiffness. In attempting hormone therapy, the potential of steroids, especially the sex steroids such as estrogen and progesterone, to be developed as a therapy for Parkinson's disease via neurotransmitter modulation of selective brain estrogen receptors was examined. However, an optimized formula was not available to specifically target brain receptors. Furthermore, an intervention study utilized gene modification and remodeling techniques such as CRISPR through human embryonic stem cells and induced pluripotent stem cells, showing promising personalized medicine strategies. The idea requires intensive investigations at a clinical level with a large cohort.

The concept of using stem cells has been successfully tested in Parkinson's disease monkey models; however, some ethical concerns arose when obtaining human embryonic stem cells in larger amounts for clinical trials. The limitation was addressed by a group of European researchers who initiated a global program in developing cell-based therapies for Parkinson's disease.

The scientists highlight the challenges to face when developing stem cell-based therapy, such as identifying the most suitable cell line, development of clinically administrable cell product, quality control and quality assurance, preclinical testing for accurate dosing, and other measures. Considering the circumstances, further interventions are essential to achieve a precise level of control in the progression of dopaminergic neurodegeneration. To date, no drug has been identified to cease the progression of neurodegeneration in Parkinson's disease.

Iron can exchange single electrons with many different substrates, leading to the generation of reactive oxygen species. Reactive oxygen species trigger oxidative stress, inducing lipid peroxidation and DNA damage that can lead to impaired cell viability and initiation of signaling pathways crucial for cell survival and death. There is evidence that the effects of neonatal iron overload in the brain intensify gradually throughout life. Iron treatment increased the content of ubiquitinated

proteins, a marker of UPS-ubiquitin system deterioration, in the hippocampus of adult rats. At the same time, no effects were observed when the analysis was performed earlier in life [115].

Studies using aged rats and mice suggest that iron accumulates and redistributes in brain regions during life without a coincident increase in ferritin, the main cellular iron storage protein in neurons, suggesting an age-related iron dyshomeostasis [116]. The deleterious effects of iron overload in the neonatal period will be revealed later in life. In humans, cognitive behavior is influenced by age-associated increases in brain iron content [117]. Based on the findings, it is suggested that iron-induced increased apoptosis later in life might lead to functional deficits observed in the animal model and patients, implicating iron in the pathogenesis of memory dysfunction associated with aging and neurodegenerative disorders [118].

Chemotherapy-induced peripheral neuropathy is the main dose-limiting adverse effect of chemotherapy drugs such as paclitaxel. Paclitaxel causes marked molecular and cellular damage, mainly in the peripheral nervous system, including sensory neurons in the dorsal root ganglia. Studies have indicated the therapeutic potential of cannabinoids, including cannabidiol, to treat peripheral neuropathies. The efficacy of PECS-101 (former HUF-101), a cannabidiol fluorinated analog, was investigated on paclitaxel-induced neuropathic pain in mice [119]. PECS-101, administered after treatment with paclitaxel, did not reverse mechanical allodynia.

However, PECS-101, at a dose of 1 mg/kg, administered along with paclitaxel treatment, caused a long-lasting relief of the mechanical and cold allodynia. PPAR γ blocked the effects, but not CB1 and CB2 receptor antagonists. The effects of PECS-101 on the relief of paclitaxel-induced mechanical and cold allodynia were not found in macrophage-specific PPAR γ -deficient mice. PECS-101 also decreased the paclitaxel-induced increase in Tnf, Il6, and Aif1 (Iba-1) gene expression in the dorsal root ganglia and the loss of intraepidermal nerve fibers. PECS-101 did not alter motor coordination, produce tolerance, or show abuse potential. PECS-101 did not interfere with the chemotherapeutic effects of paclitaxel. Thus, PECS-101, the fluorinated cannabidiol analog, could represent a therapeutic alternative to prevent mechanical and cold allodynia induced by paclitaxel potentially through the activation of PPAR γ in macrophages.

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Chapter 12

Cannabis and the Skin



Anandamide mediates transcriptional effects associated with epidermal differentiation and skin development in those cells through a CB1-dependent mechanism. Components of the endothelial cells (ECs) are found to be active in the human epidermis, where CB1 cannabinoid receptor expression is higher in more differentiated layers of the skin [1]. Also, immortalized and normal epidermal keratinocytes (KC) have fully functional ECs [2].

Cannabinoids can act independently of the cannabinoid receptors through transient receptor potential (TRP) ion channels. Researchers have identified two G proteins, coupled cannabinoid receptors 1 2. The receptors are present in the skin and serve various functions [5–7]. Through the receptors, cannabinoids affect the growth, proliferation, differentiation, and apoptosis of keratinocytes, melanocytes, adnexal structures, and fibroblasts [9, 10]. Cannabinoid receptors also exist in cutaneous nerve fibers and immune cells. Overall, cannabinoids' influence on skin homeostasis supports their therapeutic potential in dermatology.

The endocannabinoid system comprising the endogenous lipid mediators present in virtually all tissues, their G-protein-coupled cannabinoid receptors, biosynthetic pathways, and metabolizing enzymes has been implicated in multiple regulatory functions both in health and disease. Recent studies have indicated the existence of a functional ECs in the skin and implicating it in various biological processes such as proliferation, growth, differentiation, apoptosis, and cytokine mediator or hormone production of various cell types of the skin and appendages, such as the hair follicle (HF) and sebaceous gland. The main physiological function of the cutaneous ECS is to constitutively control the proper and well-balanced proliferation, differentiation, and survival, as well as immune competence and tolerance, of skin cells. The disruption of the delicate balance might facilitate the development of multiple pathological conditions and diseases of the skin.

The endocannabinoid system plays an important role in skin homeostasis in addition to broader effects on neurogenic responses such as pruritus and nociception, inflammation, and immune reactions. Numerous studies of in vitro and animal

models have provided insight into the possible mechanisms of cannabinoid modulation on pruritus, with the most evidence behind neuronal modulation of peripheral itch fibers and centrally acting cannabinoid receptors.

Clinical studies have shown a reduction in pruritus in several dermatologic ((AD), psoriasis, asteatotic eczema, prurigo nodularis, and allergic contact dermatitis (ACD)) and systemic (uremic pruritus and cholestatic pruritus) diseases. Human studies have consistently shown significant reductions in scratching and symptoms in chronic pruritus, although limited due to differences in the cannabinoids used, disease models, and delivery method. The preliminary human studies warrant controlled trials to confirm the benefit of cannabinoids for pruritus and to standardize treatment regimens and indications. In patients with chronic refractory pruritus after standard therapies, cannabinoid formulations may be considered an adjuvant therapy.

The endocannabinoid system in the skin and its role in maintaining skin homeostasis, along with the anti-inflammatory actions of cannabinoids, have raised interest in their use for treating inflammatory skin diseases. In vitro and in vivo studies of *Cannabis* have suggested it has favorable effects on regulating pain, pruritus, and inflammation, making it a potentially attractive therapeutic agent for dermatologic conditions. The research, possible cutaneous adverse effects, and future directions for cannabinoids and their use in skin cancer, acne, psoriasis, pruritus, dermatitis, scleroderma, dermatomyositis, cutaneous lupus erythematosus, epidermolysis bullosa (EB), pain, and wound healing have been examined [3].

Keratinocyte Proliferation

The epidermis is a physical and chemical barrier to the environment, provided by terminally differentiated keratinocytes [4, 5]. Epidermal differentiation begins with the migration of keratinocytes from a basal layer composed of proliferating cells. It ends with the formation of the cornified cell envelope, an insoluble protein structure found in differentiated keratinocytes [6]. The keratinizing epithelium protects terrestrial mammals from water loss and noxious physical, chemical, and mechanical insults. The barrier between the body and the environment is constantly maintained by the reproduction of inner-living epidermal keratinocytes, which undergo a process of terminal differentiation and then migrate to the surface as interlocking layers of dead stratum corneum cells. The cells provide the bulwark of mechanical and chemical protection and confer water impermeability with their intercellular lipid surroundings.

In spontaneously immortalized human keratinocytes (HaCaT cells) and in normal human epidermal keratinocytes (NHEK cells) induced to differentiate in vitro by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) plus calcium, anandamide (AEA) levels were reduced due to enhanced degradation by FAAH [7]. Moreover, in HaCaT cells exposed to AEA, there is a reduction in the formation of cornified envelopes and a reduction in the expression of keratins 1 (K1) and 10 (K10),

involucrin and transglutaminase 5 (TGase5) genes, which are all upregulated during cornification [8].

Psoriasis is a common skin disorder characterized by the hyperproliferation of keratinocytes. Although the exact pathophysiology of psoriasis is not entirely understood, the immune system and its interaction with the nervous system have been postulated and investigated as the underlying mechanism. The interaction between the two systems through the cholinergic anti-inflammatory pathway and the endocannabinoid system suggests cannabinoids as a potential addition to the antipsoriatic armamentarium [9].

Studies indicate that it exerts antineoplastic effects in multiple cancers, including melanoma. In an *in vitro* study to determine the anticancer properties of CBD in malignant melanoma cell lines (SK-MEL 28, A375, FM55P, and FM55M2), CBD was administered alone, as well as in combination with mitoxantrone (MTX) or cisplatin (CDDP). The MTT assay measured the effects of CBD on the viability of melanoma cells; cytotoxicity was determined in the LDH test and proliferation in the BrdU test. The safety of CBD was tested in human keratinocytes (HaCaT) in LDH and MTT tests. The results indicate that CBD reduces the viability and proliferation of melanoma-malignant cells and exerts additive interactions with MTX [10].

CBD produced antagonistic interaction when combined with CDDP. CBD does not produce significant cytotoxicity in the HaCaT cell line. CBD may be considered a part of melanoma multidrug therapy when combined with MTX. Special attention should be given to combining CBD with CDDP due to the antagonistic interaction observed in the studied malignant melanoma cell lines.

Cannabinoids are anti-inflammatory and have inhibitory effects on the proliferation of tumorigenic cell lines, some of which are mediated via cannabinoid receptors. Cannabinoid receptors are present in human skin, and anandamide, an endogenous CB receptor ligand, inhibits epidermal keratinocyte differentiation. Psoriasis is an inflammatory disease also characterized in part by epidermal keratinocyte hyper-proliferation.

Delta-9 tetrahydrocannabinol, cannabidiol, cannabinol, and cannabigerol were examined for their ability to inhibit the proliferation of a hyper-proliferating human keratinocyte cell line and their involvement of cannabinoid receptors. A keratinocyte proliferation assay was used to assess the effect of treatment with cannabinoids. Cell integrity and metabolic competence were confirmed using lactate-dehydrogenase and adenosine tri-phosphate assays. To determine the involvement of the receptors, specific agonists and antagonists were used in conjunction with some phytocannabinoids. Western blot and RT-PCR analysis confirmed the presence of CB1 and CB2 receptors [11].

The results revealed cannabinoids' inhibition of keratinocyte proliferation and supported a potential role for cannabinoids in the treatment of psoriasis. The cannabinoids inhibited keratinocyte proliferation in a concentration-dependent manner. The selective CB2 receptor agonists JWH015 and BML190 elicited only partial inhibition; the non-selective CB agonist HU210 produced a concentration-dependent response, and either CB1/CB2 antagonists did not block the activities of the

agonists. The results indicate that while CB receptors may have a circumstantial role in keratinocyte proliferation, they do not contribute significantly to this process.

It is well-established that genetic and environmental factors generally cause complex diseases. Gene expression is controlled by epigenetic mechanisms that cause heritable but potentially reversible changes in DNA methylation, histone modification, and RNA-associated silencing [12]. Epigenetics is thus the study of molecular mechanisms by which the environment controls gene activity independently of DNA sequence. However, even though the role of genetic abnormalities in the pathogenesis of many skin diseases has been thoroughly investigated; however, there have been few studies on the importance of epigenetics in altering the course of those diseases [13].

Variations in global DNA methylation have been reported between differentiated and undifferentiated cells, and in particular, a hypomethylation in differentiated versus undifferentiated keratinocytes has been documented [14]. Inhibition of DNA methylation and histone deacetylation has been shown to promote keratinocyte differentiation. An inverse correlation between DNA methylation and the expression of differentiating genes has been demonstrated in human keratinocytes [15]. It has also been suggested that inhibition of differentiation by AEA occurs through changes in chromatin methylation patterns and that AEA induces DNA methylation of keratinocyte-differentiating genes by increasing DNA methyltransferase (DNMT) activity via a CB1-dependent involvement of p38 and p42/p44 MAPK8. The possible epigenetic regulation of skin differentiation genes by selected phytocannabinoids that are plant-derived cannabinoids, which mimic the natural eCBs, have potential as novel therapeutics for human diseases [16].

Phytocannabinoids have anti-inflammatory properties and inhibit the growth of proliferating carcinogenic cells [17]. The compounds are lipophilic and are readily absorbed through the skin. In particular, cannabidiol accumulates only in the stratum corneum without penetrating the deeper layers [18].

The effects of the three major phytocannabinoids CBD, cannabigerol (CBG), and cannabidivarin (CBDV) on proliferating and differentiated HaCaT cells were tested and compared to those of the endogenous cannabinoid AEA as a control [7, 8]. In the initial set of dose-response experiments on K10 gene expression levels, the lowest effective dose of CBD ($p < 0.001$) and CBG ($p < 0.05$) was found to be 0.5 μM , whereas CBDV was ineffective up to 1.0 μM , previously found to be the lowest effective dose of AEA [8]. Therefore, in all subsequent experiments, CBD and CBG were used at 0.5 μM , and CBDV and AEA at 1.0 μM . By using qRT-PCR analysis, we showed a significant reduction in the expression of K10 and TGase5 genes after the treatment of differentiated HaCaT cells with 0.5 μM CBD ($p < 0.001$) or CBG ($p < 0.05$ for K10; $p < 0.001$ for TGase5), as well as with 1.0 μM AEA ($p < 0.001$).

Also, the expression of involucrin and K1 genes was significantly inhibited by CBD but not by CBG under the same experimental conditions, resembling the effect of AEA. In contrast, 1.0 μM CBDV did not change the expression of any gene tested. Based on the findings, further analyses were performed on K10 to test the effects of AEA [8]. The study was extended to investigate the most relevant effects

of AEA, CBD, and CBG on primary cells, NHEKs. The results revealed that all the treatments induced a consistent down-regulation of K10 gene expression. Unfortunately, the difficulties in growing NHEKs prevented the further extension of the analyses in the primary cells.

The molecular mechanism by which CBD and CBG affect K10 gene expression revealed that the effect of 0.5 μM CBD was reversed by 0.05 μM SR141716 ($p < 0.05$), a selective CB1 receptor antagonist, but not by 0.05 μM SR144528, a selective antagonist of CB2 receptors [19]. Additionally, 0.5 μM CPZ, a selective antagonist of TRPV1 receptors, was ineffective in keeping with the absence of TRPV1 receptors in HaCaT cells [7]. The data suggest that CBD triggered a CB1-dependent mechanism that resembled that already observed for AEA. In contrast, the effect of CBG on K10 mRNA levels was not inhibited by any of the three selective receptor antagonists, supporting the involvement of a distinct transduction pathway. Incidentally, SR141716 and SR144528 were used at concentrations previously shown to block their specific targets in HaCaT cells [8].

The methylation status of the K10 gene, using a bisulfite-based methylation-specific PCR assay, was examined. The methylation level of K10 was significantly decreased ($p < 0.001$) upon differentiation of proliferating HaCaT cells with TPA plus calcium. Both CBD and CBG significantly increased K10 gene methylation in differentiated cells ($p < 0.001$ and $p < 0.05$, respectively), thus producing the effect of AEA8. The effect of CBD was due to a CB1-dependent mechanism because it was prevented by SR141716 ($p < 0.05$). In contrast, CBG did not trigger CB1 signaling because SR141716 did not counteract the effect of this phytocannabinoid on the K10 gene.

The overall methylation levels were measured in human keratinocytes using an SSSI methylase assay. Firstly, differentiation of HaCaT cells led to a significant reduction ($p < 0.05$) in global DNA methylation; secondly, AEA ($p < 0.01$) and CBD ($p < 0.05$), but not CBG increased DNA methylation levels in differentiated cells, up to those of proliferating cells. Again, the effect of CBD was reversed by SR141716 ($p < 0.05$), indicating a CB1-dependent mechanism. The effect of CBG was not dependent on this receptor.

The effect of CBD and CBG on genomic DNA methylation through the regulation of DNMT activity was also examined. Similarly to AEA, CBD increased ($p = 0.4156$) in DNMT activity in differentiating cells. In contrast, CBG induced a small yet insignificant ($p = 0.1043$) decrease in enzyme activity in the same cells. Selective alterations in the gene expression of the various DNMTs in differentiated HaCaT cells were demonstrated, either untreated or after exposure to AEA, CBG, and CBD. In particular, it was found that DNMT1 gene expression was significantly reduced ($p = 0.0039$) in differentiated cells and was upregulated by AEA ($p = 0.1014$), CBD ($p = 0.3290$), and CBG ($p = 0.0520$). However, this did not reach statistical significance. Consistently, densitometric analysis of DNMT1 levels revealed a reduction in the enzyme protein in differentiated cells and a recovery toward that of proliferating cells after any treatment. The levels of gene expression of all the other DNMTs analyzed (DNMT3a, DNMT3b, DNMT3L) were not affected by any of the compounds tested under the same experimental conditions.

The expression of epidermal differentiation genes (keratins, involucrin, and transglutaminase) is regulated by the phytocannabinoids CBD and CBG, but not CBDV, through distinct mechanisms. The effect of CBD depended on CB1 cannabinoid receptors, similar to that reported for AEA. In contrast, CBG did not affect this or the other AEA-binding receptor subtype, the CB2 cannabinoid receptor. CBG did not affect the transcription of involucrin and K1, but it down-regulated that of K10 and TGase. In the context, it should be recalled that CBD and CBG also dose-dependently inhibit keratinocyte proliferation, although at an effective dose ($>1\text{ }\mu\text{M}$) higher than the optimal dose ($0.5\text{ }\mu\text{M}$) found here to reduce the differentiation markers K10 and TGase5 [11].

Additionally, the inhibition of epidermal differentiation elicited by CBD shares the same CB1-dependent mechanism of action as that of AEA. This appears remarkable since CBD is generally reported to have a very low affinity (in the micromolar range) for CB1 and CB2 cannabinoid receptors. However, independent investigations have revealed that it can behave as an inverse agonist or antagonist at the same receptors [20].

Cannabidiol, like AEA, might enhance the biological activity of endogenous cannabinoids by increasing their release and inhibiting their degradation. Such an entourage effect may represent an additional indirect mechanism by which CBD might modulate CB1 and CB2 signaling [21]. On the other hand; the effects of CBG on K10 gene expression were not mediated by CB1 or CB2 cannabinoid receptors. In vitro studies have shown that CBG is also an $\alpha 2$ -adrenoceptor agonist and an antagonist of 6-HT1A and TRPV1 receptors [22, 24]. The possibility that those receptors, as well as other eCBs targets like peroxisome PPAR- γ which play a role in skin biology or GPR55, might be triggered by CBG remains to be explored [23].

SR141716 also behaves as an agonist at GPR55, although there is no data on this receptor's possible involvement in the epidermis [25]. Studies have indicated that barrier recovery is delayed in CB1 knock-out mice and accelerated in CB2 knock-out mice. Additionally, CB1 activation in human keratinocytes by high doses (2.5 and $10\text{ }\mu\text{M}$) of arachidonoyl-cyclopropyl amide for 24 h increased the mRNA level of K10 at high Ca^{2+} concentrations while reducing K10 protein level under the same conditions [26].

On the one hand, knock-out animals might have developed different compensatory mechanisms that do not fully reflect the physiology of normal (wild-type) keratinocytes. On the other hand, the opposite effects of arachidonoyl-cyclopropyl amide on human keratinocytes (so-called cannabinoid paradox) at doses well above those used here might be due to complex mechanisms, which may be related to eCBs signaling mechanisms that inhibit mRNA translation, as well as to reduced cell viability and proliferation induced by eCBs and phytocannabinoids at concentrations less than $1\text{ }\mu\text{M}$ [26]. Consistent with other findings with AEA, the changes in K10 gene expression induced by CBD, but not CBG, are due to increased methylation of genomic DNA [8]. It is noteworthy that an inverse correlation between DNA methylation and the expression of differentiating genes has already been identified in human keratinocytes. However, the role of phytocannabinoids in that process is unprecedented [15].

An overall reduction in DNA methylation in differentiating keratinocytes was observed, in agreement with an early study showing that DNA methylation in human keratinocytes varies depending on the differentiation state, whereby there is lower methylcytosine content in the DNA of differentiated versus undifferentiated cells [14]. CBD reversed these changes and increased global DNA methylation in differentiated cells, thus suggesting a broader effect, not restricted to the K10 gene alone.

Melanocytes

As the key specialized cells originating from the neural crest, melanocytes play a critical role in synthesizing melanin in the epidermis, iris, hair, ears, brain, and lungs. Although melanocytes constitute a minority population in the suprabasal layers of the epidermis, they can extend multiple dendrites that facilitate their contact with multiple keratinocytes; a single melanocyte can make contact with up to 40 keratinocytes [27]. Tyrosinase is a key enzyme that catalyzes the initial steps in melanin biosynthesis. The pigment melanin imparts photoprotective benefits and acts as a free-radical scavenger [28]. Abnormal accumulation of melanin in the skin is a risk factor for melanoma.

Cannabidiol has been shown to stimulate human epidermal melanogenesis. The effects of delta-9-THC were studied and compared to CBD in human epidermal melanocytes derived from lightly-pigmented (HEMn-LP; Caucasian) and darkly-pigmented (HEMn-DP; African-American) cells over a chronic exposure of 6 days. The results revealed that both compounds displayed cytotoxicity at 4 μ M but stimulated melanin synthesis and tyrosinase activity similarly in HEMn-LP and HWMn-DP cells at nontoxic concentrations of 1–2 μ M [29].

Delta-9-THC and CBD produced a differential effect on dendricity in both cells; delta-9-THC and CBD reversibly increased dendricity in HEMn-LP cells, while there was an insignificant change in HEMn-DP cells. Delta-9-THC and CBD induced higher levels of reactive oxygen species (ROS) in HEMn-LP cells, while there was no change in the ROS levels in HEMn-DP cells. Although delta-9-THC was relatively less cytotoxic than CBD in both HEMn-LP and HEMn-DP cells, it exhibited a similar capacity to CBD to stimulate melanin synthesis and export in HEMn-LP cells which were accompanied by significant oxidative stress. HEMn-DP cells were relatively resistant to delta-9-THC and CBD, implicating the protective effects of melanin in dark-skinned individuals.

The differential impact of nicotine, the primary constituent of tobacco smoke, on melanogenesis in human melanocytes was shown to suppress melanin synthesis and tyrosinase activity in HEMn-LP cells. At the same time, it enhanced melanin synthesis and tyrosinase activity in HEMn-DP cells [30]. Nicotine was also shown to induce oxidative stress in cells from both pigmentation types and induced higher levels of melanin synthesis in primary melanocytes. Compared to tobacco smoke,

Cannabis smoke is perceived to be less harmful, although it remains in the body longer due to larger puffs, deep inhalation, and a fourfold higher breath hold time.

Primary human melanocytes possess an endocannabinoid system and their target G protein-coupled receptors CB1 and CB2. The endocannabinoid anandamide was shown to stimulate melanin synthesis and tyrosinase activity [31]. However, in one study, endocannabinoids suppressed melanin synthesis in human melanoma cells and keratinocyte co-cultures under basal and UV-irradiated conditions. The effects were shown to be mediated by the CB1 receptor [32]. The paucity of studies on the effects of delta-9-THC in normal human melanocytes prompted a study to determine whether delta-9-THC, like nicotine, might impact melanogenesis and cause adverse effects. Cannabidiol was included for comparison in the study to identify if there may be any differences in the effects of the two compounds, especially since delta-9-THC has previously been shown to diminish the melanin content of human hair follicles. In contrast, CBD was shown to stimulate the melanin content of human epidermal melanocytes [33].

Cannabinoids are metabolized by hepatic microsomal cytochrome P450 (CYP450) enzymes; delta-9-THC is primarily metabolized by isoenzymes CYP2C9 and CYP3A4 while CYP2C19 and CYP3A4 metabolize CBD. Polymorphisms in CYP2C9 have been shown to result in different bioavailability of delta-9-THC, causing higher chances of exposure to this xenobiotic. Compared to other ethnicities, Caucasians have a 35% higher prevalence rate of CYP2C9 polymorphisms that can suppress the metabolic activity of CYP2C9, which in turn enhances the bioavailability of delta-9-THC by almost two- or threefold [34].

Testing the effects on both melanin synthesis and export is important to verify the pro-melanogenic activity. A study was conducted to investigate and compare the effects of delta-9-THC and CBD on melanogenesis and oxidative stress in vitro using HEMn-LP and HEMn-DP cells. The effects of compounds on melanin export were indirectly evaluated by quantifying melanocyte dendricity, which is critical as many compounds which increase melanin synthesis may inhibit melanosome export causing depigmentation.

The results revealed that delta-9-THC produced significant cytotoxicity to HEMn-LP cells at the highest concentration of 4 μ M, where the viability was reduced by 54.47%; at lower concentrations, it significantly enhanced viability by 15.09% and 14.27% at 1 and 2 μ M, respectively. Cannabidiol induced higher cytotoxicity to HEMn-LP cells at 4 μ M with ~100% cell loss, significantly higher than cell loss at delta-9-THC (4 μ M). There was no change in the viability of cells by CBD at concentrations lower than 4 μ M.

The results indicated that delta-9-THC is less cytotoxic to both HEMn-LP and HEMn-DP cells than CBD. In HEMn-DP cells, delta-9-THC significantly lowered viability by 32.60% at 4 μ M, while at a lower concentration of 2 μ M, there was an increase in viability by 13%. Cannabidiol induced higher cytotoxicity to HEMn-DP cells at 4 μ M, with no effect at lower concentrations.

Delta-9-THC and CBD stimulated melanin synthesis in HEMn-LP cells in a concentration-dependent manner. Delta-9-THC significantly stimulated melanin synthesis by 23.52% and 36.71% at 1 μ M and 2 μ M, respectively. Cannabidiol

produced a similar trend with a significant increase of 22.76% and 44.29% at 1 μM and 2 μM , respectively. The increase in melanin synthesis by CBD at 2 μM was significantly higher by 21.53% than at 1 μM .

The intracellular tyrosinase activities were examined to determine if the stimulation of melanin synthesis is correlated to the stimulation of tyrosinase activity. The intracellular tyrosinase activities in delta-9-THC-treated cells showed a marginal increase of 12.91% at 1 μM but a significant increase of 28.59% at 2 μM compared to the control. Cannabidiol indicated a similar result as delta-9-THC, with a significant increase of 20.35% at 2 μM . These results show that the increase in melanin synthesis by delta-9-THC and CBD can be explained, at least in part, by the increase in tyrosinase activity.

The significance of melanin export as a step in the melanogenesis cycle in melanocytes has been explored. Delta-9-THC and CBD were examined for effects on melanin export. It was estimated by quantifying the total dendrite length (TDL) and the number of dendrites. The images of cells indicated a visible elongation in dendrites treated with delta-9-THC and CBD at 1 μM and 2 μM in a similar fashion. Dendritic parameters further demonstrated that delta-9-THC significantly increased TDL by 25.71% and 23.71% at 1 μM and 2 μM , respectively, compared to the control. Cannabidiol produced similar effects on TDL with significant increases of 25.02% at 1 μM and 25.25% at 2 μM . However, the compounds did not affect dendrite numbers at any concentration, indicating that in HEMn-LP cells, delta-9-THC and CBD displayed similar effects in stimulating melanin synthesis and dendricity over the concentration range of 1–2 μM .

Delta-9-THC and CBD dose-dependently stimulated melanin synthesis in HEMn-DP cells. Delta-9-THC significantly stimulated melanin synthesis by 25.53% and 41.58% at 1 μM and 2 μM , respectively. Cannabidiol significantly increased cellular melanin by 22.88% and 51.36% at 1 μM and 2 μM , respectively. The increase in melanin synthesis by CBD at 2 μM was significantly higher than CBD at 1 μM by 28.48%.

The intracellular tyrosinase activities in delta-9-THC-treated cells indicated a significant increase of 33.76% and 75.57% at concentrations of 1 μM and 2 μM , respectively, compared to the control. The increase in tyrosinase activity by delta-9-THC at 2 μM was significantly higher (41.81% higher) than at 1 μM . Cannabidiol produced similar results of dose-dependent increase with a significant increase of 25.91% and 85.33% for concentrations of 1 μM and 2 μM , respectively. The increase in tyrosinase activity by CBD at 2 μM was significantly higher (59.41%) than at 1 μM . The results indicated that the increase in melanin synthesis by delta-9-THC and CBD could be explained, at least in part, by the corresponding increase in tyrosinase activity.

The phase-contrast micrographs of HEMn-DP cells in the control group showed arborized morphology as expected for the cells; the morphology appeared to be retained in both delta-9-THC- and CBD-treated groups, although the cells treated with both compounds at 2 μM seemed to show a reduced dendrite length. The dendritic parameters indicated that delta-9-THC (2 μM) and CBD (2 μM) reduced TDL

by 15.14% and 16.65%, respectively, as compared to the control, although no statistical significance was reached. The results revealed that delta-9-THC and CBD displayed similar effects in stimulating melanin synthesis in DP cells but showed a trend toward inhibiting melanin export at a concentration of 2 μ M. Delta-9-THC and CBD did not affect the number of dendrites in the cells at any concentration.

The levels of ROS generation determined the effects of delta-9-THC and CBD on melanocyte oxidative stress. Delta-9-THC and CBD increased ROS levels in HEMn-LP cells; CBD significantly increased ROS by 29.29% at 1 μ M and 24.90% at 2 μ M. Delta-9-THC increased ROS by 14.94% and 42.13% at 1 and 2 μ M, respectively. No ROS levels were changed in HEMn-DP cells treated with delta-9-THC or CBD at any concentration.

Melanoma is the fourth most common type of cancer diagnosed in Australians after breast, prostate, and colorectal cancers. While there has been substantial progress in treating cancer in general, malignant melanoma, in particular, is resistant to existing medical therapies requiring an urgent need to develop effective treatments with lesser side effects. Studies have revealed that cannabinoids can reduce cell proliferation and induce apoptosis in melanoma cells.

As with NMSC, there are conflicting results regarding the relationship of cannabinoids to melanoma. Melanocytes produce endocannabinoid anandamide, and melanoma cells express CB1Rs and CB2Rs. One study demonstrated that the anti-growth effect of delta-9-THC on melanoma cells may be through hampering its characteristic proinflammatory microenvironment. When compared to a chemotherapy control, delta-9-THC inhibited melanoma proliferation and viability.

Kaposi's Sarcoma

Kaposi's sarcoma is the most common neoplasm caused by Kaposi's sarcoma-associated herpesvirus (KSHV). It is prevalent among the elderly in the Mediterranean, inhabitants of sub-Saharan Africa, and immunocompromised individuals such as organ transplant recipients and AIDS patients. Kaposi's sarcoma-associated herpesvirus (KSHV, also named human herpesvirus 8) is necessary but not sufficient for developing Kaposi's sarcoma. Treatments for Kaposi's sarcoma can inhibit tumor growth but cannot eliminate KSHV from the host. When the host's immune system weakens, KSHV begins to replicate again, and active tumor growth ensues. Cannabidiol exhibits promising antitumor effects and is emerging as therapeutic for this condition.

Various factors may contribute to the pathogenesis of Kaposi's sarcoma and KSHV. To evaluate how cannabinoids may affect the pathogenesis of Kaposi's sarcoma, primary human dermal microvascular endothelial cells (HMVEC) exposed to KSHV were studied. It was revealed that delta-9-THC increased the viral load in KSHV-infected HMVEC by activating the KSHV lytic switch gene, the open reading frame [48]. There was an increased efficiency of KSHV infection in the presence of low doses of delta-9-THC [35].

The results indicate that delta-9-THC can enhance KSHV infection and replication and foster KSHV-mediated endothelial transformation. It was also observed that delta-9-THC stimulated the expression of the KSHV-encoded viral G protein-coupled receptor (vGPCR) and Kaposi's sarcoma cell proliferation. Thus, cannabinoids may place individuals at greater risk for the development and progression of Kaposi's sarcoma.

A study examined the effects of CBD on the infection of endothelial cells (ECs) by KSHV and on the growth and apoptosis of KSHV-infected ECs, an *in vitro* model for the transformation of normal endothelium to Kaposi's sarcoma. While CBD did not affect the efficiency with which KSHV infects ECs, it reduced proliferation and induced apoptosis in those infected by the virus. Cannabidiol inhibited the expression of KSHV viral G protein-coupled receptor (vGPCR), its agonist, the chemokine growth-regulated protein α (GRO- α), vascular endothelial growth factor receptor 3 (VEGFR-3), and the VEGFR-3 ligand, vascular endothelial growth factor C (VEGF-C); indicating a potential mechanism by which CBD exerts its effects on KSHV-infected endothelium [36].

While KSHV usually establishes lifelong latent infection in the host, additional factors, including immunosuppression, can facilitate the transformation of infected cells into an active malignancy. KSHV produces a set of viral proteins that promote its survival and spread. The proteins can co-opt normal cellular receptors to facilitate viral entry into target cells, mimic cellular receptors to evade the immune system, and foster tumor cell proliferation [37].

The KSHV viral G protein-coupled receptor (vGPCR) contributes to the malignant transformation by immortalizing endothelial cells and inducing Kaposi's sarcoma-like lesions in a transgenic animal model [38]. vGPCR is constitutively active. *In vitro*, it can be detected in target cells during lytic infection. It is also expressed in scattered tumor cells within Kaposi's sarcoma lesions. vGPCR is highly homologous to the human CXC chemokine receptors CXCR1 and CXCR2. Ligands of the receptors, including interleukin-8 (IL-8) and growth-regulated protein α (GRO- α), can augment the effects of vGPCR. Although some cellular G protein-coupled receptor kinases may inhibit vGPCR to a small degree, and chemokines such as interferon γ -induced protein 10 (IP-10) and stromal cell-derived factor 1 α (SDF-1 α) have been reported to inhibit vGPCR signaling, no effective means to block the effects of vGPCR have advanced to clinical use.

Studies have documented the potential antitumorigenic properties of CBD in the treatment of various neoplasms, including breast cancer, lung cancer, bladder cancer, glioblastoma, and leukemia. Cannabidiol induces those effects through a variety of mechanisms and signaling pathways. For example, it inhibits the growth and metastasis of breast cancer by downregulating the expression of inhibitor of differentiation 1 (*ID-1*), limits the invasion of lung cancer cells into adjacent tissues by reducing plasminogen activator inhibitor 1 (PAI-1) levels, and induces the programmed cell death of bladder cancer cells by affecting a continuous calcium influx. In addition, CBD can enhance the inhibitory properties of delta-9-THC on the growth and survival of glioblastoma cell lines; however, the precise molecular mechanisms by which CBD mediates those effects are unclear.

KSHV-infected endothelial cells are commonly used as an *in vitro* model for Kaposi's sarcoma. Primary human dermal microvascular endothelial cells (HMVECs) were used to assess the effects of CBD on infection, proliferation, and programmed cell death. Cannabidiol inhibited proliferation and enhanced apoptosis in KSHV-infected HMVECs by blocking the expression of vGPCR, GRO- α , vascular endothelial growth factor receptor 3 (VEGFR-3), and vascular endothelial growth factor C (VEGF-C) [39]. Data support the evaluation of CBD to treat Kaposi's sarcoma.

Cannabidiol alters vascular permeability and therefore affects viral entry into endothelial cells. To examine whether CBD may enhance KSHV infection of endothelial cells, HMVECs were exposed to a green fluorescent protein (GFP)-tagged KSHV or to a mock infection in various concentrations of CBD. Under control conditions (0 μ M CBD), KSHV infected an average of 25% of HMVECs per well. No significant difference in infection efficiency was observed when the cells were pretreated with up to 10 μ M CBD. The data indicate that CBD does not modulate the infection of endothelial cells by KSHV [40].

To assess the effects of CBD on the growth of mock-infected HMVECs and KSHV-infected HMVECs (KSHV/HMVEC), models for normal endothelium and nascent Kaposi's sarcoma, respectively, an MTS-based assay was used. The assay measures changes in metabolic activity as a reflection of changes in proliferation and survival. A decrease in the percentage of proliferating mock-infected cells was found when treated with CBD at concentrations between 2.5 and 10 μ M; however, even at 10 μ M CBD, proliferation was reduced by only 20% of the controls. At concentrations between 1.5 and 10 μ M, CBD inhibited significantly more proliferation in the virus-infected HMVECs ($IC_{50} = 2.08 \mu$ M) than in the mock-infected HMVECs. At 10 μ M, CBD reduced proliferation in the virus-infected cells by 79% of the controls.

Studies have shown that CBD induces apoptosis in various tumor cells [41]. The differential effects of CBD were evaluated using the mock-infected HMVECs and KSHV-infected HMVECs on programmed cell death. While CBD induced dose-dependent apoptosis in both cell types, at 0.25–1.0 μ M, CBD induced significantly more cell death in the virus-infected HMVECs than in the mock-infected HMVECs. In the virus-infected cells, the *in vitro* EC_{50} for CBD was 1.04 μ M, while the EC_{50} of the mock-infected cells was calculated as 1.68 μ M. When treated with 2.0–10 μ M CBD, the two cell types had no significant difference in apoptosis. At the higher concentrations, CBD induced between 80% and 100% apoptosis in both mock- and virus-infected cells. The dose-response data indicate that endothelial cells infected with KSHV are more sensitive to CBD-induced apoptosis than mock-infected endothelium and indicated that CBD might preferentially kill Kaposi's sarcoma at specific concentrations of sarcoma tumor cells versus normal endothelium.

To explore the mechanism by which CBD inhibits the growth of KSHV/HMVECs and induces their programmed cell death, the effect of CBD was examined on KSHV vGPCR, a protein that immortalizes endothelial cells and promotes Kaposi's sarcoma tumorigenesis [42]. vGPCR facilitates the transformation of KSHV-infected cells and enhances the proliferation of KSHV-infected endothelium

[38]. Protein levels and localization in Kaposi's sarcoma lesions were examined by immunohistochemical analysis to confirm that vGPCR is expressed in Kaposi's sarcoma; vGPCR expression was present in irregular vascular formations and scattered spindle cells of the Kaposi's sarcoma tumor sections. The lack of staining in the isotype control-treated section illustrated antibody specificity.

The effects of CBD on the expression of vGPCR in such cells were evaluated by Western blot analysis. At low doses, delta-9-THC enhanced the expression of vGPCR in KSHV-infected HMVECs [35]. Cannabidiol altered vGPCR expression in KSHV-infected HMVECs; however, in contrast to delta-9-THC, CBD induced dose-dependent inhibition. The concentrations at which CBD preferentially induced apoptosis and reduced proliferation in the KSHV/HMVECs were those at which vGPCR expression was also inhibited in the cells, indicating a potential mechanism through which CBD exerts its anticancer effects in KSHV-associated neoplasms.

Although vGPCR is a constitutively active G protein-coupled receptor, IL-8 and GRO- α can enhance its activity [43]. The levels of the cytokines in the conditioned media of KSHV/HMVECs were evaluated after treatment with various concentrations of CBD or control using ELISA. A significant dose-dependent decrease in GRO- α in the conditioned media was observed with an *in vitro* IC₅₀ of 1.66 μ M. In contrast, CBD did not produce a statistically significant change in IL-8 levels in the conditioned media.

Upon activation, the VEGFR-3 receptor, a key molecule for lymphangiogenesis, promotes KSHV-induced infection, growth, and transformation of endothelial cells [44]. To examine the activation status of VEGFR-3, a vGPCR expression plasmid or vector control into a stable 293 cell line was transiently transfected and manipulated to express high levels of VEGFR-3 (293/VEGFR-3). VEGFR-3 was immunoprecipitated from whole cell lysates, and subsequently, Western blot analyses were performed with a phosphotyrosine antibody. Significant tyrosine phosphorylation was observed in the cells transfected with vGPCR (+) compared to those transfected with the vector control (–).

To confirm the observations in a primary cell line with endogenous VEGFR-3 expression, the experiment in HMVECs was repeated. Similarly, significantly greater VEGFR-3 activation in the HMVECs transfected with vGPCR was observed compared to those transfected with the vector control. The data indicate that vGPCR activates VEGFR-3 in 293/VEGFR-3 and primary endothelial cells. When the total VEGFR-3 levels in KSHV-infected HMVECs were evaluated, high levels of the protein under control conditions were revealed, consistent with data that demonstrate the enhancement of VEGFR-3 expression by KSHV and with high endogenous expression of VEGFR-3 in Kaposi's sarcoma [45]. Treatment with various concentrations of CBD reduced the levels of VEGFR-3 significantly, indicating another mechanism where CBD may affect the proliferation and viability of Kaposi's sarcoma through the VEGFR-3 signaling pathway.

The VEGF-C and VEGF-D, the cognate ligands of VEGFR-3, in the conditioned media of KSHV/HMVECs after treatment with various concentrations of CBD were examined by ELISA. The data demonstrate that CBD inhibits the expression of VEGF-C in KSHV/HMVECs, while VEGF-D levels remain very low with/

without CBD treatment. A significant dose-dependent decrease in VEGF-C levels in the conditioned media was observed, with an in vitro IC₅₀ of 7.87 μ M; however, CBD did not affect VEGF-D expression [46]. The data are consistent with CBD-induced reduction of VEGFR-3 levels, indicating that CBD may be an inhibitor of VEGFR-3/VEGF-C signaling in Kaposi's sarcoma.

Psoriasis

Autoimmune diseases, including psoriasis, are caused by environmental and genetic factors that lead to the overactivation of immune cells and chronic inflammation. Since oxidative stress is a common feature of these diseases, which activates leukocytes to intensify inflammation, antioxidants could reduce the severity of the diseases. In addition to activating leukocytes, oxidative stress increases the production of lipid mediators, notably endocannabinoids and eicosanoids, which are products of enzymatic lipid metabolism that act through specific receptors.

The anti-inflammatory CB₂ receptors are the predominant cannabinoid receptors in leukocytes, and endocannabinoids act as anti-inflammatory factors that regulate compensatory mechanisms in autoimmune diseases. The administration of eicosanoids in vitro leads to the differentiation of lymphocytes into T helper 2 (Th₂) cells; eicosanoids are also necessary for differentiating Th₁ and Th₁₇ cells [47]. Therefore, their antagonists and the genetic deletion of their receptors abolish inflammation in animal models of psoriasis-RA and SLE. Products of non-enzymatic lipid peroxidation, especially acrolein and 4-hydroxynonenal-protein adducts, mostly generated by an oxidative burst of granulocytes, may enhance inflammation and even act as autoantigens and extracellular signaling molecules in the vicious circle of autoimmune diseases.

Psoriasis is a chronic skin disease affecting other sites, such as joints. The disease is highly dependent on inflammation, angiogenesis, and other pathways. At each step of the psoriasis molecular pathway, different inflammatory cytokines and angiogenic growth factors are involved, such as hypoxia-inducible factor-1 α (HIF-1 α), vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), basic fibroblast growth factor (bFGF), Angiopoitin-2, interleukin-8 (IL-8), IL-17, and IL-2. Besides the growth factors and cytokines, cellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which play roles in angiogenesis and inflammation, are also involved in the pathogenesis [48].

Cannabinoids are indicated for treating psoriasis due to their potential to suppress the two main steps of psoriatic pathogenesis. JWH-133 is a synthetic cannabinoid with strong anti-angiogenic and anti-inflammatory activities. The agent can inhibit HIF-1 α , VEGF, MMPs, bFGF, IL-8, IL-17, and other cytokines and adhesion molecules in vivo and in vitro.

Cannabinoids are endogenous and exogenous chemicals with antipruritic, anti-inflammatory, and antinociceptive properties. Psoriasis and psoriatic arthritis are indications for medical *Cannabis*. To assess expert perceptions of and experience

with medical *Cannabis*, an institutional review board-approved survey was disseminated to the National Psoriasis Foundation Medical Advisory Board, with a 72% response rate (24/33). Among dermatologists, 83.3% were uncertain if psoriasis was an indication for medical *Cannabis* in the United States; similarly, 80% of rheumatologists surveyed were unsure about psoriatic arthritis.

However, 83.3% of respondents supported medical *Cannabis* use, though only 25.0% have educated patients on medical *Cannabis*, and 21.7% have recommended medical *Cannabis*. Only 79.2% reported they did not have sufficient knowledge to explain the risks and benefits of medical *Cannabis*; 16.7% of respondents had prior education on medical *Cannabis*. Importantly, 100% of the respondents expressed interest in learning about the potential uses of medical *Cannabis*, in parallel with past studies which found that an overwhelming majority of dermatologists surveyed also wanted more education on medical *Cannabis* applications (91–93%).

Reasons against recommending medical *Cannabis* included limited knowledge (60.0%), no experience (45.0%), and discomfort (20%). Beyond familiarity, 55.5% and 60% of Medical Advisory Board dermatologists and rheumatologists, respectively, believe medical *Cannabis* is safe for their patients, and no respondents believed that medical *Cannabis* is unsafe. While 21.1% of respondents previously recommended medical *Cannabis*, 33.3% stated they are likely to recommend medical *Cannabis* in the future, and 16.7% stated they are unlikely to do so, highlighting a large subset that is undecided, likely due to limited knowledge [49].

Cannabinoid receptors are expressed throughout the skin epithelium. CB1 activation inhibits human hair growth and decreases the proliferation of epidermal keratinocytes. The up-regulation of keratins K6 and K16 by psoriasis was investigated by examining whether CB1 stimulation modulates their expression in the human epidermis. Treatment of organ-cultured human skin with the CB1-specific agonist, arachidonoyl-chloro-ethanolamide (ACEA), decreased K6 and K16 staining intensity in situ. At the gene and protein levels, ACEA also decreased the K6 expression of cultured HaCaT keratinocytes, which show some similarities to psoriatic keratinocytes. The CB1-specific antagonist, AM251, partly antagonized the effects.

While CB1-mediated signaling also inhibited human epidermal keratinocyte proliferation in situ, as shown by K6/Ki-67-double immunofluorescence, the inhibitory effect of ACEA on K6 expression in situ was independent of its anti-proliferative effect. The role of K6 as a functionally important protein that regulates epithelial wound healing in mice indicates that the CB1-mediated regulation of keratin 6/16 is relevant to wound healing [50]. The results revealed that cannabinoids and their receptors constitute a clinically relevant control element of human K6 and K16 expression.

To evaluate CB2 variants association in the presence of immune-mediated disorders (IMDs) in patients with chronic HCV infection, 168 anti-HCV/HCV-RNA-positive patients were examined; 81 had signs of IMDs and 87 without. The patients were screened for the CNR2 rs35761398 single nucleotide polymorphism using a TaqMan Assay. In the IMDs group, 22 (27.2%) showed ANA positivity (titers $\geq 1:160$), 3 (3.7%) SMA positivity (titers $\geq 1:160$), 24 (29.6%) had

cryoglobulinemia, 25 (30.9%) autoimmune thyroiditis, four (4.9%) psoriasis, two (2.5%) B-cell non-Hodgkin's lymphoma, and one (1.2%) autoimmune hemolytic anemia.

Compared with the 87 patients without IMDs, the 81 with IMDs were more frequently females (65% vs. 45%, $p = 0.01$). However, no significant difference was found in the initial demographic, epidemiological, and serological, biochemical, or virological data. Instead, the prevalence of patients with the CB2-63 RR variant was significantly higher in the IMD than in the non-IMD group (49.4% vs. 24.1%, $p = 0.001$) [51, 52]. A logistic regression analysis including the CB2-63 receptor (RR vs. QR or QQ), age, and sex identified the CB2-63 RR as the only independent predictor of IMDs ($p = 0.005$).

Inflammatory granulocytes are characterized by an oxidative burst, which promotes oxidative stress and lipid modification both in affected tissues and on a systemic level. Redox signaling, involving lipid peroxidation products acting as second messengers of free radicals, plays an important role in the pathophysiology of inflammation and various stress-associated disorders.

A study to evaluate the onset of oxidative stress and alterations of enzyme-dependent lipid metabolism from redox imbalance in granulocytes and plasma obtained from patients with psoriasis vulgaris or psoriatic arthritis was examined and compared to healthy subjects. The results revealed enhanced activity of pro-oxidant enzymes nicotinamide adenine dinucleotide phosphate (NADPH) and xanthine oxidases in granulocytes with a decrease in enzymatic and non-enzymatic antioxidants in the plasma of psoriatic patients.

The nuclear factor erythroid 2-related factor 2 (Nrf2) and its regulators increased in both psoriasis forms, while heme oxygenase 1 levels were increased only in psoriasis vulgaris. The redox imbalance was associated with decreased phospholipids and free polyunsaturated fatty acids but with enhanced activity of enzymes involved in lipid metabolism (phospholipase A2, acetylhydrolase PAF, cyclooxygenases 1 and 2) and increased lipid peroxidation products 4-hydroxynonenal, isoprostanes, and neuroprostanes [53].

Increased endocannabinoids and G protein-coupled receptor 55 were observed in both forms of the disease. In contrast, expression of the cannabinoid type 1 receptor was increased only in patients with psoriatic arthritis, which is opposite to the cannabinoid type 2 receptors. The receptors were increased only in psoriasis vulgaris. Changes in protein expression promoted the apoptosis of granulocytes by increased caspases, mainly in psoriasis vulgaris. The study indicates that inhibition of the Nrf2 pathway in psoriatic arthritis promotes a redox imbalance. In addition, increased expression of CB1 receptors leads to increased oxidative stress, lipid modifications, and inflammation which promote the progression of psoriasis into the advanced, arthritic form of the disease.

UVB phototherapy is a treatment for psoriasis that increases phospholipid oxidative modifications in the skin's cell membrane. Lipidomic analysis was conducted on the keratinocytes of healthy individuals and patients with psoriasis irradiated with ultraviolet B (UVB) and treated with cannabidiol. Cannabidiol treatment of psoriatic keratinocytes produced downregulation of phosphatidylcholine,

phosphatidylserine, and upregulation of certain PEO and an SM species, SM (d42:2), and the zeta potential [54]. The results revealed that in psoriatic keratinocytes, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, and ether-linked phosphoethanolamine were down, while SM (d41:2) was upregulated. The changes were accompanied by an increase in negative zeta potential, which indicates phosphatidylserine's translocation to the membrane's outer layer.

However, UVB irradiation of psoriatic keratinocytes resulted in the upregulation of phosphatidylcholine, phosphatidylcholine plasmalogens, phosphoethanolamine, and a decrease in the negative zeta potential. The exposure of UVB-irradiated cells to CBD decreased the level of SM (d42:2). The results indicated that CBD induces pro-apoptotic mechanisms in psoriatic keratinocytes while simultaneously improving the antioxidant properties and preventing the loss of transepidermal water of keratinocytes of patients irradiated with UVB.

Psoriasis is associated with increased production of reactive oxygen species, which leads to oxidative stress. As an antioxidant, cannabidiol can provide protection. A study evaluated the effects of cannabidiol on neutrophil extracellular trap (NET) formation in psoriatic and healthy neutrophils. Important markers of NETosis were measured in healthy and psoriatic neutrophils after incubation with CBD, lipopolysaccharide (LPS), and LPS + CBD.

The percentage of neutrophils undergoing NETosis and the level of NETosis markers (cfDNA, MPO, elastase) were higher in the neutrophils and blood plasma of psoriatic patients compared to controls. After lipopolysaccharide treatment, all of the markers of NETosis, except elastase and p47 and citrullinated histones, were increased in samples from healthy subjects and psoriasis patients.

Cannabidiol reduces NETosis, mainly in psoriatic neutrophils, possibly due to its antioxidant properties. Cannabidiol reduced the concentrations of NETosis markers leading to a reduction in NETosis, which was more pronounced in psoriatic neutrophils and neutrophils treated with lipopolysaccharide in both psoriatic and healthy participants. The results indicated that psoriatic patients' neutrophils are at a higher risk of NETosis *in vitro* and *in vivo*. The anti-NET properties of CBD revealed the positive effect of CBD in the treatment of autoimmune diseases [55].

Psoriasis is characterized by dysregulated keratinocyte differentiation, but oxidative stress also plays an important role in the pathogenesis of the disease. The effect of cannabidiol was examined on the redox balance and phospholipid metabolism in ultraviolet A (UVA)/UVB-irradiated keratinocytes isolated from the skin of psoriatic patients or healthy volunteers. Cannabidiol accumulates mainly in membrane keratinocytes, especially in patients with psoriasis. It reduces the redox imbalance observed in the UV-irradiated keratinocytes of healthy subjects by decreasing reactive oxygen species generation, increasing the Trx-dependent system efficiency, and increasing vitamin A and E levels. Consequently, a reduction in lipid peroxidation products, such as 8-isoprostanes and 4-hydroxynonenal, was observed.

Cannabidiol regulates metabolic changes by modulating the endocannabinoid system disturbed by psoriasis development and UV irradiation. Cannabidiol modifies redox balance and lipid peroxidation in psoriatic patient keratinocytes following UV irradiation. The changes are largely opposed to the case of keratinocytes

from healthy subjects. A decrease in anandamide level was observed in the UV-irradiated keratinocytes of healthy controls following CBD treatment, while in keratinocytes from patients treated with CBD, the anandamide level was increased.

The palmitoylethanolamide (PEA) level was decreased in both groups treated with CBD. Cannabidiol increases CB1 receptor expression, primarily in the keratinocytes of patients, and increases CB2 receptor expression in both the psoriatic and control groups. However, CBD decreases CB2 receptor expression in UV-irradiated keratinocytes taken from patients. The UV- and psoriasis-induced activity of transmembrane transporters (multi-drug-resistance (MDR) and breast cancer resistance protein (BCRP)) is normalized after CBD treatment. Cannabidiol partially reduces oxidative stress in the keratinocytes of healthy individuals while showing a tendency to increase the oxidative and inflammatory state in the keratinocytes of patients with psoriasis, especially following UV irradiation [56].

The interest in using medical *Cannabis* for psoriasis, eczema, and acne has been growing commercially as a variety of cannabinoid preparations in topical forms such as creams, salves, lotions, and lubricants.

A 33-year-old male patient with no past medical history presented with a 1-year history of psoriasis. The patient reported a strong family history of psoriasis with his sister and father. The course of the disease began 2 years before diagnosis with erythematous, scaly plaques in sebaceous areas, which progressively worsened. Over-the-counter topical hydrocortisone steroid cream failed to control his symptoms. The primary care physician prescribed triamcinolone acetonide cream 0.1%, which reduced erythema on the patient's face, but for only a short time, and the disease spread to his trunk and other areas body.

Apremilast was recommended for follow-up; however, he was uninterested because of the cost and side effect profile and wished to pursue a natural approach. After reviewing the pros and cons of utilizing topical medical cannabinoids, the patient was provided with samples of delta-9-tetrahydrocannabinol distillate cream with medium chain triglyceride (MCT) oil, beeswax, and sunflower lecithin, delta-9-THC soap infused with scent-free hemp soap, 5 mg/mL, and hair oil with delta-9-THC distillate dissolved into jojoba oil, 5 mg/mL. The patient was advised to apply the cream and oil to patches on the body's skin, use the oil on the hair-bearing areas daily, and use soap when showering.

The patient reported improvement within 2 days of initiating this regimen. Following 2 weeks of use, the patient reported continued improvement and clearance. Two months after the beginning of treatment, the patient's wife reported that he was only using the products once a week at the first sign of a flare, that the symptoms resolved quickly, and that he did not require further medication for the next week. Seven months after initial use, the patient reported that he continues to use the products regularly for maintenance; he uses the soap to prevent the activity of areas with a high density of hair follicles and follows up with the creams every few days to control skin dryness [57].

Cannabinoids may be promising in treating psoriasis due to their potential inhibitory effects on keratinocyte proliferation. Studies indicated that the effect of cannabinoids on psoriasis might act independently of CB1 and CB2 receptors. In

human skin models, a synthetic CB1R agonist inhibited keratinocyte cell proliferation and reduced the expression of K6 and K16.

Acne Vulgaris and Seborrhea

Acne is a skin disease characterized by elevated sebum production and inflammation of the sebaceous glands. It has been demonstrated that cannabidiol exerted complex anti-acne effects by normalizing pro-acne agents-induced excessive sebaceous lipid production, reducing proliferation, and alleviating inflammation in human SZ95 sebocytes. A study explored the putative anti-acne effects of non-psychotropic phytocannabinoids ((-)-cannabichromene [CBC], (-)-cannabidivarin [CBDV], (-)-cannabigerol [CBG], (-)-cannabigerovarin [CBGV] and (-)-delta-9-tetrahydrocannabivarin [THCV]). MTT and CyQUANT assays investigated the viability and proliferation of human SZ95 sebocytes; cell death and lipid synthesis were monitored by DiIC1 (5)-SYTOX Green labeling and Nile Red staining, respectively. Inflammatory responses were investigated by monitoring expressions of selected cytokines upon lipopolysaccharide treatment (RT-qPCR, ELISA).

Up to 10 μm , the phytocannabinoids negligibly altered the viability of the sebocytes, whereas high doses ($\geq 50 \mu\text{m}$) induced apoptosis. The substances differentially modulated basal sebaceous lipid synthesis: CBC and THCV suppressed it, and CBDV had only minor effects, whereas CBG and CBGV increased it. Importantly, CBC, CBDV, and THCV significantly reduced arachidonic acid (AA)-induced acne-like lipogenesis. (-)-Delta-9-tetrahydrocannabivarin suppressed proliferation, and the phytocannabinoids exerted remarkable anti-inflammatory actions. The data revealed that CBG and CBGV might have potential in treating dry-skin syndrome, whereas CBC, CBDV, and especially THCV indicated promise to become effective anti-acne agents [58].

Cannabinoids have a multifactorial impact on sebaceous disorders. The expression of CB1 and CB2 receptors in human sebaceous glands has been demonstrated. Key enzymes that synthesize and degrade endocannabinoids are also expressed in human sebocytes. Cannabinoids may influence sebocytes via activation of the CB2Rs to inhibit lipogenic actions of arachidonic acid, linoleic acid, and testosterone resulting in decreased lipid production and sebum secretion.

The role of the cannabinoid system in pilosebaceous biology has been explored to determine the impact of endocannabinoids on the human sebaceous gland using human SZ95 sebocytes as the cell culture model. SZ95 sebocytes express CB2 but not CB1. The endocannabinoids arachidonoyl ethanolamide/anandamide and 2-arachidonoyl glycerol are present in SZ95 sebocytes and dose-dependently induce lipid production and apoptosis-driven cell death. The endocannabinoids also upregulate the expression of key genes involved in lipid synthesis, e.g., PPAR transcription factors and some of their target genes. The actions are selectively mediated by CB2-coupled signaling involving the MAPK pathway, as revealed by specific agonists/antagonists and RNA interference [59].

Cells with silenced CB2 exhibited significantly suppressed basal lipid production, indicating that human sebocytes utilize a paracrine-autocrine, endogenously active, CB2-mediated endocannabinoid signaling system for positively regulating lipid production and cell death. The involvement of cannabinoid CB2 receptor and the effect of cannabinoid CB2 receptor antagonist/inverse agonists on cutaneous inflammation were investigated. The ears of mice were topically exposed to an ether-linked analog of 2-arachidonoylglycerol (2-AG-E) or selective cannabinoid CB2 receptor agonist, {4-[4-(1,1-dimethylheptyl)-2,6-dimethoxy-phenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl}-methanol (HU-308), had early. Late ear swellings were observed at zero to 24 h and one to 8 days after exposure. Both types of responses induced by 2-AG-E were significantly suppressed by the oral administration of cannabinoid CB2 receptor antagonist/inverse agonists, [*N*-(benzo[1,3]dioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide] (JTE-907) and {*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide} (SR144528).

In contrast, JTE-907 did not affect arachidonic acid-induced swelling. Orally administered JTE-907 (0.1–10 mg/kg) and SR144528 (1 mg/kg) also significantly inhibited dinitrofluorobenzene-induced ear swelling, with increased cannabinoid CB2 receptor mRNA expression observed in the inflamed ear. The results indicate that the cannabinoid CB2 receptor is partially involved in local inflammatory responses. The cannabinoid CB2 receptor antagonist/inverse agonist has beneficial effects on ear swelling.

Acne is an inflammatory skin disease, and cannabidiol has been proven to exert universal anti-inflammatory properties. A study explored the effect of CBD on acne inflammation induced by *Cutibacterium acnes*-derived extracellular vesicles (CEVs) in keratinocytes and revealed the underlying mechanisms. *Cutibacterium acnes*-derived extracellular vesicles stimulated normal human epidermal keratinocytes (NHEKs) in the presence of CBD or vehicle. Interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)- α levels were examined by RT-PCR and ELISA. The expression of cannabinoid type-2 (CB2) receptor and transient receptor potential vanilloid type-1 (TRPV1) was detected by Western blotting. TNF- α levels in the presence of CB2 receptor antagonist (AM630) or TRPV1 antagonist (Capsazepine) were detected by RT-PCR. The activation of MAPK and NF- κ B signaling pathways and the nuclear translocation of NF- κ B p65 upon CBD treatment were analyzed by Western blotting and immunofluorescence assay, respectively.

The expression of inflammatory cytokines (IL-6, IL-8, and TNF- α) in CEVs-stimulated NHEKs was suppressed by CBD. Cannabinoid receptor 2 expressions were upregulated by CBD, whereas CBD downregulated CEVs-promoted TRPV1 expression. AM630 reversed TNF- α levels inhibited by CBD. Capsazepine exerts an inhibitory effect on CEVs-induced inflammation and has a synergistic impact with CBD. The phosphorylation of ERK1/2 and NF- κ B p65 and nuclear translocation of NF- κ B p65 were induced by CEVs but reduced by CBD [60].

The results indicated that CBD could inhibit inflammation induced by CEVs in NHEKs, which was mediated by activation of the CB2 receptor and enhanced by

the TRPV1 antagonist through inactivation of the MAPK and NF- κ B signaling pathways. Escalated sebum fabrication adds to the growth of acne. A study examined the efficacy and safety of 3% *Cannabis* seed extract cream on human cheek skin sebum and erythema content. A base plus 3% *Cannabis* seeds extract, and base (control) were prepared for a single-blinded and comparative study. Healthy males were instructed to apply the base plus 3% *Cannabis* seeds extract and base twice a day to their cheeks for 12 weeks. Measurements for sebum and erythema content were recorded at baseline, second, fourth, sixth, eighth, tenth, and twelfth week in a control room with Sebumeter and Mexameter. Adverse events were observed to determine skin irritation.

The base plus 3% *Cannabis* seeds extract was safe for volunteers. Measurements demonstrated that skin sebum and erythema content of base plus 3% *Cannabis* seeds extract treated side showed a significant decrease ($p < 0.05$) compared with base treated side. The base plus 3% *Cannabis* seeds extract produced no adverse effect. It was well tolerated for the reduction of skin sebum and erythema content [61].

Cannabigerol is a minor non-psychoactive cannabinoid. At low levels (<1% per dry weight), it serves as the direct precursor to both cannabidiol and delta-9-tetrahydrocannabinol. The extraction and purification of CBG from *Cannabis* are both challenging and expensive. However, using a novel yeast fermentation technology platform, cannabinoids such as CBG can be produced more sustainably, cost-effectively, and timely than plant-based production.

Cannabidiol has been studied extensively and produced several beneficial skin properties. A study clinically examined the in vitro activity profile of CBG and CBD on human skin. Gene microarray analysis conducted using 3D human skin equivalents demonstrates that CBG regulates more genes than CBD, including several key skin targets.

Human dermal fibroblasts (HDFs) and normal human epidermal keratinocytes were exposed in culture to proinflammatory inducers to trigger cytokine production and oxidative stress. The results demonstrate that CBG and CBD reduce reactive oxygen species levels in HDFs better than vitamin C. Cannabigerol inhibits proinflammatory cytokine (Interleukin-1 β , -6, -8, tumor necrosis factor α) release from several inflammatory inducers, such as ultraviolet A (UVA), ultraviolet B (UVB), chemical, *C. acnes*, and in some instances does so more potently than CBD [62].

A 20-subject vehicle-controlled clinical study was performed with 0.1% CBG serum, and a placebo was applied topically for 2 weeks after sodium lauryl sulfate (SLS)-induced irritation. CBG serum produced statistically significant improvement, above placebo, for transepidermal water loss (TEWL) and reduction in the appearance of redness. Cannabigerol's broad range of in vitro and clinical skin health-promoting activities demonstrates its strong potential as a safe, effective ingredient for topical use. It indicates areas where it could be more effective than CBD.

Alopecia and Hair Growth Disorders

Most cases of excessive hair loss and unwanted hair growth, such as effluvium, alopecia, hirsutism, and hypertrichosis, result in part from major disturbances in the cyclic transformation of hair follicles (HFs), mainly their switch from active growth and pigmented hair shaft production (anagen) to apoptosis-driven HF involution (catagen). Complex neuroendocrine pathways profoundly influence the switch.

The endocannabinoid system's activation of CB1 receptors by the endocannabinoid anandamide and the plant-derived (–)-delta-*trans*-tetrahydrocannabinol promotes premature catagen entry in human scalp HFs *ex vivo*. However, 2-arachidonoylglycerol, the other prototypic endocannabinoid, does not modulate HF growth *ex vivo*, indicating functional heterogeneity between different cannabinoids in human hair growth modulation [63].

Cannabidiol exerts complex sebostatic and anti-inflammatory effects on the pilosebaceous unit by activating both transient receptor potential vanilloid-4 (TRPV4) ion channels and adenosine A2A receptors on sebocytes [65]. Cannabidiol does not activate CB1 receptors but may context-dependently signal via modulating several other targets, such as GPR55, 5-HT1a, or μ -opioid receptors [64]. Topically administered CBD is likely also to reach human HFs, which express functional TRPV4 receptors, the stimulation of which induces premature catagen, whereas adenosine reportedly promotes human hair growth.

When applied at 0.1 μ M, CBD promotes hair shaft elongation but, unexpectedly, did not modulate HF cycling and hair matrix keratinocyte proliferation or apoptosis *ex vivo*. The tendency may represent a pseudo-elongation of the hair shaft, pushed out of its anchorage in the HF by tissue shrinkage during organ culture. However, 10 μ M CBD significantly suppressed hair shaft production and induced the development of catagen morphology. The action was accompanied by a significant reduction of the ratio of proliferating (Ki-67+) and a significant increase in the percentage of apoptotic (TUNEL+) hair matrix keratinocytes indicating the onset of an apoptosis-associated involution.

This hypothesis was probed in cultured ORSKs to facilitate mechanistic analyses and permit higher throughput assessments than very scarcely available scalp HFs. In line with the above HF data, CBD rapidly decreased the ORSK count in 3- to 72-h treatments; CyQUANT assay; reduced viability, and induced chiefly apoptotic cell death in a concentration- and time-dependent manner (24-h treatments; MTT assay and combined 1,1',3,3,3',3'-hexamethylindodicarbo-cyanine iodide (DiIc1 (5))-SYTOX Green labeling).

Cannabidiol activates the mostly calcium-permeable TRPV4 ion channels, stimulating catagen in human HFs *ex vivo* [66]. The catagen-promoting CBD concentration ($\geq 10 \mu$ M) induced calcium influx in cultured ORSKs, which the highly selective TRPV4 antagonist almost completely abrogated, HC067047 (1 μ M), indicating that at micromolar concentrations, CBD activates TRPV4 in human ORSKs and that the catagen-promoting effect on organ-cultured HFs may be stimulated via signaling through the catagen-inducing pathway [67].

Toll-like receptor 3 activation on human ORSKs leads to inflammasome activation and subsequent IL-1 β production, which creates a proinflammatory, catagen- and HF dystrophy-promoting signaling milieu. Since CBD exerts remarkable anti-inflammatory effects in several systems, a non-cytotoxic and non-catagen-promoting CBD concentration (0.1 μ M) impacts the production of catagen-promoting cytokines by ORSKs was examined. Cannabidiol effectively antagonized not only the toll-like receptor 3-activator polyinosinic-polycytidylic acid-induced upregulation of IL-1 β but also that of IL-6, IL-8, and tumor necrosis factor- α . Similar to sebocytes, the anti-inflammatory actions of CBD appear to primarily depend on adenosine receptor-mediated signaling because the adenosine receptor pan-antagonist CGS 15943 almost completely abrogated the CBD effects [68].

Besides potentially reducing the hair growth-inhibitory effects of the likely high level of the wounding-associated cytokines of freshly microdissected HFs compared with vehicle-treated controls, the moderate hair shaft-stimulatory effects of 0.1 μ M CBD also result from increased fibroblast growth factor-7 transcription by human dermal papilla fibroblasts.

Human scalp hair follicles are targets and sources of endocannabinoids. Studies indicated that the cannabinoid system is a key player in cell growth control. The endocannabinoid N-arachidonylethanolamide (anandamide, AEA) and the exocannabinoid delta-9-tetrahydrocannabinol dose-dependently inhibited hair shaft elongation and the proliferation of hair matrix keratinocytes and induced intraepithelial apoptosis and premature HF regression (catagen). A selective antagonist of cannabinoid receptor-1 inhibited the effects. In contrast to CB2, CB1 was expressed in a hair cycle-dependent manner in the human HF epithelium. The presence of endocannabinoids in human HF strongly suggests that human HF exploit a CB1-mediated endocannabinoid signaling system to regulate their growth negatively. Clinically, CB1 agonists may help manage unwanted hair growth, while CB1 antagonists might counteract hair loss [63].

Cannabinoids may have therapeutic potential in hair growth disorders, especially those targeting CB1R or TRP channels as antagonists. CB1 receptors are expressed in human hair follicles. CB1 receptors and cannabinoid-responsive receptors, like TRP ion channels, promote the onset of the catagen phase and suppress hair shaft elongation when activated. Agonists could have benefits for hirsutism or hypertrichosis.

Pruritus

Chronic pruritus of any origin is a frequent discomfort in daily medical practice, and its therapy is challenging. The neuronal mechanisms underlying chronic pruritus have been partly identified and offer new therapeutic strategies. For example, mast cell (MC) degranulation, activation of neuroreceptors on sensory nerve fibers, and neurogenic inflammation has been identified to be involved in the induction and

chronification of the symptom. Accordingly, controlling neuroreceptors such as cannabinoid receptors by agonists or antagonists showed high antipruritic efficacy.

It has been demonstrated that pruritus and pain have neuronal pathways with broad interactions. Specialized nerve fibers and sensory receptors transmit pruritus to the central nervous system. The developments show that highlighting the basis of pruritus offers modern neurophysiological and neurochemical therapeutic models and the possibility of treating patients with refractory itching of different origins.

The relationship between cannabinoids and itch is multifactorial. Cannabinoids inhibit mast cell degranulation and histamine release. In skin prick tests, an irritant (falscarinol) found in ginseng, carrots, and parsley, caused histamine-induced edema by acting as an antagonist of CB1 receptor on keratinocytes and increasing proinflammatory cytokines like interleukin (IL)-8 and monocyte chemotactic protein-1. Cutaneous nerve fibers contain CB1 and CB2 receptors.

Pruritus due to cholestatic liver disease can be difficult to manage and frequently intractable to various medical therapies. The efficacy of delta-9-tetrahydrocannabinol was evaluated for intractable cholestatic-related pruritus (ICRP), in which conventional (and unconventional) remedies had failed. Three patients were evaluated for plasmapheresis because of ICRP. The three patients had previously been extensively treated with standard therapies for ICRP, including diphenhydramine, chlorpheniramine, cholestyramine, rifampicin, phenobarbital, doxepin, naltrexone, UV therapy, and topical lotions. Even multiple courses of plasmapheresis were performed without any benefit for the intractable pruritus. The patients reported significant decreases in their quality of life, including lack of sleep, depression, inability to work, and suicidal ideations.

The patients started on 5 mg of delta-9-THC (Marinol) at bedtime. The three patients reported a decrease in pruritus, marked improvement in sleep, and eventually were able to return to work. The resolution of depression occurred in two of three. Side effects related to the drug include one patient experiencing a disturbance in coordination. Marinol dosage was decreased to 2.5 mg in that patient with the resolution of symptoms. The duration of the antipruritic effect is approximately 4–6 h in the three patients suggesting the need for more frequent dosing [117].

JTE-907, *N*-(benzo[1,3]dioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide, is a selective cannabinoid CB2 receptor antagonist/inverse agonist. The antipruritic activity of JTE-907 was studied in NC mice with chronic dermatitis, a model of atopic dermatitis. The oral dose of JTE-907 (1 and 10 mg/kg/day), immunosuppressant agent tacrolimus (1 mg/kg/day), and a glucocorticoid betamethasone 17-valerate (1 mg/kg/day) for 20 days suppressed the spontaneous scratching and cutaneous nerve activity of NC mice. JTE-907 (10, but not 1, mg/kg) and tacrolimus, but not betamethasone, alleviate dermatitis. Betamethasone inhibited body weight gain. The results suggest that JTE-907 suppresses spontaneous itch-associated responses of NC mice without adverse effects such as weight loss.

Cannabinoids, heat shock protein 90 (Hsp90), and nitric oxide (NO) are involved in dry skin-induced itch. Heat shock protein 90 inhibitors and cannabinoid agonists ameliorate dry skin-induced chronic itch. The contribution of the Th2 cell signaling

pathway to the antipruritic effect of the hsp90 inhibitor 17-Alilamino-17-demethoxygeldanamycin (17-AAG), nitric oxide synthase (NOS) inhibitor N ω -Nitro-L-arginine methyl ester hydrochloride (L-NAME) and cannabinoid agonist WIN-55212-2 on a dry skin-induced scratch was investigated.

Dry skin-induced chronic itching was created by topical application of AEW (acetone/diethyl ether/water). WIN-55212-2 (1 mg/kg, i.p.), L-NAME (1 mg/kg, i.p.), and increasing doses of 17-AAG (1, 3, and 5 mg/kg, i.p.) were administered to Balb/c mice (for each group, $n = 6$). After the applications, skin tissues were taken from the nape region of all mice. Gene and protein expressions of IL-13 and IL-31 were evaluated in skin tissues by RT-PCR and immunohistochemistry, respectively.

IL-13 and IL-31 mRNA expressions and immune-positive cell counts were increased in the AEW-applied groups. WIN-55212-2 reduced both of the increased cytokines levels, while L-NAME decreased only the IL-13. 17-AAG dose-dependently reduced the increased cytokine levels. IL-13 and IL-31 levels significantly decreased following the co-administration of the agents. The results indicated that increased IL-13 and IL-31 are associated with pruritus. Hsp90 inhibition and cannabinoid system activation may induce antipruritic effects through the down-regulation of the cytokines [118].

In many types of itch, the interaction between immune system cells, keratinocytes, and sensory nerves involved in the transmission of the itch is quite complex. Especially for patients with chronic itching, treatments are insufficient, and the quality of life deteriorates significantly.

A study investigates the role of the heat shock protein 90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG), cannabinoid agonist WIN-55212-2, and nitric oxide (NO) synthase inhibitor N ω -nitro-L-arginine methyl ester hydrochloride in pruritus. A serotonin (5-HT)-induced (50 $\mu\text{g}/\mu\text{L}$ /mouse, i.d.) acute and acetone-ether-water (AEW)-induced chronic itching models were created, and 17-AAG (1, 3, and 5 mg/kg, i.p.), WIN-55212-2 (1 mg/kg, i.p.), and L-NAME (1 mg/kg, i.p.) were applied to Balb/c mice. 17-AAG suppressed the scratches of mice, depending on the dose. WIN-55212-2 reduced the itching behavior, but L-NAME showed no antipruritic effect at the administered dose. The combined application of the agents in both pruritus models showed synergism in the antipruritic effect. The results showed that NO did not play a role in the antipruritic effect of WIN-55212-2 and 17-AAG. Increased plasma IgE levels with AEW treatment decreased with the administration of 17-AAG (5 mg/kg, i.p.) and WIN-55212-2. The results demonstrate that Hsp90 plays a role in the peripheral pathway of pruritus, and cannabinoid agonists and Hsp90 inhibitors can be used together in treating pruritus [119].

Cannabinoids have been used for their analgesic and euphoric effects for millennia, but the antipruritic effects of *Cannabis* have been discovered. Considering the similarities between pain and itch sensations, it was hypothesized that cannabinoid receptors might play a role in the antipruritic effects of cannabinoids. To analyze the role of the spinal cannabinoid receptors 1 and 2 in the antipruritic effects of the cannabinoid agonist WIN-55212-2, male Balb/c mice weighing 20–30 g were used.

After testing different doses of WIN-55212-2 (1, 3, and 10 mg/kg intraperitoneally), the effects of the CB1 receptor antagonist, AM-251 (1 µg/mouse administered intrathecally (IT)) and the CB2 receptor antagonist AM-630 (4 µg/mouse IT) on the antipruritic effects of WIN-55212-2 were studied using a rotarod apparatus. Scratching behavior in the mice was produced by injection of serotonin 5 µg/50 µL intradermally into the nape of the neck. Scratching of the injection site by the hind paws was video-recorded for 30 min.

WIN-55212-2 (1, 3, or 10 mg/kg) dose-dependently decreased serotonin-induced scratches. The receptor antagonist CB1 partially reversed the effects of WIN-55212-2 ($p < 0.05$), whereas CB2 had no statistically significant effect. WIN-55212-2 impaired motor function only at the highest dose given (10 mg/kg, $p < 0.05$). The findings support previous studies indicating that cannabinoids exert antipruritic effects. Moreover, the results indicated that the antipruritic effects of cannabinoids are partially mediated by spinal CB1 receptors [120].

A study determined whether the cannabinoid agonist WIN-55212-2 reduces serotonin-induced scratching behavior and whether the neurotoxic destruction of descending serotonergic and noradrenergic pathways mediates the antipruritic effect of WIN-55212-2. Scratching behavior was induced by intradermal injection of serotonin (50 µg/50 µL/mouse) into Balb/c mice. The neurotoxins 5,7-dihydroxytryptamine (5,7-DHT, 50 µg/mouse) and 6-hydroxydopamine (6-OHDA, 20 µg/mouse) were applied intrathecally to deplete serotonin and noradrenaline in the spinal cord.

The findings indicate that cannabinoids dose-dependently reduce serotonin-induced scratching behavior, and neurotoxic destruction of descending inhibitory pathways does not mediate the antipruritic effect [121]. WIN-55212-2 (1, 3, 10 mg/kg, i.p.) dose-dependently attenuated serotonin-induced scratches. Neurotoxic destruction of neither the serotonergic nor the noradrenergic systems by 5,7-DHT and 6-OHDA, respectively, had any effect on the antipruritic action of WIN-55212-2.

Uremic pruritus is a common phenomenon in patients with end-stage renal failure, and there is no effective treatment of choice for the condition. A study was conducted to evaluate the efficacy and tolerance of cream with structured physiological lipids (DMS, Derma Membrane Structure) and endogenous cannabinoids in controlling pruritus in patients on maintenance hemodialysis. Twenty-one patients with uremic pruritus participated in a trial. The patients applied the tested cream twice daily for 3 weeks. Pruritus was evaluated using two pruritus scoring methods: a standard visual analog scale and a questionnaire method. Moreover, all patients had dry skin scored according to the 5-point scale. Global pruritus and xerosis were examined before the trial, on study visits at weekly intervals, and on follow-up visits performed 2 weeks after study discontinuation.

After the 3-week therapy, pruritus was eliminated in eight (38.1%) patients. Pruritus evaluation by both scales revealed a significant reduction of pruritus scores ($p < 0.0001$) during the tested product application. At the beginning of the trial, there was no significant correlation between dry skin intensity and pruritus severity. The 3-week treatment period resulted in a complete reduction of xerosis in 17 (81%) patients, while xerosis scores were significantly reduced ($p = 0.0001$) throughout

the study. All patients very well tolerated the test product, and it effectively reduced pruritus and xerosis in hemodialysis patients [122].

The endogenous fatty acid palmitoylethanolamide is one of the members of the N-acyl-ethanolamines family. Palmitoylethanolamide was identified more than five decades ago and has been shown to reduce allergic reactions and inflammation in animals along with influenza symptoms in humans. Interest in PEA faded until the discovery that one of its structural analogs, anandamide (arachidonoyl ethanolamide), serves as an endogenous ligand for cannabinoid receptors, the molecular target of delta-9-tetrahydrocannabinol in *Cannabis*.

Since that finding, PEA has been shown to inhibit peripheral inflammation and mast-cell degranulation and exert neuroprotective and anti-nociceptive effects in rats and mice. Actions also mediated by PPAR- α activation are accompanied by a decrease in nitric oxide production, the neutrophil influx, and expression of proinflammatory proteins such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Additionally, PEA has potent immunoregulatory properties. Recent data have demonstrated that PEA may also play a key role in regulating complex systems involved in the inflammatory response, pruritus, and neurogenic and neuropathic pain [123].

The etiology of the pruritus of cholestasis is unknown, and it is inferred that the pruritogen is produced in the liver and excreted in bile. As a result of cholestasis, it accumulates in plasma. However, the problem with this approach is that the substance(s) is unknown; thus, it is impossible to reduce its serum levels specifically. Oral cholestyramine, a resin not absorbed, is associated with increased fecal excretion of certain substances, including cholesterol and bile acids.

Many patients respond to treatment with cholestyramine with a relief of pruritus, which unfortunately may be temporary but is well tolerated in general. It seems reasonable to prescribe it as an initial therapy. When pruritus is not relieved by resins, using opiate antagonists (naloxone and naltrexone) is supported by data from controlled clinical trials. Butorphanol is an agonist at the kappa opioid receptor and an antagonist at the mu-opioid receptor with minimal or absent abuse potential.

The cannabinoidergic and serotonergic systems mediate nociception; therefore, there appears to be a rationale for using those drugs to treat pruritus [124]. In uncontrolled observations, dronabinol, an agonist at the cannabinoid receptor 1, and sertraline, a serotonin reuptake inhibitor, have been reported to be associated with the relief of pruritus. The use of butorphanol spray in selective patients may be a therapeutic alternative.

Peripherally and centrally mediated pathologies that induce pruritus do not generally respond to typical allergenic and anti-inflammatory treatments. An acute allergenic murine model was used to determine whether the endogenous cannabinoid system could be targeted to treat pruritus. Complementary genetic and pharmacological approaches to target fatty acid amide hydrolase, the primary enzyme responsible for the degradation of the endocannabinoid anandamide, were evaluated in the compound 48/80 model. Subcutaneous administration of the mast cell degranulation compound 48/80 evoked an intense, concentration-dependent scratching

response. Systemic delta-9-tetrahydrocannabinol reduced the scratching response, although the effect was accompanied by hypomotility.

FAAH(–/–) mice and mice treated with the respective irreversible and reversible FAAH inhibitors, URB597 (cyclohexyl carbamic acid 3'-carbamoylbiphenyl-3-yl ester) and OL-135 [1-oxo-1-[5-(2-pyridyl)-2-yl]-7-phenylheptane], displayed comparable reductions in scratching to mice treated with common non-sedative allergenic treatments (loratadine and dexamethasone) but without affecting locomotor behavior.

The anti-scratching phenotype of FAAH-compromised mice was completely blocked by either genetic deletion or pharmacological antagonism of CB1 receptors. Neural-specific conditional FAAH knockout (FAAH-NS) mice, which have FAAH exclusively restricted to neural tissues, displayed a similar magnitude of scratching as wild-type mice. It is important that URB597 reduced compound 48/80-induced scratching in FAAH-NS mice, but it did not produce any further reduction in FAAH(–/–) mice. The findings indicate that neuronal FAAH suppression reduces the scratching response by activating CB1 receptors [125].

Dronabinol, a synthetic agonist at cannabinoid receptors, was reported to decrease the pruritus of cholestasis in an uncontrolled observation. The antipruritic effect of dronabinol resulted from an increased threshold to experience nociception by the drug. To test the hypothesis, the effect of WIN-55212-2, a cannabinoid agonist, was tested on the threshold for nociception using a tail-flick assay in rats with cholestasis secondary to bile duct resection and in sham-resected controls.

The administration of WIN-55212-2 was associated with a significant increase in the mean tail-flick latency in both groups compared to the baseline. Pruritus is a nociceptive stimulus; accordingly, drugs that increase the threshold to nociception in humans are a possible approach to treating patients with pruritus [126].

Uremic pruritus is a frequent and bothersome symptom in hemodialysis patients. Its etiology is not fully understood, and there is no specific treatment. There is reliable evidence of the association between the cannabinoid system and pruritus. A study evaluated whether genetic variations in the endocannabinoid receptor 1 gene can affect uremic pruritus. The rs12720071, rs806368, rs1049353, rs806381, rs10485170, rs6454674, and rs2023239 polymorphisms of the CNR1 gene were genotyped in 159 hemodialysis patients and 150 healthy controls using two multiplex polymerase chain reactions and the mini sequencing technique.

No statistically significant relationship was found in any of the evaluated genotypes between patients with and without uremic pruritus, even after excluding patients with diabetes and dyslipidemia. There were no differences between patients with uremic pruritus and the control group. However, in the group of all HD patients, a significantly higher incidence of GA genotype and lower incidence of GG genotype in the polymorphism rs806381s were revealed versus the control group ($p = 0.04$). It appears that polymorphisms of the CNR1 gene are not associated with uremic pruritus [127].

Therapy-resistant pruritus often fails to respond to standard measures. There has been an expression of cannabinoid receptors on cutaneous sensory nerve fibers, so cannabinoid agonists seem a rational therapeutic option for pruritus. In an open

application observation, 22 patients with prurigo, lichen simplex, and pruritus applied an emollient cream containing N-palmitoyl ethanolamine (PEA) to the affected area. In 14 of 22 patients, a good antipruritic effect was documented. The average reduction in itch was 86.4%. The therapy was well-tolerated by all patients; neither burning burn nor contact dermatitis was observed. The topical cannabinoid agonists represent a new effective, well-tolerated therapy for refractory itching of various origins. Creams with a higher concentration may be even more effective with broader indications [128].

Since itch and pain sensations share many similarities, a study investigates whether the blockade of cannabinoid 1 and 2 receptors participates in the antipruritic activity of paracetamol in mice. Cannabinoid 1 receptors have been shown to mediate the anti-nociceptive but not the hypothermic action of paracetamol. Scratching behavior was induced by intradermal serotonin injection into the rostral part of the back of the mice. After serotonin administration, scratching the injected site with the hind paws was videotaped and counted for 30 min. Serotonin-induced scratching behavior was attenuated with high-dose paracetamol (300 mg/kg).

The results indicate that, in contrast to its anti-nociceptive action but similar to its hypothermic effect, cannabinoid receptors are not involved in the antipruritic activity of paracetamol [129]. The CB1 receptor antagonist, AM-251 (1 mg/kg), and the CB2 receptor antagonist, SR-144528 (1 mg/kg), did not alter the anti-scratching behavioral effect of paracetamol.

Atopic Dermatitis

Ceramides, a class of sphingolipids containing a backbone of a sphingoid base, are the most important and effective structural component for the formation of the epidermal permeability barrier. While ceramides comprise approximately 50% of the epidermal lipid content by mass, the content is substantially decreased in certain inflammatory skin diseases, such as atopic dermatitis (AD), causing improper barrier function.

The endocannabinoid system can modulate several biological responses in the central nervous system. Studies revealed that activation of endocannabinoid receptor 1 triggers the generation of ceramides that mediate neuronal cell fate. The impact of ECS on the production of epidermal ceramide has been investigated to determine whether the ECs stimulates the generation of epidermal ceramides in an IL-4-treated *in vitro* model of skin inflammation. Using N-palmitoyl serinol (PS), an analog of the endocannabinoid N-palmitoyl ethanolamine, an IL-4-mediated decrease in cellular ceramide levels was significantly stimulated in human epidermal keratinocytes (KC) following PS treatment through both *de novo* ceramide synthesis and sphingomyelin hydrolysis pathways [69].

The studies demonstrate that an analog of endocannabinoid, PS, stimulates the generation of specific ceramide species and the total amount of ceramides via the endocannabinoid receptor CB1-dependent mechanism, thereby resulting in the

enhancement of epidermal permeability barrier function. N-palmitoyl serinol selectively increases ceramides with long-chain fatty acids (FAs) (C22-C24), which mainly account for the formation of the epidermal barrier through activation of ceramide synthase (CerS) 2 and Cer3 in IL-4-mediated inflamed KC. Blockade of cannabinoid receptor CB1 activation by AM-251 failed to stimulate the production of total ceramide and long-chain ceramides in response to PS.

JTE-907, N-(benzo[1,3]dioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide, is a selective CB2 receptor antagonist/inverse agonist. The antipruritic activity of JTE-907 was studied in NC mice with chronic dermatitis, a model of atopic dermatitis. The oral dose of JTE-907 (1 and 10 mg/kg/day), immunosuppressant agent tacrolimus (1 mg/kg/day), and a glucocorticoid betamethasone 17-valerate (1 mg/kg/day) for 20 days suppressed the spontaneous scratching and cutaneous nerve activity of NC mice. JTE-907 (10, but not 1, mg/kg) and tacrolimus, but not betamethasone, alleviates dermatitis. Betamethasone inhibited body weight gain [70].

The endocannabinoid system is important for skin homeostasis, and alterations are linked to inflammatory diseases like atopic dermatitis. Activation of cannabinoid receptor 2 decreases pruritus and inflammation in mouse models. Reduction of inactivation of endogenous cannabinoids could, therefore, be a therapeutic option for AD. A study tested the effects of a topical endocannabinoid membrane transporter inhibitor (WOL067-531, 1% gel) on pruritus and dermatitis in a canine AD model.

Nineteen beagles allergic to dust mites (DM) were randomized to receive either active ingredient or vehicle on the inguinal area while challenged epicutaneously with DM twice weekly for 28 days. Treatment was administered twice daily and started after three challenges (day eight). Dermatitis and pruritus were scored weekly by personnel blinded to treatment allocation. Dermatitis was scored using a validated scoring system, and pruritus was scored using camera recordings. After a 4-week washout, dogs were crossed over, and the study was repeated.

On days 15 and 22, dermatitis scores were significantly increased after the DM challenge in the vehicle group (16.34, $p = 0.0089$ and 7.42, $p = 0.04845$, respectively) but not in the active ingredient group ($p = 0.3177$ and $p = 0.3190$, respectively). A significant decrease in pruritus in the inguinal area and overall ($p = 0.048$ and $p = 0.032$, respectively) occurred in the active ingredient group. No adverse effects were noted [71].

Chronic inflammatory skin disorders, such as atopic dermatitis, have a significant disease burden worldwide. Although efficacious, the adverse effect profile of topical corticosteroids limits long-term use. As an alternative, cannabinoids have been shown to have therapeutic anti-inflammatory effects. A study assesses the effects of a topical cannabinoid product using a dermatitis mouse model. Thirty-five mice were randomized into treatment groups. 12-O-tetradecanoylphorbol-13-acetate was used as an irritant on one ear, with the contralateral ear serving as a control. The test product containing 0.9% cannabidiol and palmitoylethanolamide was compared with a potent topical corticosteroid.

Treatment with topical cannabinoid formulation reduced ear edema by 51.27% at 24 h and 65.69% at 48 h post application. Alternatively, mometasone reduced ear edema by 89.82% at 24 h and 98.25% at 48 h. Natural reduction (control) in-ear edema was 26.32% at 24 h and 44.21% at 48 h. Both test groups resulted in significantly decreased edema when compared with baseline ($p < 0.05$), as well as compared with the negative control group ($p < 0.05$). Significant reduction in ear edema, a marker for localized cutaneous inflammation, could be attributed to the anti-inflammatory properties of cannabinoids. Although the effects were less robust than topical corticosteroid use, cannabinoid formulations have therapeutic promise for dermatitis [72].

Using acute and chronic inflammation animal models, a study was performed to investigate the effects of cannabinoid receptor agonists on skin inflammation. The effectiveness of the synthesized cannabinoid receptor 1 agonist was determined using *in vitro* assays. Markers for epidermal permeability barrier function and skin inflammations were measured and histological assessments were performed for evaluation. Topical application of cannabinoid receptor 1-specific agonist significantly accelerated the recovery of epidermal permeability barrier function and produced anti-inflammatory activity in both acute and chronic inflammation models. Histological assessments confirmed the anti-inflammatory effects. The results indicated that topical application of cannabinoid receptor 1-specific agonist could be beneficial for alleviating the inflammatory symptoms in chronic skin diseases, including atopic dermatitis [73].

Studies have reported cannabidiol's effectiveness in relieving pain and easing inflammation in atopic dermatitis without major health risks. The effectiveness of cannabidiol and aspartame formulation, JW-100, in relieving signs and symptoms of AD was examined in a double-blinded placebo-controlled interventional study randomizing patients to one of three treatment groups: JW-100 (CBD plus aspartame), CBD only, or placebo topical formulations. The Investigator's Static Global Assessment (ISGA) score was used to document any changes in AD resulting from the applied interventions at 14 days.

Fifty-seven patients completed the trial and were included in the final analysis. The ISGA score of the patients at baseline was 2.56, 2.24, and 2.24 for the JW-100, CBD, and placebo groups, respectively. After 2 weeks of treatment, the ISGA score was reduced by 1.28, 0.81, and 0.71 for the JW-100, CBD, and placebo groups, respectively. The JW-100 cohorts demonstrated statistically significant ISGA score reduction ($p = 0.042$). 50% of patients in the JW-100 group achieved an ISGA score of clear or almost clear (0 or 1) with at least a 2-grade improvement from baseline after treatment ($p = 0.028$). Only 20% and 15% of patients in the CBD-only and placebo groups reported ISGA scores of clear or almost clear (0 or 1). JW-100 topical formulation containing CBD and aspartame produced statistically significant improvements in AD following 14 days of topical application [74].

Atopic dermatitis is characterized by an impaired epidermal barrier function combined with a chronic Th2-type inflammatory response and intense pruritus. An experimental mouse model for Th2-type contact hypersensitivity (CHS) to

fluorescein isothiocyanate (FITC) was used to investigate the potential role of cannabinoid 1 receptor in the pathophysiology of mouse atopic-like dermatitis.

The swelling ear responses, transepidermal water loss, Th2-type skin inflammatory responses, and serum IgE levels were evaluated. Mice lacking CB1 receptors globally (*Cnr1*($-/-$)) or specifically in keratinocytes (*KC-Cnr1*($-/-$)) and wild-type (WT) control mice were sensitized and challenged with FITC. *Cnr1*($-/-$) and *KC-Cnr1*($-/-$) produced enhanced CHS responses to FITC and delayed epidermal barrier repair compared with WT mice. Levels of mRNA for IL-4, thymic stromal lymphopoietin (TSLP), and CCL8, and eosinophil activity, were significantly increased in inflamed ear tissue of FITC-challenged *Cnr1*($-/-$) and *KC-Cnr1*($-/-$) mice.

Cannabinoid 1 receptor-deficient keratinocytes secreted increased levels of TSLP, a proinflammatory mediator that drives Th2-type skin inflammation in atopic dermatitis under basal and Th2-type inflammatory conditions. The results indicate that CB1 receptors in keratinocytes help to maintain epidermal barrier homeostasis and attenuate Th2-type allergic inflammatory responses [75].

Impaired skin barrier function facilitates the penetration of allergens and, subsequently, stronger sensitization responses. It has been demonstrated that cannabinoid receptors are expressed in healthy and diseased skin and are important mediators in the skin. A study was designed to investigate the expression of the cannabinoid receptors type 1 and 2 and the cannabinoid-related receptors G protein-coupled receptor 55 (GPR55), transient receptor potential vanilloid 1 (TRPV1), and ankyrin 1 (TRPA1), peroxisome proliferator-activated receptors alpha (PPAR α), and serotonergic receptor 1a (5-HT1aR) in keratinocytes of healthy dogs and dogs with AD.

Samples of skin tissues were collected from seven healthy controls (CTRL-dogs) and eight dogs with AD (AD-dogs). The tissue samples were processed using an immunofluorescence assay with antibodies, and the immunolabelling of the receptors studied was evaluated. The keratinocytes of the CTRL- and the AD-dogs showed immunoreactivity for all the receptors investigated with a significant upregulation of CB2R, TRPA1, and 5-HT1aR in the epidermis of the AD-dogs. The presence of cannabinoid and cannabinoid-related receptors in healthy keratinocytes suggested the possible role of the ECS in epidermal homeostasis. At the same time, their overexpression in the inflamed tissues of the AD dogs indicated the involvement of the ECS in the pathogenesis of atopic dermatitis [76].

A study examined the distribution of cannabinoid receptors 1 and 2 in the skin, including hair follicles, sweat, and sebaceous glands, of healthy dogs and dogs with atopic dermatitis and to compare results with positive control samples for CB1 (hippocampus) and CB2 (lymph nodes). Skin samples were taken from five healthy dogs and five dogs with atopic dermatitis and popliteal lymph node and hippocampus samples from five dog cadavers.

Cannabinoid receptors 1 and 2 were immunohistochemically localized in formalin-fixed, paraffin-embedded sections of the tissue samples. In the skin samples of healthy dogs, CB1 and CB2 immunoreactivity were detected in various types of cells in the epidermis and the dermis, including perivascular cells with mast

cell morphology, fibroblasts, and endothelial cells. In skin samples of dogs with atopic dermatitis, CB1 and CB2 immunoreactivity was stronger than in skin samples of healthy dogs. In positive control tissue samples, CB1 immunoreactivity was detected in all hippocampus areas, and CB2 immunoreactivity was detected in B-cell zones of lymphoid follicles [77].

The molecular level of the effects of cannabidiol was characterized on primary human keratinocytes using a combination of RNA sequencing (RNA-Seq) and sequential window acquisition of all theoretical mass spectrometry (SWATH-MS). Functional analysis revealed that cannabidiol regulated pathways involved in keratinocyte differentiation, skin development, and epidermal cell differentiation. Cannabidiol induced the expression of several NRF2 target genes, with the heme oxygenase 1 (HMOX1) gene and the protein most upregulated by cannabidiol. CRISPR/Cas9-mediated genome editing, RNA interference, and biochemical studies demonstrated that the induction of HMOX1, mediated by cannabidiol, involved nuclear export and proteasomal degradation of the transcriptional repressor BACH1. Notably, we showed that the effect of BACH1 on HMOX1 expression in keratinocytes is independent of NRF2.

The study identifies BACH1 as a molecular target for cannabidiol in keratinocytes. It sets the basis for using topical cannabidiol for treating different skin diseases, including atopic dermatitis and keratin disorders [78]. In vivo studies revealed that topical cannabidiol increased the levels of HMOX1 and the proliferation and wound-repair associated keratins 16 and 17 in the skin of mice.

To investigate the therapeutic effect of CBD-ointment on the skin of chronic diseases and their outcome scars. A spontaneous, anecdotal, retrospective study of 20 patients with the two most frequent skin disorders: psoriasis ($n = 5$), atopic dermatitis ($n = 5$), and resulting outcome scars ($n = 10$). The subjects were instructed to administer topical CBD-enriched ointment to lesioned skin areas twice daily for 3 months.

Based on skin evaluations (hydration, TEWL, elasticity), clinical questionnaires (SCORAD, ADI, PASI), and supported by photographic data and investigators' clinical assessment, the results indicated that topical treatment with CBD-enriched ointment significantly improved the skin parameters, the symptoms and also the PASI index score. No irritant or allergic reactions were documented during the period of treatment [79].

Mast cells (MCs) contribute to inflammatory diseases. Since endocannabinoids are important neuroendocrine mass cell modifiers, an investigation was conducted to determine how stimulation/inhibition of cannabinoid 1 receptors affects the biology of human skin mast cells in situ. The investigation was conducted in the mass cell-rich connective tissue sheath of organ-cultured human scalp hair follicles by quantitative Immunohistomorphometry, ultrastructural, and quantitative PCR techniques using CB1 agonists and antagonists, CB1 knockdown, or CB1 knock-out mice.

Kit+ MCs within the connective tissue sheath of human hair follicles express functional CB1 receptors, with pharmacological blockade or gene silencing significantly stimulating both the degranulation and the maturation of mass cells from

resident progenitor cells in situ (i.e., enhanced the number of tryptase+, FcεRIα, or chymase+ connective tissue sheath-MCs). Cannabinoid 1 receptor agonists counteracted the MC-activating effects of classical mass cell secretagogues. Similar phenomena were observed in CB1 knockout mice, attesting to the *in vivo* relevance of the MC-inhibitory mechanism.

Using human hair follicle organ culture as an unconventional, clinically relevant model system for studying the biology of mass cells in situ revealed that the endocannabinoid system tightly controls normal skin mass cells. This limits excessive activation and maturation of mass cells from resident progenitors via “tonic” CB1 stimulation by locally synthesized endocannabinoids. The excessive numbers and activation of mass cells in allergic and other chronic inflammatory skin diseases partially arise from resident intracutaneous mass cell progenitors, for example, because of insufficient CB1 stimulation [80].

In addition to cannabinoids’ effect on pruritus, cannabinoids produce anti-inflammatory effects that aid in managing atopic dermatitis. Mice that lack CB1 receptors have an impaired ability to repair their epidermal barrier. Those animals had elevated levels of IL-4 and CCL8, suggesting CB1 receptors may help maintain the epidermis’s barrier function and accentuate a T-helper 2 (Th2) cell response.

Allergic Contact Dermatitis

Cannabinoid receptor 2 activations were shown to reduce the production of the monocyte chemotactic protein-2 (MCP-2) chemokine in polyinosinic-polycytidylic acid [poly-(I:C)]-stimulated human keratinocyte (HaCaT) cells, an *in vitro* model of allergic contact dermatitis (ACD). A study was conducted to determine if non-psychotropic cannabinoids, such as cannabidiol, produced similar effects in the experimental model of ACD.

HaCaT cells were stimulated with poly-(I:C), and the release of chemokines and cytokines was measured in the presence of CBD or other phytocannabinoids (such as cannabidiol acid, cannabidivarin, cannabidivarinic acid, cannabichromene, cannabigerol, cannabigerolic acid, cannabigevarin, tetrahydrocannabivarin, and tetrahydrocannabivarinic acid) and antagonists of CB1, CB2, or transient receptor potential vanilloid type-1 (TRPV1) receptors. HaCaT cell viability following phytocannabinoid treatment was also measured.

The cellular levels of endocannabinoids [anandamide, 2-arachidonoylglycerol] and related molecules (palmitoylethanolamide, oleoylethanolamide) were quantified in poly-(I:C)-stimulated HaCaT cells treated with CBD and revealed that in poly-(I:C)-stimulated HaCaT cells, CBD elevates the levels of AEA and dose-dependently inhibits poly-(I:C)-induced release of MCP-2, interleukin-6 (IL-6), IL-8, and tumor necrosis factor-α in a manner reversed by CB2 and TRPV1 antagonists 6-iodopravadoline (AM630) and 5'-iodo-resiniferatoxin (I-RTX), respectively, with no cytotoxic effect [81].

In mice, 2,4-dinitrofluorobenzene induces allergic contact dermatitis (CAD), which, in a late phase, is characterized by mast cell (MC) infiltration and angiogenesis. Palmitoylethanolamide (PEA), an endogenous anti-inflammatory molecule, acts by down-modulating MCs following activation of the cannabinoid CB2 receptor and peroxisome proliferator-activated receptor- α (PPAR- α). A study examined whether PEA reduces the features of the late stage of contact allergic dermatitis, including MC activation, angiogenesis, and itching. Palmitoylethanolamide produced an anti-inflammatory effect in the early stage of contact allergic dermatitis.

After sensitization to DNFB, female C57BL/6 J mice underwent three DNFB challenges at days 5, 12, and 19. Treatments were administered at each challenge and for two more days. Contact allergic dermatitis was expressed as an increase in ear thickness between challenged and un-challenged mice. Palmitoylethanolamide (5 mg/kg/i.p.) reduced the DNFB-induced increase, the number of MCs per tissue area, and the expression of VEGF and its receptor Flk-1. The effects were reversed by co-administration of AM630 (1 mg/kg/i.p.), a CB2 antagonist, but not GW6471 (1 mg/kg/i.p.), a PPAR- α antagonist. Palmitoylethanolamide reduced the number of ear scratching 48 h after the DNFB challenge. The effect was reversed by both CB2 and PPAR- α antagonists indicating the involvement of both receptors [82].

Delta-9-tetrahydrocannabinol exerts its biological effects partly through the G-protein-coupled CB1 and CB2 receptors. Delta-9-THC also has CB1 and CB2 receptor-independent effects. Because of this immune-inhibitory potential, delta-9-THC and other cannabinoids are the potential treatment of inflammatory skin diseases. The mechanism of the anti-inflammatory activity of delta-9-THC and the role of CB1 and CB2 receptors has been examined. Immunohistochemical analyses for infiltrating immune cells and studying the influence of delta-9-THC on the interaction between T cells, keratinocytes, and myeloid immune cells *in vitro* were conducted. The impact of topically applied delta-9-THC was evaluated on DNFB-mediated allergic contact dermatitis in wild-type and CB1 and CB2 receptor-deficient mice.

Topical delta-9-THC application effectively decreased contact allergic ear swelling and myeloid immune cell infiltration in wild-type and CB1 and CB2 receptor-deficient mice. Delta-9-THC inhibited the production of IFN γ by T cells; decreased the production of CCL2 and IFN γ -induced CCL8 and CXL10 by epidermal keratinocytes, thereby limiting the recruitment of myeloid immune cells *in vitro* in a CB1 and CB2 receptor-independent manner. The topically applied delta-9-THC attenuates contact allergic inflammation by decreasing keratinocyte-derived proinflammatory mediators that orchestrate myeloid immune cell infiltration independent of CB1 and CB2 receptors [83].

Epidermal keratinocytes (KCs) and cannabinoid receptors regulate inflammatory responses in a mouse model for allergic contact dermatitis, the contact hypersensitivity (CHS) response to the obligate sensitizer 2,4-dinitrofluorobenzene. The study investigated the cellular and molecular mechanisms of CB1 receptors' attenuation of CHS responses to 2,4-dinitrofluorobenzene. A conditional gene-targeting approach was used to identify the relative contribution of CB1 receptors on epidermal KCs to control CHS responses.

To determine the underlying cellular and molecular mechanisms that regulate inflammatory responses in the effector phase of CHS, investigations on inflamed ear tissue and primary KC cultures were conducted using morphologic, molecular, and immunologic methods. Mice with a KC-specific deletion of CB1 receptors developed increased and prolonged CHS responses, associated with enhanced reactive epidermal acanthosis and inflammatory KC hyperproliferation in the effector phase of CHS.

In vitro, primary cultures of CB1 receptor-deficient KC released increased amounts of CXCL10 and CCL8 after stimulation with IFN- γ compared with controls. In vivo, contact allergic ear tissue of CB1 receptor-deficient KCs showed enhanced expression of CXCL10 and CCL8 compared with controls. CCL8 was established as the proinflammatory chemokine regulated by CB1 receptors that promote immune cell recruitment to allergen-challenged skin. The results demonstrate that CB1 receptors are functionally expressed by KCs in vivo and help limit the secretion of proinflammatory chemokines that regulate T-cell-dependent inflammation in the effector phase of CHS [84].

Palmitoylethanolamide is an anti-inflammatory mediator that enhances the activation by anandamide of cannabinoid receptors and transient receptor potential vanilloid type-1 channels and directly activates peroxisome proliferator-activated receptor- α (PPAR- α). In mice, 2,4-dinitrofluorobenzene-induced allergic contact dermatitis in inflamed ears is partly mediated by the chemokine monocyte chemoattractant protein-2 and accompanied by elevation of AEA levels.

A study examined whether PEA is produced during DNFB-induced CAD and if it has any direct protective action in keratinocytes in vitro. Eight- to ten-week-old female C57BL/6 J wild-type and CB (1)/CB (2) double knockout mice were used to measure PEA levels and the expression of TRPV1, PPAR- α receptors, and enzymes responsible for PEA biosynthesis and degradation. Human keratinocytes (HaCaT) cells were stimulated with polyinosinic polycytidylic acid [poly-(I:C)], and the expression and release of MCP-2 were measured in the presence of PEA and antagonists of its proposed receptors.

2,4-Dinitrofluorobenzene increased ear skin PEA levels and upregulated TRPV1, PPAR- α , and a PEA-biosynthesizing enzyme in ear keratinocytes. In HaCaT cells, poly-(I:C) stimulation elevated both PEA and AEA levels. Exogenous PEA (10 μ M) inhibited poly-(I:C)-induced expression and release of MCP-2 in a way reversed by antagonism at TRPV1, but not PPAR- α . Palmitoylethanolamide (5–10 mg/kg, intraperitoneal) also inhibited DNFB-induced ear inflammation in mice in vivo, in a way attenuated by TRPV1 antagonism. As an endogenous protective agent against DNFB-induced keratinocyte inflammation, palmitoylethanolamide could be considered therapeutic against CAD [85].

Cannabinoid 2 receptors are present in immune cells, including mast cells, which participate in 2,4-dinitrofluorobenzene-induced allergic contact dermatitis (ACD). A study investigates if the inhibition of mast cell degranulation was involved in the anti-ACD effect of electroacupuncture at ST36 via cannabinoid 2 receptors. Sprague–Dawley rats were sensitized and challenged with DNFB following electroacupuncture stimulation for 1 week. Ear swelling, serum IgE levels, local

cytokine production, and mast cell infiltration were evaluated. Additionally, rat peritoneal mast (RPM) cells were isolated and cultured for detection of cannabinoid 2 receptors expression, mitogen-activated protein kinase signaling activation, and mast cell degranulation (including β -hexosaminidase and histamine release) in the presence or absence of cannabinoid 2 receptors antagonists.

Electroacupuncture treatment inhibited ear swelling, suppressed IgE and cytokine production, decreased the number of mast cells, and curbed mast cell degranulation which was associated with the inhibition of p38 phosphorylation in DNFB-induced ACD. Importantly, EA enhanced the expression of cannabinoid 2 receptors mRNA and protein in the RPMs. Cannabinoid 2 receptor antagonist AM630 but not cannabinoid 1 receptor antagonist AM251 effectively reversed the suppressive effect of electroacupuncture on p38 activation, mast cell infiltration, and degranulation. The findings support the hypothesis that electroacupuncture promotes cannabinoid 2 receptors expression in mast cells, followed by inhibition of the p38 MAPK pathway, potentially resulting in the anti-ACD effect of electroacupuncture [86].

The possible involvement of 2-arachidonoylglycerol, an endogenous ligand for the cannabinoid receptors 1 and 2, in contact dermatitis in mouse ears was investigated. The level of 2-AG was markedly elevated in the ear following a challenge with oxazolone in sensitized mice. The swelling following the challenge was suppressed by either the administration of SR144528, a CB2 receptor antagonist, immediately after sensitization or SR144528 upon the challenge. The effect of AM251, a CB1 receptor antagonist, was marginal in either case. The CB2 receptor and its endogenous ligand 2-AG are closely involved in the sensitization and elicitation phases of oxazolone-induced contact dermatitis.

It was also found that Langerhans cells (MHC class II(+)) contain a substantial amount of CB2 receptor mRNA, and keratinocytes (MHC class II(-)) do not. After a challenge with oxazolone, the expression of mRNAs for proinflammatory cytokines was markedly suppressed by treatment with SR144528. The amount of 2-AG in the ear of the mice dramatically increased following repeated challenges with oxazolone, and treatment with SR144528 attenuated both the recruitment of eosinophils and ear swelling in chronic contact dermatitis induced by repeated challenge with oxazolone. The results indicated CB2 receptor and 2-AG stimulative roles in the sensitization, elicitation, and exacerbation of allergic inflammation.

The skin irritant polyynes faltarinol (panaxynol, carotatoxin) is found in carrots, parsley, celery, and *Panax ginseng*. Faltarinol was isolated from the endemic Sardinian plant *Seseli praecox* in a search for new cannabinoid receptor ligands. Faltarinol exhibits binding affinity to receptors 1 and 2, but selectively alkylates the anandamide binding site in the CB1 receptor ($K_i = 594$ nM), acting as a covalent inverse agonist in CB1 receptor-transfected CHO cells. Purified faltarinol was repeatedly isolated for biological characterization, and one new polyynes was characterized. In human HaCaT keratinocytes, faltarinol increased the expression of the pro-allergic chemokines IL-8 and CCL2/MCP-1 in a CB1 receptor-dependent manner.

Moreover, faltarinol inhibits the effects of anandamide on TNF- α -stimulated keratinocytes. In vivo, faltarinol strongly aggravated histamine-induced edema

reactions in skin prick tests. Both effects were obtained with the CB1 receptor inverse agonist rimonabant, indicating the potential role of the CB1 receptor in skin immunopharmacology. The data suggest the anti-allergic effects of anandamide and that falcarinol-associated dermatitis is due to antagonism of the CB1 receptor in keratinocytes, leading to increased chemokine expression and aggravation of histamine action [87].

Systemic Sclerosis

The role of the cannabinoid pathway in the induction and propagation of systemic sclerosis was evaluated in a mouse model of diffuse systemic sclerosis induced by hypochlorite injections. BALB/c mice were injected subcutaneously every day for 6 weeks with PBS, or hypochlorite was treated intraperitoneally with either WIN-55212-2, an agonist of the cannabinoid receptors 1 and receptors 2, with JWH-133, a selective agonist of CB2, or with PBS [88].

Histological and biochemical assessment of skin and lung fibrosis and the proliferation of fibroblasts, purified from diseased skin, were studied by thymidine incorporation. Injections of hypochlorite induced cutaneous and lung fibrosis and increased the proliferation rate of fibroblasts isolated from fibrotic skin, splenic B cell counts, and levels of anti-DNA topoisomerase-1 autoantibodies. ELISA detected autoantibodies, and spleen cell populations were analyzed by flow cytometry.

Studies of CB2-deficient mice confirmed the influence of CB2 in the development of systemic fibrosis and autoimmunity. It indicates that the CB2 receptor is a potential target for treating SSc because it controls skin fibroblast proliferation and the autoimmune reaction. Treatment with WIN-55212-2 or the selective CB2 agonist JWH-133 prevented the development of skin and lung fibrosis, reduced fibroblast proliferation, and the development of autoantibodies.

Cannabinoids modulate various key functions, especially cardiovascular and endothelial functions. The endocannabinoid pathway abrogates the activation of endothelial cells isolated from sinusoidal vessels of the human liver and human coronary arteries. It prevents ischemia/reperfusion damage in the liver, the heart, and the brain [89].

Natural and synthetic cannabinoids display immunomodulatory effects on the proliferation and apoptosis of T and B lymphocytes, the activation of macrophages, and the production of cytokines and chemokines. Most of the effects are CB2 receptor-dependent. Studies also indicate that CB2 receptor agonists counteract liver fibrogenesis [88]. Cannabinoid 2 receptors are also present in the skin, and their activation prevents bleomycin-induced dermal fibrosis in the mouse.

Endothelial cells, fibroblasts, and immune cells are dysregulated in systemic sclerosis and are also the targets of cannabinoids, their role in systemic sclerosis was examined in specific 6-week-old pathogen-free female BALB/c and C57BL/6 CB2+/+ mice. To obtain single mutant mice with a targeted mutation of the *Cnr2*

gene on an inbred congenic genetic background, heterozygous *Cnr2*+/- mice were backcrossed with wild-type C57Bl/6 J animals over 10 generations. Heterozygous mice from the N10 generation were intercrossed with homozygous *Cnr2*-/- animals (CB2-/- mice).

The BALB/c mice were randomly distributed into experimental and control groups ($n = 21$ per group). One hundred microliters of HOCl-generating solution were injected subcutaneously into the shaved back of the mice every day for 6 weeks. Control groups received injections of 100 μ L sterilized PBS (PBS mice). The agents were prepared extemporaneously. HOCl was produced by adding 166 μ L NaClO solution (2.6% as active chlorine) to 11.1 mL KH₂PO₄ solution (100 mmol/L, pH 7.2).

HOCl and PBS BALB/c mice were randomized and treated simultaneously by intraperitoneal injections either with WIN-55212-2, a non-selective CB1, and CB2 agonist, or JWH-133, a selective CB2 agonist, or vehicle alone for 6 weeks ($n = 14$ per group). Cannabinoid agonists were given 5 days a week from Monday to Friday. The doses increased each week: WIN-55212-2 was started at 0.5 mg/kg per day the first week, and then 1, 2, 3, 4, and 5 mg/kg per day the following weeks; JWH-133 was started at 1 mg/kg per day, and then 1.5, 2, 2.5, 3, and 4 mg/kg per day. WIN-55212-2 and JWH-133 were reconstituted with DMSO, aliquoted, and stored as stock solutions at 1 mg/mL at -20°C .

Ten-week-old C57BL/6 CB2-/- and CB2+/+ mice were randomly distributed into experimental and control groups ($n = 5$ per group). The experimental procedure was similar to that of BALB/c mice, except that C57BL/6 CB2+/+ and CB2-/- mice were sacrificed after 3 weeks of subcutaneous injections. Subcutaneous injections of HOCl in BALB/c mice induced an increase in dermal thickness and the concentration of acid- and pepsin-soluble type I collagen in the skin versus injections of PBS ($p < 0.0001$ in both cases). Histopathological analysis confirmed dermal fibrosis.

To determine if the activation of cannabinoid receptors affects the development of dermal fibrosis in the model of systemic sclerosis, mice exposed to HOCl were simultaneously treated with WIN-55212-2. WIN-55212-2 reduced the dermal thickness and the accumulation of collagen-induced by HOCl ($p = 0.0007$ for dermal thickness and $p = 0.0006$ for collagen concentration in the skin versus untreated mice exposed to HOCl). The results were confirmed by histopathological analysis of skin biopsies stained with hematoxylin, eosin, and picro-sirius red staining, which showed a decrease in dermal thickness in HOCl-BALB/c mice treated with WIN-55212-2.

JWH-133 significantly reduced the dermal thickness and the accumulation of collagen in the skin of HOCl mice ($p = 0.0004$) for dermal thickness and ($p = 0.005$) for collagen concentration in the skin versus untreated HOCl mice. The results confirmed by histopathological analysis indicated that the selective activation of CB2 is sufficient to reduce the fibrotic process triggered by HOCl.

In addition to skin fibrosis, HOCl-treated BALB/c mice developed lung fibrosis revealed by histopathological analysis and the higher concentration of type I

collagen in the lungs of HOCl mice as compared with mice treated with PBS ($p = 0.0002$). WIN-55212-2 abrogated the development of lung fibrosis induced by HOCl, as shown by histopathological analysis and by the weaker accumulation of type I collagen in the lungs ($p = 0.0002$) for WIN-treated versus untreated HOCl mice.

JWH-133 also reduced the concentration of type I collagen in the lungs compared with untreated HOCl mice ($p = 0.002$). The histopathological analysis of lung biopsies stained with hematoxylin and eosin and picro-sirius red staining confirmed the data, which produced decreased fibrosis in BALB/c mice submitted to HOCl injections and simultaneously treated with JWH-133. Immunohistochemistry analysis of lung tissue sections from the BALB/c mice injected with HOCl, revealed an inflammatory infiltrate mostly consisting of T lymphocytes. WIN-55212-2 and JWH-133 reduced the pulmonary T-cell infiltrate triggered by HOCl.

Skin fibroblasts isolated from HOCl mice displayed a higher proliferation rate than fibroblasts obtained from mice injected with PBS ($p = 0.0004$). By contrast, the proliferation rate of fibroblasts isolated from HOCl mice treated with WIN-55212-2 was lower than that of fibroblasts isolated from mice injected with PBS ($p = 0.02$). The rate of proliferation of skin fibroblasts was also reduced when HOCl mice were treated with the selective CB2 agonist JWH-133, compared with that of fibroblasts isolated from HOCl mice without any treatment ($p = 0.02$) for HOCl mice treated with JWH-133 versus HOCl mice and untreated with JWH-133; ($p = 0.13$) for HOCl mice treated with JWH-133 versus PBS mice treated with JWH-133.

Because fibroblasts from HOCl mice displayed an abnormal phenotype with an excessive rate of proliferation, additional experiments were performed to assess whether WIN-55212-2 and JWH-133 could directly decrease the rate of fibroblast proliferation *in vitro*. Skin fibroblasts from HOCl mice were incubated with 10, 20, or 40 $\mu\text{mol/L}$ WIN-55212-2, JWH-133, or PBS. WIN-55212-2 reduced the proliferation rate of fibroblasts *in vitro* in a dose-dependent manner ($p = 0.022$) for each concentration of WIN-55212-2 tested versus PBS. For JWH-133, the doses of 10 and 20 $\mu\text{mol/L}$ did not reverse the pro-proliferative of HOCl treatment, but at a dose of 40 $\mu\text{mol/L}$ JWH-133 significantly abrogated the rate of proliferation of fibroblasts from HOCl mice ($p = 0.914$, $p = 0.171$, and $p = 0.032$) for the respective concentrations of 10, 20, and 40 $\mu\text{mol/L}$ of JWH-133 versus PBS. Thus, cannabinoid agonists counteracted the pro-proliferative effect of HOCl on fibroblasts *in vivo* and *in vitro* and prevented skin and lung fibrosis.

The effect of the activation of cannabinoid receptors on the immune system was examined in humans and mice. Diffuse systemic sclerosis is characterized by B-cell activation and the production of AAbs. Exposure to HOCl for 6 weeks increased the total number of splenic B220+ B cells compared with PBS-injected mice ($p = 0.011$). No significant difference was observed in the numbers of CD11b+, CD4+, or CD8+ spleen cells between HOCl and PBS-injected mice.

Activation of the cannabinoid pathway by WIN-55212-2 or by the selective CB2 agonist JWH-133 prevented the increase in splenic B-cell numbers in HOCl mice ($p = 0.003$) for HOCl mice treated by WIN-55212-2 versus HOCl mice; ($p = 0.003$) and for HOCl mice treated by JWH-133 versus HOCl mice. The effect of

WIN-55212-2 and JWH-133 on the specific autoimmune response to DNA-topoisomerase 1 that characterizes the cutaneous diffuse systemic sclerosis phenotype was examined.

Mice exposed to HOCl developed anti-DNA topoisomerase 1 IgG Abs ($p = 0.002$) versus PBS. No significant levels of anti-DNA IgG Abs, anticardiolipin IgG Abs, or rheumatoid factors were detected in the sera of the mice. Mice treated with WIN-55212-2, or JWH-133 did not develop anti-DNA topoisomerase 1 IgG Abs after exposure to HOCl compared with untreated mice ($p = 0.002$) for HOCl mice treated by WIN-55212-2 versus HOCl mice; ($p = 0.03$) for HOCl mice treated by JWH-133 versus HOCl mice. The non-selective cannabinoid agonist WIN-55212-2 and the selective CB2 agonist JWH-133 counteracted the pro-proliferative effects of HOCl on B cells. It prevented the selective autoimmune response to DNA-topoisomerase 1.

To further evaluate the role of CB2 receptors in the development of systemic sclerosis, subcutaneous injections of a HOCl-generating solution or PBS were administered to CB2 $^{-/-}$ mice. Because of the rapid establishment of the disease in CB2 $^{-/-}$ mice, the animals were sacrificed after 3 weeks of treatment to maximize the differences. Subcutaneous injections of HOCl every day for 3 weeks induced a significant dermal thickness compared with PBS-injected mice both in CB2 $^{-/-}$ and CB2 $+/+$ mice ($p < 0.0001$) for CB2 $^{-/-}$ mice and ($p = 0.0002$) for CB2 $+/+$ mice. The dermis was thicker in CB2 $^{-/-}$ mice than in CB2 $+/+$ mice ($p = 0.029$). The extension of the induced fibrosis compared to that observed in CB2 $+/+$ mice was submitted to the same regimen. The results were confirmed by histopathological analysis of skin biopsies, which showed more skin fibrosis in CB2 $^{-/-}$ mice than in CB2 $+/+$ mice submitted to HOCl injections.

The concentration of acid- and pepsin-soluble type I collagen in the skin extracts of both CB2 $^{-/-}$ mice and CB2 $+/+$ mice was higher than in their respective controls injected with PBS ($p = 0.029$) for CB2 $^{-/-}$ mice and ($p = 0.016$) for CB2 $+/+$ mice versus their respective controls. The accumulation of collagen in the skin was higher in CB2 $^{-/-}$ mice than in CB2 $+/+$ mice ($p = 0.029$). In addition to skin fibrosis, CB2 $^{-/-}$ mice exposed to HOCl subcutaneously for 3 weeks developed lung fibrosis, as shown by the higher concentration of type I collagen in the lungs compared with PBS-injected CB2 $^{-/-}$ mice ($p = 0.016$) and by histopathological analysis. CB2 $+/+$ mice displayed slight lung fibrosis, as shown by a few foci of fibrosis on histological analysis and by a 32% increase in type I collagen content of the lung. However, after 3 weeks of treatment, this increase did not reach significance ($p = 0.413$) versus PBS-injected CB2 $+/+$ mice.

To determine if CB2 modulates the growth of fibroblasts isolated from fibrotic skin areas of mice, skin fibroblasts isolated from HOCl-CB2 $^{-/-}$ were examined. The mice displayed a higher proliferation rate than fibroblasts obtained from CB2 $^{-/-}$ mice injected with PBS ($p = 0.032$). Moreover, the proliferation rate of fibroblasts isolated from HOCl-CB2 $^{-/-}$ mice was higher than that of fibroblasts from HOCl-CB2 $+/+$ mice ($p = 0.032$).

The consequences of CB2 gene silencing on the immune system's activation in the systemic sclerosis model were evaluated. Subcutaneous injections of HOCl

induced a significant increase in the total numbers of splenic B220+ B cells in CB2^{-/-} mice compared with PBS-injected CB2^{-/-} mice ($p = 0.016$). However, no significant difference was observed in the numbers of splenic B cells in HOCl-CB2^{+/+} mice compared with PBS-injected CB2^{+/+} controls ($p = 0.86$) after only 3 weeks of HOCl injections.

The effect of the inhibition of CB2 signaling on the specific autoimmune response that characterizes the diffuse cutaneous form of systemic sclerosis was evaluated. As previously observed in BALB/c mice, no significant levels of anti-DNA IgG Abs, anticardiolipin IgG Abs, or rheumatoid factors were detected in the sera of CB2^{+/+} or CB2^{-/-} C57BL/6 mice after 3 weeks' HOCl exposure. CB2^{+/+} and CB2^{-/-} mice developed anti-DNA topoisomerase 1 IgG AAbs ($p = 0.032$) for CB2^{+/+} mice and ($p = 0.016$) for CB2^{-/-} mice versus the respective PBS-injected control mice. The anti-DNA topoisomerase 1 IgG Abs level was higher in HOCl-CB2^{-/-} mice than in HOCl-CB2^{+/+} mice ($p = 0.032$).

Cutaneous Melanoma

Cutaneous melanoma is treated with complete surgical resection. Adjuvant radiation can improve local control for patients with a high risk of local recurrence after surgery. High-risk features include head and neck desmoplastic primaries, thick or ulcerated non-desmoplastic primaries, close or positive resection margins, and locally recurrent disease. Cutaneous melanoma has a high risk of regional lymph node metastasis, depending upon the depth of invasion. It is estimated that 30% of lymph node metastasis occurs when the depth of invasion is more than 2 mm and up to 50% when the invasion is more than 4 mm.

Although the global incidence of cutaneous melanoma is increasing, survival rates for patients with metastatic disease remain at less than 10%. Targeting autophagy is one way to promote cancer cell death in chemotherapy-resistant tumors. Treatment with delta-9-tetrahydrocannabinol results in the activation of autophagy, loss of cell viability, and activation of apoptosis. In contrast, co-treatment with chloroquine or knockdown of Atg7, but not Beclin-1 or Ambra1, prevented delta-9-THC-induced autophagy and cell death in vitro. Administration of Sativex to mice bearing BRAF wild-type melanoma xenografts substantially inhibited melanoma viability, proliferation, and tumor growth paralleled by an increase in autophagy and apoptosis compared with standard single-agent temozolomide [90].

A study investigated the efficacy of a standardized *Cannabis sativa* extract alone and in combination with a single radiation dose (6 Gy) in B16F10 mouse melanoma cells in an extract dose-dependent manner. The *Cannabis* extract at three concentrations of 25, 12.5, and 6.25 $\mu\text{g/mL}$ alone for 72 h, or in combination with radiation for 24 h incubation after the extract treatment +48 h incubation after radiation exposure, were evaluated for cell viability of melanoma cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The cells were also treated with

6.25 µg/mL extract alone for 72 h before analyzing *Cannabis*-induced cell death by flow cytometry.

Administration of the extract alone or alongside radiation substantially inhibited melanoma cell viability and proliferation in the extract dose response-dependent manner. The inhibition of melanoma cell viability was paralleled by an increase in necrosis but not apoptosis when melanoma cells were treated with the extract alone. Radiation alone did not have any antiproliferative effects, and radiation also did not synergize the antiproliferative effects of the extract when the extract and radiation were combined. The study's results also indicate that B16F10 mouse melanoma cells are radioresistant [91]. The data suggest that *Cannabis* extract may have significant health and physiological implications for the treatment of melanoma.

Cannabis-based medicines are effective adjunctive drugs in cancer patients. Metastasis is the main cause of death in patients with melanoma. Tau and Stathmin proteins are the key proteins in cancer metastasis. An investigation was conducted on the effect of a standardized *Cannabis* extract on cell migration and Tau and Stathmin gene expression in the melanoma cell line.

Melanoma (B1617) was treated for 48 h with various concentrations of standardized *Cannabis* extract. Cells with no treatment were considered the control group. A quantitative RT–real-time PCR assay followed the study. Relative gene expression was calculated by the $\Delta\Delta\text{Ct}$ method. Migration assay was used to evaluate cancer metastasis. Tau and stathmin gene expression were significantly decreased compared to the control group. Cell migration was also significantly reduced compared to controls [92].

Synthetic cannabinoids have been implicated in inhibiting cancer cell proliferation, reducing tumor growth, and reducing metastasis. A study on the effects of treatment with a cannabinoid derivative on malignant melanoma tumors was conducted in a murine model. Murine B16F10 melanoma tumors were established subcutaneously in C57BL/6 mice that were treated with intraperitoneal injections of the vehicle twice per week (control-group 1, $n = 6$), Cisplatin 5 mg/kg/wk. (group 2; $n = 6$), and cannabidiol, 5 mg/kg twice per week (group 3; $n = 6$). Tumors were measured, and volume was calculated as $(4\pi/3) \times (\text{width}/2)^2 \times (\text{length}/2)$. Tumor size and survival curves were measured.

A significant decrease in tumor size was detected in the mice treated with CBD compared to the control group ($p = 0.01$). The survival curve of melanoma tumors treated with CBD increased compared to the control group ($p = 0.04$). The growth curve data from experimental animal models indicated that natural and synthetic cannabinoids downregulate inflammatory responses mediated by the immune cells responsible for AD chronicity and progression and survival curve of melanoma tumors treated with Cisplatin was significantly decreased and increased, respectively, when compared to the control and CBD-treated groups. Mice treated with Cisplatin demonstrated the longest survival time. However, the quality of life and movement of CBD-treated mice were observed to be better [93].

Studies report that *Cannabis* exerts antineoplastic effects in multiple cancers, including melanoma. An in vitro study reveals the anticancer properties of CBD

administered alone and in combination with mitoxantrone or Cisplatin to malignant melanoma cell lines SK-MEL 28, A375, FM55P, and FM55M2. The safety of CBD was tested in human keratinocytes (HaCaT) in LDH and MTT tests. The MTT assay measured the effects of CBD on the viability of melanoma cells; cytotoxicity was determined in the LDH test and proliferation in the BrdU test.

The results indicate that CBD reduces the viability and proliferation of melanoma-malignant cells and exerts additive interactions with mitoxantrone. Cannabidiol produced antagonistic interaction when combined with Cisplatin. Cannabidiol does not cause significant cytotoxicity in the HaCaT cell line. There is an indication for CBD as a part of melanoma multi-drug therapy when combined with mitoxantrone. Attention should be given to combining CBD with Cisplatin due to the antagonistic interaction observed in the studied malignant melanoma cell lines [10].

The overactivation of tyrosinase, a rate-limiting enzyme in melanogenesis, induces hyperpigmentation. The defatted extract of *Cannabis* seed is known to have inhibitory effects on melanogenesis; however, the effective components in the extract have not been identified. Three phenethyl cinnamamides in *Cannabis* seed extract were prepared by purification and chemical synthesis and assessed for their inhibitory effect on melanogenesis in B16F10 melanoma cells.

A comparison of the anti-melanogenesis and anti-tyrosinase activity of *Cannabis* seeds solvent fractions reveals that the ethyl acetate fraction possessed the greatest potential for suppressing melanogenesis in melanoma cells by decreasing tyrosinase activity. The three phenethyl cinnamamides, *N-trans*-caffeoyltyramine, *N-trans*-coumaroyltyramine, and *N-trans*-feruloyltyramine, were present abundantly in the ethyl acetate fraction prepared, and their anti-melanogenesis and anti-tyrosinase activities in melanoma cells were evaluated.

N-trans-caffeoyltyramine and *N-trans*-feruloyltyramine inhibited alpha-melanocyte stimulating hormone (α -MSH)-induced melanogenesis without cytotoxicity, while *N-trans*-coumaroyltyramine inhibited melanogenesis with cytotoxicity. The IC₅₀ values of *N-trans*-caffeoyltyramine, *N-trans*-feruloyltyramine, and *N-trans*-coumaroyltyramine for inhibition of α -MSH-mediated tyrosinase activation were 0.8, 20.2, and 6.3 μ M, respectively. *N-trans*-caffeoyltyramine possessed the strongest anti-melanogenesis activity among the three phenethyl cinnamamides evaluated. The inhibitory effect of *N-trans*-caffeoyltyramine was verified by determining the melanin content and tyrosinase activity in melanoma after treating the cells with synthetic compounds [94].

B16 mice melanoma cells exposed to cannabidiol are affected by four different concentrations (0.2 mg/mL, 0.04 mg/mL, 0.008 mg/mL, and 0.0016 mg/mL) of 99% CBD oil. The results demonstrate that CBD significantly inhibited melanoma cell growth in vitro at 0.2 mg/mL and 0.04 mg/mL [95].

Dermatomyositis

Research has identified anti-inflammatory properties in both endocannabinoids and phytocannabinoids. A study investigated whether the anti-inflammatory effects of a non-psychoactive cannabinoid called ajulemic acid could target the cytokines pathogenic in cutaneous dermatomyositis. Ajulemic acid has a low affinity for the cannabinoid 1 receptor, which is found in the nervous system and mediates delta-9-THC's psychoactive effects. Ajulemic acid has a 65-fold higher affinity for the cannabinoid 2 receptor than the cannabinoid 1 receptor [96]. The cannabinoid 2 receptor is found on immune cells and plays a natural role in resolving inflammation [97]. Preferential binding of the cannabinoid 2 receptor enables ajulemic acid to preserve delta-9-THC's anti-inflammatory properties without the psychological stimulatory effects, making it a potentially useful therapy for treating autoimmune diseases.

Prior studies of ajulemic acid in vitro with human blood and synovial cells indicated anti-inflammatory properties. Ajulemic acid reduced the production of the inflammatory mediators IL-1 β and IL-6, induced T-cell apoptosis, and increased anti-inflammatory eicosanoids, prostaglandin J2, and lipoxin A4. Furthermore, multiple clinical trials of ajulemic acid administered orally found no serious adverse or *Cannabis*-like neurological effects [98].

The anti-inflammatory properties of ajulemic acid were examined on the pathogenic cytokines in cutaneous dermatomyositis. The key inflammatory cytokines in dermatomyositis are type I IFNs and tumor necrosis factor- α (TNF α). It was suggested that ajulemic acid could suppress the production of these cytokines from the immune cells of dermatomyositis patients in vitro. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of 18 dermatomyositis patients. The cells were treated with 0, 3, 10, and 15 $\mu\text{mol/L}$ ajulemic acids for 1 h and then cultured with lipopolysaccharide for TNF- α stimulation or CpG oligonucleotides for IFN- α and IFN- β stimulation. After 18 h, the supernatant was separated from the cells. ELISA quantified TNF α , IFN- α , and IFN- β production.

The baseline and multi-dosage post-simulation gene expression was compared through a linear mixed model, adjusted for within-subject correlations. All expression values were log-transformed before the analysis. Samples that did not produce baseline minimum cytokine levels without ajulemic acid were excluded from the analysis. PMBC viability studies found that 98.2%, 97.4%, and 95.7% of cells were viable after incubation with 3, 10, and 15 $\mu\text{mol/L}$ ajulemic acids compared with 99.3% of cells incubated without ajulemic acid.

Moderate and high ajulemic acid concentrations significantly suppressed TNF- α secretion. In contrast, all ajulemic acid doses significantly reduced IFN- α production. Ajulemic acid suppressed TNF- α secretion from lipopolysaccharide-stimulated cells at concentrations of 10 $\mu\text{mol/L}$ ($n = 16$) ($p = 0.0002$) and 15 $\mu\text{mol/L}$ ($n = 14$) ($p < 0.0001$) but not at three $\mu\text{mol/L}$ ($n = 16$) ($p = 0.4727$) compared with cells not incubated with ajulemic acid ($n = 17$). As opposed to untreated cells ($n = 8$), ajulemic acid suppressed secretion of IFN- α at all tested concentrations (3 $\mu\text{mol/L}$, $n = 7$;

10 $\mu\text{mol/L}$, $n = 4$; 15 $\mu\text{mol/L}$ $n = 3$) ($p \leq 0.0007$). In the samples tested, all ajulemic acid doses decreased IFN- β production.

Similar to the results observed with systemic sclerosis, a phase 2 trial of patients with skin-predominant dermatomyositis demonstrated symptom severity index scores markedly improved with oral lenabasum [85]. The pathogenesis may be explained by lenabasum's ability to significantly suppress secretions of TNF- α , Interferon- α -(IFN- α), and IFN- β in peripheral mononuclear cells of patients with dermatomyositis [86]. Another randomized control trial demonstrated that lenabasum was administered over 12 weeks.

Cutaneous Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a challenging disorder. The diversity of its clinical features is matched by the complexity of the genetic, hormonal, and environmental factors that caused it and the array of autoantibodies with which it is associated. It is a chronic autoimmune disease with multiple clinical manifestations that differ from one patient to another. Systemic lupus erythematosus is an extremely heterogeneous disease in all aspects. However, recent studies have demonstrated characteristic induction of type I interferon-regulated genes (IFN-signature), which is linked to a more severe disease activity with organ failure in patients with SLE [99].

A loss of tolerance toward self-nuclear Ags characterizes systemic lupus erythematosus. Systemic induction of type I IFNs plays a pivotal role in SLE, a major source of type I IFNs being the plasmacytoid dendritic cells (pDCs). Several genes have been linked with susceptibility to SLE in genome-wide association studies. The part of one such gene, α/β -hydrolase domain-containing 6 (ABHD6), in the regulation of IFN- α induction in SLE patients, revealed a regulatory role of ABHD6 in human pDCs through modulating the local abundance of its substrate, the endocannabinoid 2-arachidonyl glycerol, and elucidated a cannabinoid receptor 2 mediated regulatory role of 2-AG on IFN- α induction by pDCs. Also identified was an ABHD6^{High} SLE endophenotype wherein reduced local abundance of 2-AG relieves the CB2-mediated steady-state resistive tuning on IFN- α induction by pDCs, thereby contributing to SLE pathogenesis [100].

Autoimmune diseases, including systemic lupus erythematosus, are caused by the overactivation of immune cells and chronic inflammation. Since oxidative stress is a common feature of these diseases, which activates leukocytes to intensify inflammation, antioxidants could reduce the severity of the diseases. In addition to activating leukocytes, oxidative stress increases the production of lipid mediators, notably endocannabinoids and eicosanoids, which are products of enzymatic lipid metabolism that act through specific receptors. The anti-inflammatory CB2 receptors are the predominant cannabinoid receptors in leukocytes. Endocannabinoids

act as anti-inflammatory factors that regulate compensatory mechanisms in autoimmune diseases.

While the administration of eicosanoids *in vitro* leads to the differentiation of lymphocytes into T helper 2 (Th2) cells, eicosanoids are also necessary for differentiating Th1 and Th17 cells. Therefore, their antagonists and the genetic deletion of their receptors abolish inflammation in animal models of SLE. On the other hand, Products of non-enzymatic lipid peroxidation, such as acrolein and 4-hydroxynonenal-protein adducts, mostly generated by an oxidative burst of granulocytes, enhance inflammation and even act as autoantigens and extracellular signaling molecules in the vicious circle of autoimmune diseases [47].

Plasmacytoid dendritic cells compose 0.2–0.5% of circulating leukocytes but play a significant role in mounting host immune responses. Elevated and chronic activation of pDC is implicated in an autoimmune disease like systemic lupus erythematosus. Delta-9-tetrahydrocannabinol is a well-characterized cannabinoid with potent anti-inflammatory activity. The psychotropic effects of delta-9-THC are mediated through cannabinoid receptor 1 expressed in the central nervous system. The immunomodulatory effects of delta-9-THC result from delta-9-THC binding to CB1 and CB2 on immune cells.

Synthetic CB2-selective agonists have been developed to explore immune modulation by cannabinoids without the psychotropic effects. Studies have determined that the CB2-selective agonists, JWH-015 and JWH-133, have comparable efficacy to delta-9-THC in modulating IFN α and TNF α responses by primary human pDC. Treatment with JWH-133 and JWH-015 inhibited CpG-induced IFN α and TNF α responses by pDC. Further, the phosphorylation of IRF7, TBK1, NF κ B, and IKK γ , key events in pDC activation, were suppressed by delta-9-THC, JWH-133, and JWH-015. Likewise, the phosphorylation of AKT at the S473 and T308 residues were differentially modulated by treatment with delta-9-THC and both JWH compounds, demonstrating the potential for CB2-targeted therapeutics for the treatment of inflammatory conditions involving aberrant pDC activity [101].

The endocannabinoid system plays a key role in many physiological and pathological conditions. Its dysregulation has been described in several rheumatological and autoimmune diseases. Plasma and peripheral blood mononuclear cells of patients with cutaneous lupus erythematosus and age- and sex-matched healthy subjects were examined. Quantitation of eCB levels highlighted that plasma levels of 2-arachidonoylglycerol were significantly increased in cutaneous lupus erythematosus patients compared to HS ($p = 0.0059$). The highest 2-AG levels were associated with lower disease activity among cutaneous lupus erythematosus patients. No differences were found in *N*-arachidonylethanolamine and its congeners, *N*-palmitoylethanolamine and *N*-oleoylethanolamine concentrations between the two groups.

Moreover, gene expression analysis of metabolic enzymes and receptor targets of eCBs and investigation of functional activity and protein expression of selected components of the eCB system disclosed a deranged 2-AG metabolism in patients with cutaneous lupus erythematosus. The expression and functional activity of

2-AG biosynthetic enzyme DAGL were selectively enhanced in PBMCs of cutaneous lupus erythematosus patients compared to healthy subjects.

Delta-9-THC and CBD downregulate immune responses by modulating the endocannabinoid system, a modulation that is relevant for treating autoimmune diseases such as systemic lupus erythematosus. Evidence of the immunomodulatory effect of Cannabidiol in SLE was found to be one of the main modulators of the immune response. Cannabidiol decreased the number of Th1 and Th17 proinflammatory cells and the production of the proinflammatory cytokines, interleukin (IL)-1, IL-12, IL-17, interferon (IFN)- γ , and tumor necrosis factor- α , in mouse models of MS and DMT1 [102].

Synthetic cannabinoid-like molecules have shown anti-inflammatory properties, with agonist or antagonist activity on CB1, CB2, TRPV1, PPAR- α , and PPAR- γ receptors. Data from experimental animal models indicate that natural and synthetic cannabinoids downregulate inflammatory responses mediated by the immune cells responsible for AD chronicity and progression.

Epidermolysis Bullosa

Epidermolysis bullosa was challenging to manage because of skin fragility; the repeated wound healings produce itching, pain, limited mobility, and recurrent infections. Epidermolysis bullosa (EB) is characterized by intense pain related to its pathology and care-based interventions. Opioid-based therapies underpin pain care in EB; however, they cannot provide adequate analgesia in many patients. Cannabinoid-based medicines have been confirmed for pain conditions of various etiologies that provide a different dimension for pain alleviation in EB [103].

Three patients with EB were prescribed pharmaceutical-grade sublingually administered cannabinoid-based medicines comprising delta-9-tetrahydrocannabinol and cannabidiol. The three patients reported improved pain scores, reduced pruritus, and reduced analgesic drug intake. A 64-year-old woman diagnosed with junctional EB generalized intermediate (JEB-gen intermed) suffered from refractory pain for over 20 years, reporting pain on the visual analog scale. Her daily pain therapy included 3 $\times$ 1000 mg paracetamol, 2 $\times$ 10 mg oxycodone extended-release (ER), 1 $\times$ 10 mg codeine-phosphate, 2 $\times$ 25 mg amitriptyline, and 1 $\times$ 5 mg/g topical morphine (applied to the painful right heel). The regimen could not provide satisfactory analgesia.

Ten years prior, she attempted treatment with an inhaled CBM; however, she experienced only short-lived analgesia (< 45 min) and the presence of euphoria and dizziness. Subsequently, an orally administered CBM liquid was prescribed with no adequate pain relief. Sublingual CBM oil (20 mg/mL CBD, 13 mg/mL delta-9-THC) was started at 0.5 mg CBD and 0.325 mg delta-9-THC, 4 $\times$ daily and increased stepwise up to 2.5 mg CBD and 1.625 mg delta-9-THC, 4 $\times$ daily. The patient reported VAS scores ranging between 1/10 and 4/10.

At 3 months, she was weaned off oxycodone-ER. At 6 months, oxycodone immediate release (IR) was used for dressing changes only. During the following 2 years, treatment with topical morphine was replaced with a combination of 1 mg CBD and 0.65 mg delta-9-THC daily to the painful heel. The patient was also weaned off amitriptyline and reported a moderate reduction of pruritus expressed by a lower pruritus frequency and reduced urges to scratch. The CBM reported an increased appetite and maintained that the treatment provides adequate pain relief.

Another case of a 41-year-old man diagnosed with JEB-gen intermed was treated for pain, for over 10 years, with 1 g paracetamol, 4 × 200 mg ibuprofen, 20 mg oxycodone-IR, and 5 mg/g topical morphine daily. The patient reported a VAS for the pain of 9/10 and started treatment with a sublingual CBM of 20 mg/mL CBD and 13 mg/mL delta-9-THC. After 1 month, he reached a dose of 3 mg CBD and 1.95 mg delta-9-THC, 4 × daily, and reported a VAS for the pain of 3/10. After commencing the CBM treatment, the patient reported a reduction in the frequency and intensity of his pruritus and a reduced urge to scratch. He was weaned off oxycodone-IR, and topical morphine was stopped.

After 6 months, due to increased wound pain, supplementary treatment was started with 5 mg oxycodone-IR 3 × daily, subsequently inducing a self-reported distorted sense of time and delayed reaction time. On the grounds of drug-drug interaction, the CBM was reduced to nocturnal doses, alleviating the symptoms and maintaining his pain relief. After 2 months, the patient stopped CBM treatment as not affordable. He was admitted to the hospital after a subsequent exacerbation of skin ulcerations and pain. His clinical team resorted to treatment with prednisolone 15 mg daily for 2 weeks, which provided only moderate analgesia. His pain treatment remains unresolved.

A thirty-six-year-old man, diagnosed with recessive dystrophic EB generalized severe (RDEB-gen sev), had a history of complications including chronic pain, pruritus, obstipation, pseudosyndactyly, squamous cell carcinoma (SCC) of his hands, and multiple amputations. His pain treatment consisted of topical morphine, oxycodone-ER, oxycodone-IR, amitriptyline, paracetamol, etoricoxib, and locally injected dexamethasone. He experienced sedative side effects from amitriptyline, delayed wound healing by topical morphine, and obstipation due to oxycodone-ER, oxycodone-IR, and etoricoxib.

Unable to tolerate the side effects, he experimented with CBM-*flos* (the dried flower of the female *Cannabis* plant) by way of combustion and inhalation. He inhaled CBM-*flos* and his opioid-induced obstipation resolved. During routine clinical follow-up, he reported an improvement in pain treated with paracetamol. After 6 months, worsening tumor pain in both hands required supplementary analgesia. As EB's pain recommendations indicated, the use of strong opioids, which were contraindicated due to his susceptibility to side effects, sublingual CBM oil comprising 20 mg/mL CBD and 13 mg/mL delta-9-THC, was started. After 1 week, he reported a 40% reduction in pain intensity [104].

Both the sublingual CBM and intrapulmonary CBM were continued for 2 years. The combination of intrapulmonary and sublingual CBMs surpassed previous pain treatments. It reduced the severity of pruritus and his urge to scratch. Later, he

entered terminal care as a sequela of metastasized cutaneous SCC and continued intrapulmonary and sublingual CBM administration, combined with 10 mg amitriptyline and 10 mg prednisolone daily.

Pain in EB significantly impacts the quality of life and day-to-day functioning [105]. In addition to nociceptive pain associated with blistering and wounds, peripheral nerve damage objectified in RDEB and postulated in JEB adds plausibility to reported high pain scores in the EB types [106]. Neuropathies limit the central role of opioids for EB pain as they may be less effective for this type of pain according to systematic analysis.

The persistent inflammatory condition in RDEB skin may also contribute to central sensitization to painful stimuli, which is challenging to objectify through diagnostic techniques and does not respond adequately to targeted therapies. The different aetiologies of pain in EB require tailored interventions. Therefore, pain care is optimized through combined drug treatments and psychological interventions. However, the limited effectiveness of conventional analgesics stresses that the gold standard for pain care in EB has yet to be established, motivating clinicians to consider alternative treatments for EB pain from various aetiologies.

Studies on CBM treatments show varying levels of success with moderate-quality evidence supporting CBM efficacy for chronic pain [107]. Three patients were prescribed CBMs, comprising delta-9-THC and CBD, by way of sublingual administration, with good effects. However, two patients had used at least one other administration form, of which one had administered self-acquired CBMs. In general, the difficulty of measuring clinical outcomes of CBM treatments is characterized by cannabinoid compositions. This has led to a call for the production and distribution of standardized, pharmaceutical-grade CBM compositions, and administration forms that can increase the predictability of dosing and effects to reduce the hazards of over- and under-dosing in the clinical setting.

Cannabinoids mimic the actions of endocannabinoids, endogenous ligands, which play a key role in synaptic transmission. Pain modulation has been a central point of discussion, explained, among other reasons, by the actions of cannabinoids on neuronal circuits through cannabinoid-binding receptor-dependent and independent pathways [108]. Cannabinoid receptors 1 and 2 are expressed on presynaptic terminals of primary afferent pain circuits, brain areas processing nociception, including the central-medial thalamic nuclei, periaqueductal grey, and raphe nuclei, and are colocalized with μ -opioid receptors in the spinal cord junction for peripheral nociceptive neurons. The role of CB2 antinociception has also been implied in inflammatory and neuropathic pain models, likely due to the interaction of the endocannabinoid system with endorphin/enkephalin, vanilloid/transient receptor potential, and inflammatory systems [109].

Cannabinoid receptors 1 and 2 have been localized in human skin, and CB2 activation on keratinocytes is described to produce antinociception through the peripheral release of endogenous opioids. Pruritus entails the largest physical and psychological burden for children with EB, and the reported diminished frequency and intensities of pruritus in the cases are notable as the antipruritic effects of CBMs have been postulated in several dermatological conditions [110].

Delta-9-THC, like the endocannabinoids 2-arachyldonylglycerol and anandamide, is a partial agonist of CB1 and CB2 and has been shown to stimulate β -endorphin production, allowing for opioid sparing in clinical practice [111]. In contrast to delta-9-THC, CBD has a low affinity for CB1 and CB2 and, at high doses, does not produce psychotropic effects. Interestingly, CBD can antagonize the undesired effects of delta-9-THC, such as sedation and intoxication, while concurrently improving desirable effects like analgesia.

Wound Healing and Skin Aging

Skin inflammatory diseases result from complex events that include dysregulation and abnormal expression of inflammatory mediators or their receptors in skin cells. A study investigated the potential effect of a *Cannabis* ethanolic extract standardized in cannabidiol as an anti-inflammatory agent in the skin. The extract inhibited the release of mediators of inflammation involved in wound healing and inflammatory processes occurring in the skin. The mode of action involved the impairment of the nuclear factor-kappa B (NF- κ B) pathway since the extract counteracted the tumor necrosis factor-alpha-induced NF- κ B-driven transcription in both skin cell lines. *Cannabis* extract and cannabidiol showed different effects on the release of interleukin-8 and vascular endothelial growth factor, which are mediators whose genes depend on NF- κ B.

The effect of cannabidiol on the NF- κ B pathway and metalloproteinase-9 (MMP-9) release paralleled the effect of the extract, thus making cannabidiol the major contributor to the effect observed. The down-regulation of genes involved in wound healing and skin inflammation was in part due to the presence of cannabidiol. The findings provide insights into the potential effect of *Cannabis* extracts against inflammation-based skin diseases [112].

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wound healing and skin inflammation was partially due to the presence of cannabidiol [112].

To evaluate the anti-inflammatory and gingival wound healing activities of *Cannabis*, the cellular bioactivities of *Cannabis* extract and CBD were determined by the inhibition of TNF- α and IL-1 β in LPS-induced murine macrophage, RAW 264.7, cells. ELISA was used while a scratch test assay was performed during the wound healing activity in human gingival fibroblast (HGF-1) cells; the cytotoxicity was also evaluated by MTT assay.

The *Cannabis* extract and CBD significantly decreased TNF- α release by up to $91.05 \pm 2.91\%$ and $50.78 \pm 7.21\%$ of LPS activity, respectively, in a dose-dependent manner, compared to 10 $\mu\text{g/mL}$ hydrocortisone ($61.67 \pm 3.79\%$). The *Cannabis* extract and CBD also significantly decreased IL-1 β release in dose-dependent response, up to $78.03 \pm 3.34\%$ and $85.87 \pm 1.11\%$ of LPS activity, respectively, compared to 5 $\mu\text{g/mL}$ hydrocortisone ($80.81 \pm 3.55\%$). The mean percentage of closure of the wound area was $27.92 \pm 1.21\%$ when exposed to 5 $\mu\text{g/mL}$ of *Cannabis* extract and $33.49 \pm 1.67\%$ when exposed to 0.5 $\mu\text{g/mL}$ CBD compared to $24.34 \pm 2.29\%$ for non-treated control [113].

Stem cell therapy promotes tissue regeneration and wound healing. Efforts have been made to prime stem cells to enhance their regenerative abilities. Cannabidiol and delta-9-tetrahydrocannabinol are defined as immunomodulators. Tests of two sources of stem cells primed with CBD or delta-9-THC demonstrate improved regenerative abilities.

Human adipose-derived stem cells (ASCs) and bone marrow-derived stem cells (BMDSCs), not obtained from the same individual, were treated with low (300 nM) or high (3 μM) concentration CBD. Porcine ASCs and BMDSCs were isolated from a single pig and treated with either low or high concentrations of CBD or delta-9-THC. Transwell migration and MTT proliferation assays were performed on the human ASCs and BMDSCs. Also, a transwell migration assay was performed on the porcine ASCs and BMDSCs.

A wound-healing scratch assay in porcine primary fibroblasts (PFs) was performed and co-cultured with the cannabinoid-treated ASCs. Cannabidiol priming at low concentration induces migration by 180% ($p < 0.01$) in porcine ASCs, and by 93% ($p < 0.02$) in porcine BMDSCs. In porcine stem cells, delta-9-THC priming at low concentration induces migration by 91.6% ($p < 0.01$) in ASCs but by only 44.3% ($p < 0.03$) in BMDSCs. Compared to PFs co-cultured with untreated ASCs, PFs co-cultured with low CBD-primed ASCs had 75% faster-wound closure at 18 h ($p < 0.01$). Cannabidiol and delta-9-THC priming of ASCs and BMDSCs, particularly at lower doses, enhances several regenerative parameters, suggesting that the major *Cannabis* constituents improve stem cell-based therapies [114].

Venous leg ulcers are highly prevalent lower limb integumentary wounds that remain challenging to heal despite evidence-based compression therapies. Many adjuvant treatments have been studied, but none have demonstrated enough efficacies to gain adoption into treatment guidelines. Global attention on *Cannabis*-Based Therapies is increasing and has been driven by quantum scientific advancements in understanding the endocannabinoid signaling system.

Topical *Cannabis*-based medicines (TCBM) represent a treatment paradigm for venous leg ulcers in terms of promoting wound closure. Fourteen complex patients with 16 recalcitrant leg ulcers were treated with Topical *Cannabis*-based medicines and compression bandaging to both wound bed and peri-wound tissues. The cohort had a mean age of 75.8 years. It was medically complex, as reflected by a mean M3 multimorbidity index score of 2.94 and a mean Palliative Performance Scale score of 67.1%.

Complete wound closure, defined as being fully epithelialized, was achieved among 11 patients (79%) and 13 wounds (81%) within a median of 34 days. The three remaining patients demonstrated progressive healing trends but were lost to follow-up. The treatments were well tolerated, and no significant adverse reactions were experienced. The rapid wound closure of previously non-healing venous leg ulcers among elderly and highly complex patients suggests that Topical *Cannabis*-based medicines may become effective adjuvants in conjunction with compression therapy; also an indication that they have a broader role within the integumentary system and wound management [115].

Non-Uremic Calciphylaxis (NUC) is a rare condition that often manifests as intractable and painful integumentary wounds, afflicting patients with a high comorbidity burden. The Endocannabinoid System is a ubiquitous signaling system theorized to be dysregulated within wound beds and associated peri-wound tissues. Preclinical research has shown that the dominant chemical classes derived from the *Cannabis* plant, cannabinoids, terpenes, and flavonoids, interact with the integumentary ECS to promote wound closure and analgesia.

A prospective open-labeled cohort study was conducted involving two elderly Caucasian females with recalcitrant NUC leg ulcers of more than a month. Topical *Cannabis*-based medicines (TCBM) composed of cannabinoids, terpenes, and flavonoids were applied daily to the wound until complete wound closure was achieved. Wounds were photographed regularly, and the digital images were subjected to planimetric analysis to objectively quantify the degree of granulation and epithelization.

Analgesic utilization was tracked as a surrogate/proxy for pain scores. The cohort had a mean M3 multimorbidity index score of 3.31. Complete wound closure was achieved in a mean of 76.3 days. Additionally, no analgesics were required after a mean of 63 days. The treatments were well tolerated, with no adverse reactions. The positive results demonstrated in challenging wounds, such as NUC, among highly complex patients indicated that TCBM has an even broader role within integumentary and wound management [116].

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Chapter 13

Antiviral Activities of *Cannabis*



Despite the history of scientific evidence regarding plants and their products in prophylactics and therapeutics, their applications in healthcare systems are only now gaining momentum. Plants contain bioactive compounds that target certain viruses to cure or prevent viral diseases and infections. They provide a rich resource of antiviral drugs. Identifying the antiviral mechanisms in plants has shed light on where they interact with the life cycle of viruses, such as viral entry, replication, assembly, and release.

Viruses are responsible for many diseases, including cancer; complex syndromes such as Alzheimer's disease, diabetes, and hepatocellular carcinoma have been associated with viral infections. Plant-based therapies can be beneficial against some notable viral pathogens, including coronavirus, Coxsackie virus, dengue virus, enterovirus, hepatitis B virus, hepatitis C virus (HCV), herpes simplex virus (HSV), human immunodeficiency virus (HIV), influenza virus, measles virus, and respiratory syncytial virus. There are numerous bioactive compounds in plants that possess biodynamic properties [1].

When a host is infected with a virus, the host marshals innate and adaptive immunity against the replication and spread of the virus; if innate and adaptive immunity successfully suppresses viral replication, specific lifelong immunity may result. If the virus outpaces the host containment effort, the host might succumb to tissue damage, and inflammation will occur due to exposure of cells to harmful stimuli.

The primary physical manifestations of inflammation are pain, swelling, heat, and loss of function in the affected area. The processes involve the major cells of the immune system, including monocytes, macrophages, neutrophils, basophils, dendritic cells, mast cells, T-cells, and B-cells. Examination of a range of inflammatory lesions demonstrates the presence of specific leukocytes in any given lesion, indicating that the inflammatory process is regulated in such a way as to ensure that the appropriate leukocytes are recruited. The events are, in turn, controlled by a host of extracellular molecular regulators, including members of the cytokine and

chemokine families that mediate both immune cell recruitment and complex intracellular signaling control mechanisms that characterize inflammation.

The innate response is non-specific, while the secondary adaptive response is specific for the pathogen or closely related species. The cellular components include innate immune cells like the monocytes/macrophages, neutrophils, natural killer cells, and adaptive immune cells like B- and T-lymphocytes are coordinated for an effective response. Cytokines are a dedicated group of biochemicals involved in the coordination and include interferons, interleukins, and chemokines responsible for synchronizing the initiation, regulation, and termination of immune responses [2]. Immune cells such as macrophages and lymphocytes produce a group of small polypeptides called cytokines which exerts their function by engaging respective cell-surface receptors. Depending on their biological functions, they are labeled as either proinflammatory or anti-inflammatory cytokines.

Cannabis and its derivatives are credited for treating various medical conditions, including pain in adults, chemotherapy-induced nausea and vomiting, and spasticity associated with multiple sclerosis. Many medical applications of *Cannabis* and cannabinoids are related to their anti-inflammatory activity, which is a double-edged sword under certain conditions; particularly in viral infections, the anti-inflammatory activity of delta-tetrahydrocannabinol (THC) may jeopardize host immune responses to acute viral infections that could lead to disease progression.

Nonetheless, delta-9-THC may be beneficial in viral infections where the host inflammatory response is symptomatic, although its psychoactive and addictive potential is a significant limitation to its therapeutic development. In neurons, innate antiviral/pro-resolution responses include the activation of NOS-1, inhibition of Ca^{2+} activity by cannabinoids, increased viral replication, and disease.

Both endogenous and exogenous cannabinoids can influence the course of infections in vitro and in vivo. Exogenous cannabinoids or receptor antagonists may influence many cellular and systemic host responses. The anti-inflammatory activity of cannabinoids may compromise host inflammatory responses to acute viral infections but may be beneficial in persistent infections. Cannabinoids may act either through the CB1 or the CB2 receptor, which are found on distinct cell types. The CB1 receptor is found on neurons, some astrocytes, and skeletal muscle cells; neurons are frequently the target of viral infection.

Engagement of the CB1 receptor by its endogenous or exogenous agonists may inhibit the release of Ca^{2+} from intracellular or extracellular stores. Since many important intracellular proteins are Ca^{2+} -dependent for activation, signal transduction through the CB1 receptor may impair the secondary pathways and profoundly influence the ability of viruses to replicate in neurons. The response of cells expressing the CB2 receptor may influence not only the responses in that cell but may alter the course of the host's innate and adaptive immune response to the pathogen, suppressing inflammation and the development of virus-specific cellular and humoral responses.

Immune Response

The endocannabinoid system regulates several physiological processes, including sleep and the immune response. It is known that the use of endocannabinoid system medications increases the susceptibility to infections because of the impact on the modulation of the immune system. The use of *Cannabis* and its influence on the course of an infection, whether it was caused by bacteria, viruses, parasites, or fungi, has been indicated in the control and elimination of infectious agents such as bacteria, viruses, and some protozoa [3].

Sepsis is a systemic infection as the result of an inadequate immune response. A murine study model involving the injection of lipopolysaccharide (LPS) is how the role of CB1 and CB2 during sepsis was discovered. Where the activation of CB1 is concerned, this allows the good functioning of innate immunity parameters, e.g., CB1-deficient mice are unable to develop fever, have a lower expression of TLR4 in the liver and spleen when compared to wild-type mice, and their peritoneal macrophages do not secrete proinflammatory cytokines [4].

Concerning CB2, a study performed on CB2 knockout mice revealed a higher mortality rate, a higher concentration of proinflammatory cytokines such as TNF- α , interleukin (IL)-6, and a higher HMGB1 level when compared to WT mice under sepsis; after administering GW405833, a CB2 agonist, the cytokines and the mortality rate were decreased [5]. Another study determined that the activation of CB2, using the agonist HU-308, can prevent additional damage during sepsis, in this case, at the intestinal level [6].

Furthermore, mice with endotoxemia, induced by LPS and treated with the CB2 agonist URB597 and the inhibitor of the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), JZL184, showed a reduced number of adherent leukocytes in the submucosal and axial veins, reducing the endothelial interaction of leukocytes and thus preventing inflammatory damage, in addition to improving intestinal capillary perfusion, an idea further reinforced when the mice were treated with the antagonist AM630, resulting in an unmodified number of leukocytes [5, 6]. CB1 is, thus, important in developing an efficient innate immune response, whereas CB2 prevents additional inflammatory damage during sepsis. The studies support that the ECS could be used as an effective therapeutic target in sepsis treatment.

Hepatitis Virus

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV). It affects approximately 150 million people globally; a significant number are chronically infected and will eventually develop liver cirrhosis or cancer [7]. It can also develop into metabolic disorders such as insulin resistance and steatosis. However, the moderate use of *Cannabis* may be helpful for patients with hepatitis C because it

stabilizes weight loss and nausea. Long-term daily use, however, has been associated with fibrosis progression [8]. ECS activation by the CB1 agonist ACEA promotes HCV replication in hepatocyte cultures. CB1 upregulation and high levels of 2-AG during hepatic diseases have been documented [9].

Liver steatosis is common in human immunodeficiency virus (HIV)-hepatitis C virus-co-infected patients. It was found that *Cannabis* use is negatively associated with insulin resistance in the general population and HIV-HCV-co-infected patients. Given the link between insulin resistance and steatosis, a study was conducted to determine whether *Cannabis* use in a population was associated with a reduced risk of steatosis. Among the study patients ($n = 838$), 40.1% had steatosis. Fourteen percent reported daily *Cannabis* use, 11.7% regular use, and 74.7% no use or occasional use. Daily *Cannabis* use was associated with a reduced prevalence after adjusting for body mass index, hazardous alcohol consumption, and current or lifetime use of lamivudine/zidovudine [10].

It has been demonstrated that CB1 and CB2 receptors regulate the progression of experimental liver fibrosis. The impact of *Cannabis* smoking on fibrosis progression rate (FPR) in patients with chronic hepatitis C (CHC) was evaluated in 270 consecutive untreated patients with CHC of known duration undergoing a liver biopsy [11]. Fibrosis stage, steatosis, and activity grades were scored according to the Metavir system. Patients were categorized as non-*Cannabis* users (52.2%), occasional users (14.8%), or daily users (33.0%), and the relationship between *Cannabis* use and fibrosis progression rate (FPR) or fibrosis stage was assessed. Daily *Cannabis* smoking is significantly associated with fibrosis progression during CHC. Patients with ongoing CHC should be advised to refrain from regular *Cannabis* use.

Viral hepatitis B (HBV) and hepatitis C pose major health problems that can lead to severe liver damage resulting in liver cirrhosis and cancer. There is a vaccine for HBV but not for HCV. The therapeutic potentials of *Cannabis* and its metabolites against HBV and HCV revealed that cannabidiol is active against HCV but not against HBV in vitro [12]. CBD inhibited HCV replication by 86.4% at a concentration of 10 μM with EC₅₀ of 3.163 μM in a dose–response assay. The findings suggest that CBD could be further developed and therapeutically against HCV.

The endocannabinoid system is implicated in many chronic liver diseases, including hepatitis C viral infection. *Cannabis* consumption is associated with fibrosis progression in chronic hepatitis C (CHC) patients. Anandamide and 2-arachidonoyl glycerol were quantified in HCV patients and healthy control samples. Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) activities were assessed by [³H]AEA and [³H]2-AG hydrolysis, respectively. Gene expression and cytokine release were assayed by TaqMan PCR and ELISpot, respectively.

AEA and 2-AG levels were increased in the plasma of HCV patients but not in liver tissues. Hepatic FAAH and MAGL activity was not changed. In peripheral blood mononuclear cells (PBMCs), ECs inhibited IFN- γ , TNF- α , and IL-2 secretion. Inhibition of IL-2 by endogenous AEA was stronger in PBMC from HCV patients [13]. In hepatocytes, 2-AG induced the expression of IL-6, -17A, -32, and

COX-2 and enhanced activation of hepatic stellate cells co-cultivated with PBMC from subjects with CHC.

Herpes Simplex Virus

Herpes simplex virus (HSV) has been described into two types: HSV-1, transmitted by oral contact and mainly causing orolabial herpes, and HSV-2, transmitted through sexual contact with an infected person, causing genital herpes. The WHO has estimated that 400 million people worldwide are infected with HSV-2. It has been suggested that cannabinoids can suppress host resistance to HSV [14]. Based on this, the effect of delta-9-THC on HSV-2 infections was evaluated [15, 16]. The studies in vitro, using virus-infected Vero cells pretreated for 24 hours with delta-9-THC, indicated a higher extracellular incidence of the virus that correlated with the dissolution of the cellular membrane and inhibited the synthesis, maturation, and cellular transport of specific HSV-2 glycoproteins and macrovacuoles in the cytoplasm that contained virus aggregates [17].

The results suggested that treatment with cannabinoids facilitates the exit of the virus from infected cells, thereby facilitating viral dispersion [18]. In addition, delta-9-THC has been found to inhibit macrophage-extrinsic anti-HSV activity [19]. Experiments in vivo, using mice and guinea pigs, reported that delta-9-THC decreased host resistance to HSV-2 vaginal infection, as observed by the greater frequency and severity of genital lesions, higher mortality, and higher virus titers in vaginal secretions, and reduced IFN titers indicating that cannabinoids induce a decreased resistance to HSV-2 [20–22].

In another context, an association between HSV and Kaposi sarcoma, known as Kaposi sarcoma-associated herpes virus (KSHV), causes Kaposi sarcoma in individuals with HIV. KSHV is very persistent in immune-suppressed hosts, promoting tumor growth. The lack of efficacious therapies has precipitated the use of compounds like CBD, a non-psychoactive cannabinoid shown to induce apoptosis in endothelial cells infected with KSHV, as well as inhibiting endothelial growth factors, e.g., viral G protein-coupled receptor (vGPCR), chemokine growth-regulated protein α (GRO- α), vascular endothelial growth factor receptor (VEGFR)-3, and VEGF-C [23].

Human Immunodeficiency Virus

People with HIV/AIDS use *Cannabis* more frequently to improve their appetite, decrease nausea, or control pain; however, some consequences should be considered. Animal models have demonstrated that NK cell activity becomes suppressed at high delta-9-THC doses [24]. Delta-9-THC suppresses cellular immune functions, and further impairment in HIV-infected individuals is expected. A hybrid

model based on human peripheral-blood leukocytes implanted into SCID mice (the huPBL-SCID model) revealed that, in the presence of delta-9-THC, HIV replication was increased 50-fold in the systemic viral load. However, there was no difference in the percentage of CD4+ T cells, and the expression of CCR5 and CXCR4 (chemokine receptors, both essential co-receptors for HIV infection) was increased. The number of IFN- γ -producing cells was decreased [25].

In other cases, the negative effects of infection, such as HSV, are enhanced by the administration of delta-9-THC. When there is coinfection, such as by the Friend leukemia virus, those effects are even greater than the individual effect, i.e., the rate of infection development and mortality was increased [23]. The activation of CB2 could be an alternative in active antiretroviral therapy, as it can attenuate neurocognitive disorders associated with HIV infection by inhibiting viral replication, regulate inflammation by reducing the permeability of the hematoencephalic barrier and leukocyte infiltration, and suppress the activity of neurotoxic proteins (e.g., Tat and HIV-1gp120), thereby decreasing neuronal damage [26]. It also prevents Tat from promoting monocyte migration through the hematoencephalic barrier [27].

Influenza Virus

The administration of delta-9-THC could be harmful to a host infected with the influenza virus. As mentioned in studies on mice, delta-9-THC administration after an immune challenge with influenza virus A/PR/8 resulted in increased viral loads, higher hemagglutinin 1 expression, and diminished CD4+ and CD8+ lymphocyte and macrophage recruitment into the lungs [28]. Some effects were observed opposite when CB1 and CB2 knockout mice were infected, i.e., increased CD4+ lymphocyte recruitment, IFN- γ levels, and lung inflammation, higher than in control WT mice [29]. Other immune parameters affected include cytokine secretion by CD4+ T cells and NK cells, besides a lower overall percentage of subpopulations of antigen-presenting cells present in the lungs of infected mice [30]. The results demonstrate that delta-9-THC administration diminishes the immune response against the influenza virus.

Coronavirus

Coronavirus disease (COVID-19) is an acute disease that produces infection by SARS-CoV-2 with a mortality of approximately 3.7%. The leading cause of COVID-19 mortality is respiratory failure due to acute respiratory distress syndrome. A two-phase immune response often characterizes the disease progression of COVID-19. An extract fraction from *Cannabis* Arbel strain (FCBD) substantially reduced (dose-dependently) interleukin (IL)-6 and -8 levels in an alveolar epithelial (A549) cell line. FCBD contains cannabidiol, cannabigerol (CBG),

tetrahydrocannabivarin (THCV), and multiple terpenes. Treatments with FCBD and an FCBD formulation using phytocannabinoid standards (FCBD:std) reduced IL-6, IL-8, *C-C Motif Chemokine Ligands (CCLs)* 2 and 7, and angiotensin I converting enzyme 2 (ACE2) expression in the A549 cell line.

Treatment with FCBD induced macrophage (differentiated KG1 cell line) polarization and phagocytosis in vitro and increased CD36 and type II receptor for the Fc region of IgG (FcγRII) expression. FCBD treatment also substantially increased IL-6 and IL-8 expression in macrophages. FCBD:std., while maintaining anti-inflammatory activity in alveolar epithelial cells, reduced phagocytosis and proinflammatory IL secretion in macrophages compared to FCBD. The phytocannabinoid formulation may show superior activity versus the *Cannabis*-derived fraction for the reduction of lung inflammation [40].

Cannabinoids have been indicated to be immune modulators in changing the balance between pro- and anti-inflammatory cytokines [41]. Cannabinoids can also influence macrophage activity. For example, delta-9-tetrahydrocannabivarin inhibits nitrite production and IL-1β protein levels in lipopolysaccharide-activated macrophages [44]. Further, delta-9-tetrahydrocannabinol can inhibit macrophage phagocytosis by 90% [45]. Cannabidiol is also suggested to have anti-inflammatory effects in various conditions [42]. For example, CBD increases intracellular calcium levels in rheumatoid arthritis synovial fibroblasts and reduces the production of IL-6 and IL-8. Because CBD shows anti-inflammatory activity and is approved by the United States Food and Drug Administration (FDA) for treating children with intractable epilepsy for seizure reduction, it has been suggested that CBD might alleviate COVID-19-related inflammation [42].

Cannabidiol has been shown to down-regulate angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) enzymes, crucial viral gateways in the oral, lung, and intestinal epithelia constituting important routes of SARS-CoV2 invasion. By down-regulating ACE2 and TMPRSS2 enzymes, cannabidiol can be used as a preventative strategy in COVID-19 infection to limit SARS-CoV2 entry into susceptible hosts [43].

Like delta-9-tetrahydrocannabinol, the well-studied cannabinoid, CBD, decreased lung inflammation in a murine model of acute lung injury potentially through the inhibition of proinflammatory cytokine production by immune cells and suppressing exuberant immune responses [46]. CBD inhibits the production of proinflammatory cytokines like interleukin (IL)-2, IL-6, IL-1α and β, interferon-gamma, inducible protein-10, monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, and tumor necrosis factor-α that have been associated with SARS-CoV2-induced multi-organ pathology and mortality [47]. In a murine model of chronic asthma, CBD reduced proinflammatory cytokine production, airway inflammation, and fibrosis [39].

Moreover, CBD can effectively inhibit the JAK-STAT pathway, including the production and action of type I interferons, without leading to addiction, heart rate or blood pressure, and adverse effects on the gastrointestinal tract and cognition [47]. In simian immunodeficiency virus (SIV)-infected rhesus macaques, we reported delta-9-THC-mediated attenuation of IFN-stimulated gene expression in

the intestine. Like CBD, chronic delta-9-THC administration blocked inflammation-induced fibrosis in lymph nodes of chronically SIV-infected RMs [48]. Unlike delta-9-THC, CBD has a high margin of safety and is well tolerated pharmacologically, even after treatments of up to 1500 mg/day for 2 weeks in both animals and humans, which suggests its feasibility in reducing SARS-CoV2-induced lung inflammation/pathology and disease severity [47].

Cannabidiol is powerful antioxidant acting on PPAR γ (NR1C3), 5-HT1A, adenosine A2A, and transient receptor potential channel receptors to produce a range of anti-inflammatory and immunomodulatory effects. Cannabidiol and other cannabinoids exert activities through the interaction with the nuclear PPARs. The PPARs belong to the family of nuclear hormone receptors, and steroids and lipid metabolites regulate their activity. Three different PPAR isoforms (PPAR α , PPAR β , also called δ , and PPAR γ) have been identified, and they have been shown to regulate the expression of genes related to lipid and glucose homeostasis and inflammatory responses [31].

PPAR γ agonism in resident alveolar macrophages significantly limits pulmonary inflammation and promotes host recovery following respiratory viral [32]. It has been demonstrated that during acute pneumonia, alveolar macrophages largely express PPAR γ . PPAR γ activation is also responsible for the control of cytokine over-secretion with consequent amelioration of tissue damage. It is likely that in addition to directly causing an improvement in lung function, cannabidiol could counteract the onset of the cytokine storm from resident macrophages.

Prophylactic or therapeutic administration of PPAR γ agonists has reduced morbidity and mortality during influenza A virus infection [33]. Not coincidentally, the opportunity of thiazolidinediones repurposing for treating COVID-19 patients has been suggested based on their activity on PPAR γ receptors [34]. Full PPAR γ agonists bear several unwanted side effects that could limit their clinical applicability in COVID-19 infection. The use of thiazolidinediones indeed suffered a back-box warning from the US FDA, given the risk of cardiovascular complications such as acute myocardial infarction, heart failure, and stroke. Being a weak PPAR γ agonist, cannabidiol may overcome the limitations and be voided of the side effects [35, 36].

Pharmacological activities have been tested in various pathological conditions, including respiratory diseases resembling COVID-19-induced respiratory distress. Acute lung injury refers to a characteristic form of parenchymal lung disease featured by bilateral pulmonary infiltrates, alveolar-capillary vasculitis with neutrophil infiltration, and proinflammatory cytokines release, comparable to COVID-19. By acting at adenosine A2A receptors, cannabidiol caused a marked amelioration of the reduced pulmonary function. There was a significant decrease in lung resistance and elastance due to the reduction of leukocyte migration into the lung, accompanied by a marked inhibition of proinflammatory cytokines (TNF- α and IL-6) and chemokine (MCP-1/MIP-2/CXCL2) release [37].

It has been shown that a subset of COVID-19 survivors can develop post-infectious sequelae with persistently impaired lung function and pulmonary fibrosis. PPAR γ receptors represent a potential therapeutic target in fibrotic lung diseases, given their ability to regulate fibroblast/myofibroblast activation and collagen

secretion in murine models [38]. Cannabidiol has also been shown to reduce pulmonary inflammation and fibrosis in animal models of asthma [39]. It is therefore conceivable that cannabidiol, a PPAR γ receptor agonist, could potentially limit the start of late-onset pulmonary fibrosis in COVID-19-recovered patients.

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Chapter 14

Alkaline Phosphatase Stimulation



Alkaline phosphatase (AP) emerged after the discovery of a phosphatase abundantly expressed in prostate tissue that featured an acidic pH optimum. The role of alkaline phosphatase, including its natural substrates, is substantially understood from studies of the inborn-error-of-metabolism, hypophosphatasia. Alkaline phosphatase is the most frequently assayed enzyme in medicine because of its importance in the detection, follow-up, and investigation of skeletal diseases. There is also interest in alkaline phosphatase because it can be given therapeutically to patients with hypophosphatasia who lack the enzyme, and it has been investigated for its role in vascular calcification and as a treatment for sepsis-associated acute kidney injury.

CDB and Methylsulfonylmethane

Besides potential health benefits, ingestion of cannabidiol (CBD) poses risks for several side effects, of which hepatotoxicity and CBD/herb–drug interactions are of particular concern. The interaction potential between the cannabidiol-rich *Cannabis* extract (CRCE) and methylsulfonylmethane (MSM), a popular dietary supplement, was investigated in the mouse model. Male C57BL6/J mice, 8 weeks of age, received MSM-containing water (80 mg/100 mL) ad libitum for 17 days. During the last 3 days of treatment, the mice received three doses of CRCE administered in sesame oil via gavage (123 mg/kg/day).

The administration of MSM alone did not result in any evidence of liver toxicity and did not induce the expression of mouse cytochrome P450 (CYP) enzymes. Administration of CRCE produced significant ($p < 0.05$) increases in *Cyp1a2*, *Cyp2b10*, *Cyp2c29*, *Cyp3a4*, *Cyp3a11*, *Cyp2c65*, and *Cyp2c66* messenger RNA. However, the effect was not amplified by MSM/CRCE co-treatment. Similarly, no evidence of liver toxicity was observed in the MSM/CRCE-dosed mice [1].

***Cannabis* and Alcohol Co-administration**

Alcoholism has been linked to problems with male reproductive function. The combined effects of alcohol, *Cannabis*, and tobacco were compared in a study of 35 rats. The rats were assigned randomly into seven groups, A–G: A was administered distilled water, and B–G were either administered alcohol orally (30 mL 40% alcohol) or exposed to either smoke from ignited tobacco (exposure to smoke from 0.7 g tobacco for 5 min) or *Cannabis* (exposure to smoke from 0.7 g tobacco and *Cannabis* for 5 min): B (orally administered alcohol), C (exposed to the smoke from tobacco), D (exposed to smoke from *Cannabis*), E (treated with alcohol and exposed to smoke from tobacco), F (treated with alcohol and exposed to smoke from *Cannabis*), and G (treated with alcohol and exposed to smokes from tobacco and *Cannabis*).

Assays were conducted on the testicular homogenate after 14 days of treatment. There was a significant increase in the activity of alkaline phosphatase, concentrations of cholesterol, glutathione reductase, and malondialdehyde in the treated rats by the co-administration of alcohol with *Cannabis* and tobacco compared with the control group. The biochemical and histoarchitectural change was due to oxidative damage attributable to the synergistic effects. The combined treatment also caused degeneration and morphological distortions of testicular cells. The high binding energy of tetrahydrocannabinol (THC) ligand to prostate acid phosphatase may be a prediction that the ligand can have an inhibitory effect on the function of enzymes in the prostate [2].

***Cannabis* and β -Carotene**

The consumption of *Cannabis* and β -carotene (BC) as a supplement and the potential changes in the chemical and biological properties of β -carotene after exposure to *Cannabis* smoke was investigated. The results revealed that the β -carotene exposure to *Cannabis* smoke underwent 98.8% degradation and suffered a loss of its antiradical activity. The major degradation products identified were 3-hydroxy-2,4,4-trimethylpentyl)2-methylpropanoate and (2-ethyl-3-hydroxyhexyl)2-methylpropanoate compounds. The compounds were found at higher levels in the exhalations of colorectal cancer patients and are similar to the toxic products associated with the lipid peroxidation of polyunsaturated fatty acids.

Toxicological assays using micro-crustacean *Artemia salina* indicated that β -carotene was non-toxic, while the BC degraded by *Cannabis* smoke had a toxicity of $LC_{50} = 397.35 \mu\text{g/mL}$. In Wistar rats, females treated with β -carotene, degraded by *Cannabis* smoke (BCCSS), showed whitish liver spots, altered liver weight, and bilirubin and alkaline phosphatase levels a decrease in the number of leukocytes associated with atypical lymphocytosis. In male rats, there was an increase in the number of leukocytes when compared to the control group. In the histopathological analysis, the cortical region of the kidneys showed the presence of discrete

amorphous eosinophilic material (cylinders) in the lumen of the proximate and distal convoluted tubules. In general, the β -carotene in contact with *Cannabis* smoke undergoes chemical changes and exhibits toxicity to rats and *Artemia salina* [4].

Cannabis and Dill Seeds on Gut Health

The effect of different doses of *Cannabis* seeds alone or in combination with dill seed against antibiotic growth promoter was investigated on performance, serum biochemicals, and gut health of broiler chickens for 42 days. Broiler chicks were grouped randomly into six treatments and fed with basal diet (BD) along with different levels of seeds, viz., T1 (BD), T2 (BD + 0.2% HS), T3 (BD + 0.2% HS + 0.3 DS), T4 (BD + 0.3% HS), T5 (BD + 0.3% HS + 0.3 DS), and T6 (BD + 0.025% bacitracin methylene disalicylate). The performance traits such as feed intake, body weight gain, feed conversion ratio, and carcass traits like cut-up parts, giblet, and abdominal fat yield remained unaffected due to dietary treatments for the overall trial period.

The average feed intake in the early phase (0–3 weeks) reduced significantly in the treatment groups than both controls (T1 and T6). Serum protein concentration remained unchanged, whereas a significant reduction in serum lipids like triglyceride, low-density lipoprotein (LDL), and total cholesterol concentration was noticed due to the dietary inclusion of the seeds. The serum enzymes aspartate aminotransferase (AST) and alanine transaminase (ALT) concentrations depleted significantly in the treated groups, except at higher seed doses (T5); however, alkaline phosphatase levels were unaffected.

Coliform count in the caecum and jejunum reduced linearly with the seed inclusion, whereas dose-dependent proliferation of *lactobacilli* was evident in the caecum and jejunum of the treated birds. No effect was observed on the villus height and crypt depth of the jejunal mucosa. The dietary supplementation of *Cannabis* and dill seeds did not affect the growth performance and carcass traits; however, it positively altered the serum lipid profile and improved gut health, thereby enhancing the overall performance of the broiler chickens [5].

Cannabis and Physical Health

Cannabis is considered to have a minimal effect on physical health. A study was conducted to compare the laboratory profiles of treatment-seeking patients who were *Cannabis* dependent and drug users who concurrently used other substances with non-users. Medical records of patients whose urine was tested for *Cannabis* within a year were accepted for the study. The inclusion criteria for the study group were a co-morbid diagnosis of *Cannabis* dependence according to DSM-IV TR criteria, a positive urine drug screen for *Cannabis*, and at least one biochemical or

hematological examination report during the treatment period. The subjects who underwent all the tests but were negative for any psychoactive substance with no past or current history of substance use were placed in the control group [6].

Fifty-one subjects fulfilled the inclusion criteria for the study group, and thirty subjects were considered controls. There was no significant difference between the subjects' and control groups' demographic profiles. The patients' mean duration of *Cannabis* use was 9.53 ± 8.06 years. Serum levels of bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase, total protein, alkaline phosphatase, erythrocyte sedimentation rate (ESR), and eosinophil counts were raised in 13.7%, 15.6%, 33.3%, 17.6%, 37.2%, 75%, and 5.8% of the subjects, respectively. The two groups showed significant differences in serum alkaline phosphatase and hemoglobin levels. The relative monocyte count was lower than normal in 92% of cases. Physical complaints were reported in 98% of subjects.

Subcutaneous administration of delta-9-tetrahydrocannabinol to rabbits produced dose-related cumulative toxicity. Five groups of three New Zealand albino rabbits received 28 daily treatments with isotonic saline and sesame oil with 15.9, 45.0, or 153.4 mg/kg/day of delta-9-THC dissolved in the sesame oil. Dose-related dermal responses included erythema, edema, ulceration, and nodule formation. The severities of erythema and ulceration were generally maximal during the first week of treatment, but edema and nodule formation were most severe after days 12 and 14, respectively. Some of the granulomatous nodules contained an oily substance and exhibited liquefactive necrosis.

The rabbits survived the treatment, but body weights, liver weights, and liver glycogen levels were decreased in a dose-related manner. Maximal body weight effects occurred after day 19. Hemochemical changes occurred only in the rabbits treated with 153.4 mg/kg/day, including decreased blood sugar, alkaline phosphatase, and increased serum potassium. The cumulative body weight changes significantly decreased hepatic glycogen levels, and reduced blood sugar and alkaline phosphatase values have indicated drug-induced metabolic changes [14]. Hematology parameters were normal throughout the treatment period. No drug-related pathological lesions occurred in internal organs.

Delta-9-tetrahydrocannabinol was administered subcutaneously to female New Zealand white-strain rabbits for 13 days. The animals were randomly divided into six groups of five: untreated controls, vehicle (undiluted propylene glycol)-treated, and delta-9-THC treatment at dose levels of 100, 30, 10, and 3 mg/kg/day. The animals survived for the duration of the study. There were variations in various hematologic values, but they were all within the normal range for the laboratory. The delta-9-THC-treated rabbits did not gain significant body weight, which appears to be due to decreased food consumption.

Blood chemistry evaluations indicated decreased serum levels of potassium, glucose, blood urea nitrogen, alkaline phosphatase, and albumin/globulin (A/G) ratio and increased cholesterol levels of the treated animals. A significant increase in bilirubin values was observed in the animals of the 3- and 10-mg/kg groups. The injection site in the skin of the delta-9-THC-treated rabbits indicated signs of erythema and subcutaneous abscesses. There was a reduction in the absolute and percent of

body weight of the liver and absolute weight of the lungs of the treated animals; no histopathologic alterations were observed. The subcutaneous delta-9-THC treatment for 13 days in rabbits up to a dose level of 100 mg/kg/day did not produce any significant toxicity, except anorexia and local dermal irritation [15].

Delta-9-tetrahydrocannabinol is the most commonly abused neuroactive substance among young adults. The effects of *Cannabis* on the cytoarchitecture of some organs and the blood of rats were investigated. Twenty-one male Sprague Dawley rats were fed different percentages of *Cannabis* chow (0%, 5%, and 10%) for 7 weeks. The rats were subjected to intermittent cognitive function tests and sacrificed after the seventh week. The blood, brain, and other tissues were analyzed for total brain protein and nitric oxide concentration, blood chemistry, and histopathology.

The results revealed a dose-dependent decline in cognitive function and a decrease in the brain's total protein and nitric oxide. Histopathology revealed significant hypertrophy in the heart, hypercellularity in neuronal cells, prominent sinusoid cytoarchitecture of the hepatocytes, and vascular congestion in the seminiferous tubules of the testes. There was a significant difference in the plasma alanine aminotransferase (ALP), ALT, and AST levels between the controls and the *Cannabis*-treated groups. *Cannabis* use causes cellular damage through the mediation of imbalance and altered cytoarchitecture, which may affect the overall health of dependent users [3].

Cannabidiol for Enhancing Osteoblastic Differentiation

The potential of cannabidiol for enhancing osteoblastic differentiation in U2OS and MG-63 cells was evaluated. Cannabidiol increased the expression of angiopoietin 1 and the enzyme activity of alkaline phosphatase in U2OS and MG-63. Invasion and migration assay results indicated that the cell mobility was activated by cannabidiol in U2OS and MG-63. Western blotting analysis indicated that the expression of tight junction-related proteins such as claudin 1, claudin 4, occludin 1, and ZO1 was increased by cannabidiol in U2OS and MG-63.

Alizarin Red S staining analysis indicated that calcium deposition and mineralization were enhanced by cannabidiol in U2OS and MG-63. Western blotting analysis indicated that the expression of osteoblast differentiation-related proteins such as distal-less homeobox 5, bone sialoprotein, osteocalcin, type I collagen, Runt-related transcription factor 2 (RUNX2), osterix (OSX), and alkaline phosphatase was time-dependently upregulated by cannabidiol in U2OS and MG-63.

Cannabidiol-regulated osteoblastic differentiation in U2OS and MG-63 strengthens the protein–protein interaction among RUNX2, OSX, or the phosphorylated p38 mitogen-activated protein kinase (MAPK); cannabidiol increased angiopoietin 1 expression and p38 MAPK activation for osteoblastic differentiation in U2OS and MG-63, indicating that cannabidiol might provide a novel therapeutic option for bone regeneration [7].

***Cannabis* and Spermatogenesis**

Daily administration of *Cannabis* extract (12.5 mg/kg body wt. for 30 days) produced a complete arrest of spermatogenesis in dogs. Distinct degenerative effects were produced from extensive fibrosis and exfoliation of the seminiferous elements. RNA, protein, and sialic acid contents of the testis and epididymis were reduced after the administration of *Cannabis* extract, whereas testicular cholesterol and enzyme phosphatase were elevated. Serum transaminases were slightly elevated, and the alkaline phosphatase and hemoglobin/hematocrit values were in the normal range.

A host physiological examination of the liver did not indicate any damage. Reduced androgen production was reflected in low levels of sialic acid in the testis and epididymides and shrunken Leydig cell nuclei and luminal epididymal epithelium. The extract did not cause severe damage to the vital organs. However, it produced an effective inhibition of spermatogenesis in male dogs in 30 days and thus induced an antifertility state. The possibility of an adverse effect of frequent marijuana use on male reproductive organ functioning in men is undeniable [8].

Cannabidiol and Liver Tests

It has been revealed that cannabidiol products may produce elevations in liver tests (LTs). A study was conducted to compare the prevalence of elevated LT in an adult population of self-administered CBD with the normal and general adult population prevalences. An app-based, 21CFR Part 11 decentralized clinical study platform (ValidCare Study) was used to securely automate consent inclusion/exclusion criteria and collect the data, including demographic information, medical history, reasons for taking, dosage, current medications dosage, adverse effects, and efficacy. In a decentralized, observational study, adults 18–75 years of age taking CBD orally for a minimum of 30 days were recruited from 12 individual CBD product companies. They were sent the standard CBD regimen from the company of their choice. At the end of 30 days, LTs were obtained. Follow-up LTs were offered to the participants with elevated alanine transaminase (ALT) values. A total of 28,121 individuals were invited to participate in a study, 1475 enrolled, and 839 (female: 65.3%, male: 34.7%) completed the study.

Full-spectrum *Cannabis* oil was used by 55.7%, CBD-isolate by 40.5%, and broad spectrum by 3.8%. The mean $\pm$ SD daily dose of CBD was 50.3 + 40.7 mg. The prevalence of elevated ALT was 9.1%, aspartate aminotransferase (AST) 4.0%, alkaline phosphatase 1.9%, total bilirubin 1.7%, with 85.5% of the ALT elevations $<2 \times$ the upper limit of normal (ULN) with only 0.3% having ALT levels $>3 \times$ ULN. The prevalence of ALT and AST elevations (9.1% and 4.0%) was not significantly different from adult general population prevalences (8.9% and 4.9%).

There was no significant association between CBD dosage and LT values. Thirty-three individuals with elevated ALT levels had follow-up LT performed, with 21

having normal LT, eight having the same severity of ALT elevation, and four having an increase in severity, one of which ultimately became normal. Self-medication of CBD is not associated with an increased prevalence of LT elevation. Most LT elevations are likely due to the conditions/medications for which the individuals are taking CBD [9].

The safety and pharmacokinetics of various doses of plant-derived cannabidiol were examined in a randomized, blinded, placebo-controlled trial. Dogs were randomized to five groups balanced in body weight and sex ($n = 4$ dogs/group) and received a CBD (1, 2, 4, or 12 mg/kg; from *Cannabis* extract) or placebo oil formulation orally, daily for 28 days. Blood samples were collected at various time points to estimate the 24-hour pharmacokinetic profiles of CBD and the selected metabolites, 7-carboxy-CBD and 7-hydroxy-CBD. The outcome was assessed through daily observations, veterinary examinations, CBC, and serum analysis.

Repeated CBD administration was well tolerated by the dogs, with no clinically important changes in safety outcomes. Veterinary examinations revealed no clinically abnormal findings, and adverse events were mild. Relative to placebo administration, CBD at 12 mg/kg/d resulted in hypersalivation and higher serum alkaline phosphatase activity. Within each CBD dose group, repeated administration increased total systemic exposure to CBD 1.6- to 3.3-fold. The 24-hour trough plasma CBD concentrations were also dose-dependent, reaching a steady state after 2 weeks of administration [10].

Liver function tests were performed on 500 young service members with a history of drug abuse. Serum glutamic oxaloacetic transaminase (SGOT) level was abnormal in 66% of the 68 patients with a history of parenteral drug abuse. Forty-one percent of 432 patients with a history of only non-parenteral drug abuse also had elevated SGOT levels. A high incidence of liver disease in parenteral drug abusers is well established. The magnitude of the problem in non-parenteral drug abusers has not been noted previously. Liver biopsy specimens in 34 of the patients revealed either classic viral hepatitis or mild nonspecific hepatitis. It could be concluded that hepatitis may be a common sequel to epidemic non-parenteral drug abuse [16].

The influence of naltrexone on liver function in heroin addicts was examined concerning the metabolizing function by using antipyrine clearance and cellular damage by monitoring plasma levels of hepatic enzymes. The clearance of antipyrine was not affected by naltrexone treatment, and the use and withdrawal of benzodiazepines and alcohol did not change the parameter. There was no relationship between changes in hepatic plasma enzymes and antipyrine half-life. Mean plasma levels of hepatic enzymes did not significantly modify the course of treatment with naltrexone [17].

Plasma testosterone, FSH, and LH levels were obtained from 25 healthy consecutive heterosexual male *Cannabis* smoking university students. The values were within the normal range, and the means did not differ significantly from those of the 13 normal controls. The data suggested that the casual *Cannabis* smoker (at least once weekly with an average of five, one joint per week) has normal plasma testosterone levels for the time of day and the laboratory [18].

Cannabidiol and Sublesional Bone Loss

The therapeutic effect of cannabidiol on sublesional bone loss in a rat model for spinal cord injury (SCI) was investigated. Adult male rats were exposed to surgical spinal cord transection and treated with CBD for 14 consecutive days. It was observed that CBD treatment elevated the serum levels of osteocalcin, reduced the serum levels of collagen type I cross-linked C-telopeptide, and enhanced the bone mineral density of tibiae and femurs. The treatment of SCI rats with CBD enhanced bone volume, trabecular thickness, and trabecular number reduced trabecular separation in proximal tibiae and increased ultimate compressive load, stiffness, and energy to the max force of femoral diaphysis. The treatment of the SCI rats with CBD upregulated mRNA expression of alkaline phosphatase and osteoprotegerin and downregulated mRNA expression of receptor activator of NF- κ B ligand and tartrate-resistant acid phosphatase in femurs. The treatment also enhanced the mRNA expression of *wnt3a*, *Lrp5*, and *ctnnb1* in femurs [11].

Osteoarthritis is a degenerative joint disease that results from the breakdown of joint cartilage and underlying bone. Due to the antioxidants and anti-inflammatory action, the phytochemical constituents of many vegetable varieties could represent a new frontier for the treatment of patients with osteoarthritis. A human pilot study examined the effects of pasta enriched with hemp seed flour on osteoarticular pain and bone formation markers in patients in post-arthroplasty rehabilitation. The effect of hemp seed extract on bone metabolism in vitro was also investigated. A clinical study was conducted to verify the feasibility of pain symptom reduction in patients with osteoarthritis undergoing arthroplasty surgery and the effect of hemp seed extract on the Wnt/ β -catenin and ERK1/2 pathways, alkaline phosphatase, RANKL, RUNX-2, osteocalcin, and COL1A on Saos-2.

After 6 weeks of the consumption of hemp seed pasta, there was greater pain relief compared to the regular pasta control group (-2.9 ± 1.3 cm vs. -1.3 ± 1.3 cm; $p = 0.02$). There was a significant reduction in serum BALP observed in the participants consuming the hemp seed pasta compared to the control group (-2.8 ± 3.2 μ g/L vs. 1.1 ± 4.3 μ g/L; $p = 0.04$). In the Saos-2 cell line, hemp seed extract also upregulated Wnt/ β -catenin and Erk1/2 pathways ($p = 0.02$ and $p = 0.03$) and osteoblast differentiation markers (e.g., ALP, OC, RUNX2, and COL1A) and downregulated RANKL ($p = 0.02$), compared to the control. The study demonstrated that hemp seed could improve pain symptoms in patients with osteoarthritis undergoing arthroplasty surgery and improve bone metabolism in humans and in vitro [12].

Delta-9-THC and the Anticancer Drug Irinotecan

The use of *Cannabis sativa* and the interactions of the main constituent, delta-9-tetrahydrocannabinol, with conventional drugs, especially cytostatics, were studied. The concomitant intake of delta-9-THC on liver function in male Wistar rats treated

with the anticancer drug irinotecan (IRI) and the toxic effects associated with this exposure were evaluated. IRI was administered once intraperitoneally (at 100 mg/kg of the body weight (b.w.)), and delta-9-THC was administered per os repeatedly for 1, 3, and 7 days (at 7 mg/kg b.w.).

Functional liver impairments were studied using biochemical markers of liver function (aspartate aminotransferase [AST], alanine aminotransferase [ALP], alkaline phosphatase [AP], and bilirubin) in rats given a combined treatment, single IRI, single delta-9-THC, and control groups. Using common oxidative stress biomarkers, along with the measurement of primary DNA damage in hepatocytes, the degree of impairments caused at the cellular level was also evaluated.

Delta-9-THC produced a time-dependent enhancement of acute toxicity in IRI-treated rats, which was confirmed by body and liver weight reduction. Although single delta-9-THC affected ALP and AP levels more than single IRI, the liver function markers measured after the administration of a combined treatment mostly did not significantly differ from the control. Combined exposure increased oxidative stress responses in 3- and 7-day treatments, compared to single IRI. Single IRI produced the most serious DNA damage at all time points. Continuous 7-day oral exposure to delta-9-THC caused an increased mean value of comet tail length compared to its shorter treatments. Concomitant intake of delta-9-THC slightly affected the levels of IRI genotoxicity at all time points but not consistently [13].

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Index

A

- Acne vulgaris, 587–589
- Acute effects, 78, 125, 155, 186, 201, 204, 214, 220, 238, 393
- Acute pain, 266, 275–276, 280, 282
- Addictive morbidity, 207–210
- Administration, 13, 14, 66, 67, 70, 73, 75, 102, 113, 114, 116, 119, 121, 125, 130–132, 134, 135, 140, 142–144, 146–149, 151, 152, 156, 162–165, 167, 172, 187–189, 192, 194–196, 199, 200, 202, 204, 205, 209, 210, 214, 218, 219, 223, 228, 234, 236, 251–255, 257, 259, 260, 262–264, 267, 268, 270, 280, 284, 285, 287, 288, 290, 291, 293, 313, 317, 333, 345–346, 356, 365, 366, 369, 372, 374, 378–380, 392, 403, 411–413, 415, 417, 432, 446, 451, 454, 455, 457–459, 461, 463, 466, 468–470, 472, 478, 484, 486, 496, 524, 526, 527, 530, 538, 539, 541–543, 555, 582, 588, 593, 595–597, 605, 610, 611, 615, 618, 634–636, 641, 644, 646, 647, 649
- Adolescent group, 228–235
- Adult electronic cigarette, 327–328
- Adverse effects, 24, 85, 140, 186, 224, 225, 236, 252, 280, 282, 284, 289, 307, 310, 340, 350, 363, 373, 375, 378–380, 390, 391, 393, 400, 515, 524, 526, 531, 532, 554, 570, 576, 589, 592, 598, 635, 646
- Affective processing, 104–106, 165
- Alcohol, 77, 102, 197, 295, 303, 385, 435, 530, 642
- Alcohol exposure, 156, 311, 345
- α 2A-adrenoceptor, 135–136
- Alkaline phosphatase stimulation, 641–649
- Alkaloids, 11, 23–25, 552, 556
- Allergic contact dermatitis (ACD), 570, 602–606
- Alopecia, 590–591
- Alzheimer's diseases (AD), 79, 109, 132, 154–155, 259, 295, 372, 515, 519, 522, 525, 526, 528, 535, 541–544, 629
- Amphetamines, 103, 295, 342, 541, 546, 555–558
- Amyotrophic lateral sclerosis, 294–295, 515, 522, 528
- Analgesia, 193, 194, 249–256, 258–264, 266–280, 282–295, 428, 432, 446, 541, 616, 617, 619, 621
- Angiogenesis, 427, 431, 434, 447, 454–456, 462, 468, 480, 482, 483, 491, 492, 582, 603
- Anti-allergy, 14, 295, 606
- Anticancer drug, 440, 453, 648–649
- Anti-inflammatory, 12–14, 27–29, 61, 63, 65, 73, 74, 80, 82, 83, 102, 130, 134, 148, 195, 197, 233, 266, 275, 286, 289, 294, 318, 366, 368, 380, 395, 406, 410, 413, 415–416, 448, 477, 481, 488, 519, 521, 522, 527, 529, 530, 536, 570–572, 582, 587, 588, 590, 591, 595, 598, 599, 602–604, 613–616, 619, 620, 630, 635, 648
- Antinociceptive effects, 254, 255, 261–263, 266, 268, 269, 272–273, 282, 553
- Antioxidation, 14

- Antipsychotic, 61, 63, 147, 148, 186,
190–196, 198, 202–206, 212, 214, 215,
217–219, 221, 222, 238, 240, 241, 287,
403, 527, 530, 538, 539
- Antiviral activities, 12, 13, 15–21,
26–28, 629–637
- Anxiety, 61–63, 75, 77–79, 103, 114, 115,
127, 130, 140, 154, 163, 165, 166, 168,
171, 185–188, 192, 195, 200, 205–209,
211–214, 216, 220, 226, 230, 234, 235,
238, 251, 252, 260, 269, 270, 273, 287,
289, 304, 305, 330, 332, 337, 338, 347,
375, 382, 384, 385, 387, 393, 396, 402,
403, 405, 513, 521, 523, 525–527, 530,
546, 549
- Apigenin, 12–14, 39, 83
- Apoptosis, 84, 162, 317, 356, 369, 412,
427–429, 431, 433–437, 439–444,
446–451, 453–462, 464–470, 473–477,
480–486, 490–494, 496–499, 501, 502,
520, 533, 537, 538, 543, 544, 552, 558,
560, 569, 578–581, 584, 587, 590, 591,
606, 610, 611, 613, 633
- Astrocytes, 135, 194, 233, 409, 465, 466,
529, 630
- Atopic dermatitis, 592, 597–602
- Attention memory, 162, 186, 329
- Audio/visual/verbal memories, 112, 125–128,
144, 150, 158, 163, 168, 212, 223, 226,
383, 387, 389, 390, 393
- Auditory, 197–198, 201, 213, 214, 293, 345
- Auditory response, 293
- Autophagy, 427, 443, 444, 453–455, 458, 459,
462, 463, 465, 467, 469, 483–486,
490–494, 502, 534, 554, 610
- B**
- Baicalein, 12, 14
- Behavior, 105, 188, 249, 303, 366, 429,
515, 593
- Behavioral disturbances, 224–225, 308
- β-Carotene (BC), 642–643
- Bioactive components, 11–29, 59–85
- Biosynthetic pathways, 12, 63–64, 81,
569
- Bipolar disorder, 115, 145, 150–153, 206–207,
219, 230, 372, 528
- Body mass index (BMI), 68–70, 72, 76, 232,
239, 307, 309, 327, 352, 406, 407,
473, 632
- Bone cancer, 428, 495–496
- Botanical description, 1–5
- The brain, 61, 63, 64, 67–69, 74, 77, 102–106,
112, 120, 126, 134, 137, 140, 143, 146,
147, 150, 153, 158, 161, 164–167,
185–189, 191, 196, 206, 207, 210, 215,
222, 225, 231, 237, 249, 251, 253, 254,
267, 268, 272, 275, 276, 282, 285, 286,
291, 308, 312, 326, 330, 332, 333, 355,
381, 383, 386, 394, 395, 428, 432, 445,
446, 513, 515, 519–522, 530, 534, 537,
539, 540, 544, 545, 547, 549, 551, 554,
557, 559, 645
- Breast cancer, 14, 427, 431, 433, 434, 437,
438, 443, 444, 446, 447, 463, 479,
488–494, 502, 503, 579, 586
- Breastfeeding, 138–140, 225, 305, 309, 318,
339, 343, 348
- C**
- Cancer, 14, 24, 62, 63, 83, 84, 238, 251, 256,
259, 264–266, 272, 304, 305, 365, 369,
372, 402, 427–503, 519, 541, 570, 571,
578, 579, 610, 611, 629, 632
- Cannabichromene (CBC), 5, 41, 61, 102, 250,
436, 438, 441, 442, 519, 602
- Cannabidiol, 131, 185, 250, 305, 363, 434,
439, 519, 571, 641
- Cannabidiolic acid, 41, 59, 63, 367, 463, 494,
519, 543
- Cannabigerol (CBG), 29, 41, 102, 250,
365–369, 435, 436, 441, 442, 482, 519,
571, 572, 589, 602, 634
- Cannabinaceae, 1–9
- Cannabinoid hyperemesis, 290–291, 343–344
- Cannabinoid receptors, 60, 61, 63–65, 78, 107,
116, 126, 132, 133, 135, 137, 146, 149,
158, 159, 166, 185, 188, 191–193,
198–201, 216, 228, 229, 231, 233, 237,
240, 250, 251, 253, 254, 256, 258–261,
266, 267, 273–275, 278, 288, 289, 291,
292, 295, 308, 309, 313, 316, 355, 364,
365, 368, 369, 375, 378, 379, 381, 395,
396, 399, 406, 408, 409, 411, 415,
428–434, 439, 440, 442, 445–447,
455–457, 459, 461, 463–466, 470–472,
476, 479, 480, 483, 485–490, 495–496,
498, 502, 517–523, 525, 529, 537, 540,
545, 551, 569–571, 574, 582, 583, 588,
592, 593, 595–600, 602–608, 614,
615, 618
- Cannabinolic acid, 42, 59
- Cannabis* and schizophrenia, 112,
145–150, 153

- Cannabis* and tobacco use, 324, 326, 336, 351, 353, 499
- Cannabis* exposure, 225, 229, 281, 305–317, 393, 407, 413, 429, 534, 549
- Cannabis* in Adolescence, 140–145
- Cannabis indica*, 1, 6
- Cannabis* laws, 356, 371
- Cannabitol, 14, 43
- Capsaicin, 271, 275, 276, 281, 290–291
- Capsaicin-induced pain, 275, 281
- Cardiac effects, 241
- Carotid artery occlusion, 131–132
- Catalepsy, 15, 210, 239, 254, 283, 285–288, 538, 539
- Catechin, 17, 18, 24, 25
- Catechol-O-methyl transferase (COMT), 136–137, 187, 190, 231, 234, 530, 545–548
- Cell-cycle progression, 452–453, 478, 492
- Cervical cancer, 427, 453, 474, 487–489
- Chemical constituents, 39–52
- Child behavior problems, 332
- Child development, 320–322, 329–331
- Child maltreatment, 334–335
- Children's dental health, 350
- Chronic effects, 103, 132, 187, 190, 393–394
- Chronic pain, 6, 59, 80, 167, 185, 213, 250, 252, 255, 256, 258, 265, 266, 268, 275, 280, 295, 365, 373, 402, 404–406, 520, 617
- Chronic SNS, 76–77
- Circadian rhythm, 70–71
- Cocaine, 11, 78, 103, 167, 207–209, 215, 226, 249, 295, 296, 319, 329–330, 332, 335, 338–339, 342, 344–345, 350, 354, 539, 546, 554–555
- Cognitive endpoints, 328
- Cognitive functions, 73, 79–80, 101, 104, 112, 113, 115, 124, 128, 132, 142, 143, 149, 151–154, 160, 166, 170, 189, 201, 212–214, 228, 229, 232, 321, 326, 356, 375, 384, 385, 393, 397–398, 546, 645
- Cognitive impairments, 85, 102, 108, 112–115, 127, 137, 139, 141, 143, 147–149, 151, 152, 158, 161, 192, 198, 210–211, 220, 221, 262, 283, 305, 338, 372, 382–386, 390, 391, 394, 395, 403, 515, 538
- Cognitive performance, 103, 107, 115, 121, 122, 124, 127, 137, 142, 143, 147, 150, 152, 158, 160, 161, 168, 198–201, 226, 252, 255, 312, 384, 388, 389, 391
- Colorectal cancer (CRC), 427, 434–438, 452, 457, 479–483, 578, 642
- Complementary therapies, 549–550
- Components of plants, 11–29
- Coronavirus, 16, 22, 629, 634–637
- Creatine kinase, 240–241
- Cutaneous lupus erythematosus, 614–616
- Cutaneous melanoma, 483, 610–612
- Cyclooxygenase-2, 64, 137–138, 463, 494, 595
- Cytochrome P450, 22, 414–415, 576, 641
- Cytotoxicity, 20, 27, 84, 318, 441, 443, 456–457, 460, 461, 488, 493, 539, 542, 553, 571, 575, 576, 612, 620
- D**
- Delta-8-tetrahydrocannabinol (delta-8-THC), 166, 268, 479, 481
- Delta-9-tetrahydrocannabinol (delta-9-THC), 5, 15, 60, 102, 185, 250, 304, 363, 428, 519, 571, 633, 648–649
- Delta-9-tetrahydrocannabinolic acid, 5, 13, 59, 63, 367, 519
- Delta-9-tetrahydrocannabivarin, 5, 165–166, 519, 635
- Demographics, 120, 139, 146, 153, 193, 197, 225, 312, 324, 326, 343, 346–349, 371, 374, 385–387, 390, 398, 399, 500, 584, 644, 646
- Dependence, 67, 78, 108, 112, 133, 138, 160, 207–209, 224–225, 228, 264, 269, 289, 295, 324, 325, 328, 334, 349, 351, 353, 366, 370, 372, 378, 392, 393, 395, 402–404, 443, 491, 520, 528, 643
- Depression, 62, 77–79, 111, 113, 115, 116, 135, 153, 163, 186, 187, 205–206, 216, 230, 249, 251, 266–268, 273, 295, 304, 305, 314, 322, 327, 332, 337, 338, 369, 372, 373, 375, 382, 385, 387, 393, 396, 402–405, 430, 513, 515, 525, 528, 535, 540, 541, 546, 549, 553, 592
- Dermatomyositis, 570, 613–614
- Dill seeds, 643
- Distribution, 5–9, 22, 23, 64, 68, 69, 82, 115, 240, 265, 275, 282, 285, 308, 386, 405, 417, 445, 499, 501, 535, 547, 548, 600, 618
- Dopaminergic gene polymorphism, 545–549
- Driving, 128, 134, 154, 210, 211, 286, 293, 335, 480, 548
- Drug addiction, 116, 167, 171, 219, 393, 530, 556

Drug-seeking behavior, 132–133, 313
 Dysregulation, 109, 133, 171, 199, 220, 230,
 231, 233, 314, 327, 328, 344, 394, 441,
 443, 615, 619

E

Electroencephalography (EEG), 76, 112, 129,
 146, 164, 169–170, 198
 Electrophysiological effects, 149, 228, 241
 Emotional endpoints, 328
 Emotional impairments, 131–132
 Encephalograms, 349–350
 Endocannabinoids, 60, 104, 185, 250, 303,
 364, 428, 517, 576
 Endocannabinoids in the circulation, 65–67,
 72, 76, 81
 The endocannabinoid system, 60–62, 66, 78,
 109, 116, 140, 142, 152, 185, 187,
 190–193, 196–197, 199, 215, 226, 231,
 250, 273, 276–280, 285, 291–293, 303,
 308, 316, 321, 326, 364–365, 369, 411,
 414, 428, 429, 432, 440, 445, 476, 487,
 493, 495, 517–521, 525, 526, 528, 529,
 536, 569–571, 585, 590, 597, 598, 602,
 615, 616, 618, 621, 631, 632
 Endometrial cancer, 487
 The entourage effect of the
 phytochemicals, 62–63
 Epidermolysis bullosa, 277, 570, 616–619
 Executive function, 199, 214, 314, 329, 331,
 344, 383–385, 393, 400
 Executive functioning, 79, 115, 123, 124, 150,
 154, 168, 331, 382, 383, 386–392, 394
 Exercise, 60, 64, 71–73, 78, 81, 373, 550
 Explicit psychotic disorder, 212
 Eye disorders, 338–339

F

FAAH inhibitors, 79, 171, 172, 233, 291–292,
 529, 596
 Female reproduction, 278–280
 Fetal birth weight, 352
 Fetal brain development, 310, 329, 344, 350
 First-episode psychosis, 125–126, 150, 192,
 193, 197, 203–205, 221, 222, 230–232
 Fisetin, 17
 Flavonoids, 12–19, 21, 22, 25, 29, 62, 83, 185,
 277, 621
 Flower, 1–6, 13, 15, 59, 82, 162–163, 280, 617
 Functional abnormalities, 303, 309, 394–395
 Functional MRI, 106–108, 112, 137, 170, 202,
 236, 383, 387

G

Gender differences, 127, 147,
 167–169, 272–273
 Genetic involvement, 312–317
 Genetics, 2, 85, 108, 138, 153, 156, 160, 188,
 189, 200, 206, 211, 217, 225, 231, 236,
 250, 277, 278, 285, 303, 306, 311,
 316–317, 321, 355–356, 411, 427, 429,
 437, 453–455, 464, 468, 477, 485, 486,
 513, 514, 516, 530, 531, 546–549, 553,
 555, 559, 572, 582, 595, 596, 607,
 614, 615
 Genistein, 19
 Genome, 2, 12, 16, 18, 28, 429, 601
 Glioblastoma, 438, 447, 455, 464–470,
 498, 579
 Glioma, 427, 431, 433, 434, 439, 446, 447,
 454, 455, 461, 464–470, 485, 537
 Glucose uptake, 67, 72, 397–398
 Glucosinolates, 21, 22
 Glutamate N-methyl-D-aspartate, 135, 191,
 193, 259, 292–293, 541
 Glycine receptors, 271
 Glycosides, 12, 13, 17, 19, 21–23, 27, 28, 39
 Gut health, 643

H

Hair growth disorders, 590–591
 Health effects, 12, 83, 304–305, 372, 528
 Heart rate, 71, 136, 158, 164, 165, 169–170,
 252, 527, 635
 Hepatitis virus, 19, 631–633
 Hepatocellular carcinoma (HCC), 477,
 484–486, 629
 Herpes simplex virus (HSV), 12, 15, 18, 22,
 29, 629, 633, 634
 Heterosynaptic eCB-mediated
 potentiation, 109–111
 History of *Cannabis*, 1–9, 531
 HIV, 16, 18, 19, 24, 26, 29, 169, 252, 258,
 496, 629, 632–634
 Hodgkin's lymphoma (HL), 475
 Homosynaptic eCB-mediated potentiation,
 108–111, 154, 155
 HPA axis activation, 75–77
 Human immunodeficiency virus, 24,
 629, 632–634
 Hyperalgesia, 258, 269, 275–276, 281, 537
 Hyperemesis syndrome, 343–344
 Hypothalamic-pituitary-adrenal (HPA) axis,
 70, 74–77, 197, 230, 231, 289, 333, 336
 Hypothermia, 15, 253, 254, 258, 259, 269,
 283, 285–288, 293

I

Immune function, 256, 304, 317, 335–338, 407, 520, 633
 Immune response, 73, 327, 369, 406, 408, 409, 412, 414, 417, 463, 478, 479, 488, 522, 615, 616, 630, 631, 634
 The immune system, 60, 61, 295, 317–318, 412, 432, 445, 465, 519, 537, 571, 579, 608, 609, 629, 631
 Immunomodulation, 428
 Immunotherapy, 502
 Implicit psychotic disorder, 212
 Infant neurobehavioral development, 330–332
 Inflammation, 13, 16, 22, 61, 63, 73–74, 197, 240, 250, 251, 275, 278, 286, 289, 309, 368, 397, 398, 403, 405, 411–413, 415, 427, 428, 521, 569, 570, 582, 584, 587, 588, 591, 595, 597–600, 603–605, 614, 615, 619, 620, 629, 630, 634–637
 Influenza virus, 14, 17, 19, 629, 634
 Innovative therapies, 559–560
 Integumentary wounds, 276–277, 620, 621
In utero exposure, 329–330
 Irinotecan, 431, 486, 648–649

K

Kaempferol, 12, 16, 17, 47, 83
 Kaposi's sarcoma, 578–585, 633
 Keratinocyte proliferation, 570–575, 583, 586, 590

L

Lactation, 138, 305, 342–343
 Late pregnancy, 76, 341
 Leukemia, 440, 496–499, 579, 634
 Lignans, 25–27, 83, 84
 Liver tests (LT), 646–647
 Locomotion, 210, 232, 285–288, 293, 538, 542, 553
 Long-term potentiation (LTP) in learning, 108–109
 Lung cancer, 427, 441, 449, 455, 470–472, 488, 499–502, 579
 Luteolin, 12, 14, 15, 83

M

MAGL inhibitor, 292
 Maternal age, 318–319, 339
 Maternal choline, 350–351
 Maternal use, 345, 352
 Medical use, 24, 101, 154, 195, 196, 256, 295, 371, 375

Melanocytes, 483, 569, 575–578
 Melanoma, 431, 434, 436, 459, 481, 483–484, 500, 502, 571, 575, 576, 578, 610–612
 Memory, 61–63, 79, 82, 85, 102, 103, 107–110, 112–116, 118, 119, 121–129, 131, 132, 134, 136, 138–145, 147–158, 160–163, 165–170, 172, 186, 194, 195, 197, 200, 201, 206, 209, 210, 212–214, 223, 225, 226, 228, 229, 233–236, 260–262, 295, 312, 313, 321, 325, 326, 329, 330, 344, 356, 366, 383, 384, 386–393, 400, 520, 523, 524, 531, 538, 544, 545, 560
 Mental health, 143, 153, 155, 158, 186, 187, 189–190, 197, 227, 303–306, 315, 316, 372, 528, 545
 Metabolic dysregulation, 231, 239–240
 Methylsulfonylmethane, 641
 Microglia function, 134–135
 Migraine pain, 289–290
 Mismatch negativity (MMN), 129, 197–198
 Morphine, 11, 249, 253, 254, 262, 264, 266–269, 289, 295, 552–554, 616, 617
 Morphological differences, 2, 120, 146
 Motor impairment, 210, 293, 529, 538
 Motor skills, 128, 393
 Multiple sclerosis (MS), 6, 59, 61–63, 113, 256, 274, 294, 363–417, 430, 519, 522, 528, 531, 533, 537, 630
 Myeloma, 477, 478
 Myrcene, 48, 251, 270–271, 457
 Myricetin, 17

N

Naringenin, 14, 19
 Nausea, 59, 62, 63, 75, 154, 185, 186, 256, 269, 290, 304, 320, 337, 344, 347, 372, 373, 394, 403, 431, 444, 475, 496, 522, 524, 630, 632, 633
 Neonatal, 305, 307, 312, 315, 319, 320, 323, 330, 337, 339–342, 347, 379, 544, 545, 559, 560
 Neurobiological alterations, 138–140
 Neuroblastoma, 441, 464–470
 Neurodevelopmental outcomes, 305–312, 337, 350
 Neurological effects, 24, 101–172
 Neuropathic pain (NP), 59, 255–261, 266, 267, 270, 272, 275, 282, 286, 290, 294, 363, 369–381, 404–406, 519, 520, 533, 560, 618
 Neuropsychological functioning, 125–126, 149, 150, 168

O

Offspring use, 327, 349
 Olfactory neuroepithelium cells, 126–127
 Opiates, 269, 295, 546, 552–554, 595
 Opioids, 81, 189, 204, 256–259, 263–269,
 277, 279, 281, 285, 291, 303, 313, 316,
 325, 332, 335, 338–339, 342, 348–351,
 353, 399, 403, 417, 435, 475, 517, 541,
 552–554, 595, 617–619
 Oral cancer, 479
 Origins, 5–9, 107, 266, 275, 412, 442, 448,
 470, 493, 591, 592, 597
 Osteoarthritis, 79, 80, 273, 274, 648
 Osteoblastic differentiation, 645

P

Pain, 61, 103, 185, 250, 304, 363, 430, 513,
 570, 648
 Pain management, 251, 264–266, 277, 280,
 281, 283, 403–406
 Pancreatic cancer, 431, 432, 444, 446, 454,
 457–464, 488
 Parkinson's disease, 294, 295, 365, 513–560
 Parkinson's patients, 238–239, 517, 525,
 528, 533
 Paternal use, 310, 311, 321, 336, 352
 Pathological injury, 261
 Pathophysiology of psychosis, 190–196
 Persistent pain, 261, 282
 Phenols, 22, 25, 49, 83–84
 Physical health, 157, 158, 643–645
 Physical impact, 398–404
 Physiological outcomes, 305–312
 Plectasin, 20, 21
 Post-operative pain, 281–285
 Postpartum, 307–309, 318, 319, 324, 325, 340,
 342, 346–349, 351, 353, 356, 475
 Postpartum exposure, 335–338
 Post-traumatic stress disorder, 535
 Pregnancy, 138–140, 225–227, 252, 278,
 303–312, 316, 318–326, 328–330,
 332–342, 345–354, 356, 379, 404, 407,
 475, 487, 558
 Pregnancy risk assessment, 319–322, 339,
 346, 348
 Prenatal, 138, 188, 303
 Prenatal *Cannabis* exposure, 226, 331
 Prenatal choline, 329
 Prenatal drug use, 354
 Prenatal exposure, 305, 311, 312, 319,
 321–323, 325–328, 331–334, 336, 338,
 344, 345, 349–350, 355

Prenatal exposure to delta-9-THC, 305, 311,
 325–326, 338
 Prenatal tobacco exposures, 331, 332
 Pre-operative pain, 251, 268, 281–285
 Processing speed, 103, 115, 124, 126, 153,
 373, 374, 382–393, 399, 400, 416
 Prostate cancer, 427, 431, 434, 437, 440, 447,
 475–477, 479, 490, 537
 Proteins and peptides, 20–21
 Pruritus, 277, 569, 570, 591–599,
 602, 616–618
 Psoriasis, 570, 571, 582–587, 601
 Psychiatric morbidity, 207–210
 Psychological impact, 398–404
 Psychosis, 80, 85, 112, 126, 145–148, 150,
 153, 187–193, 197, 199, 201–203,
 205–207, 209, 211, 213–225, 227–237,
 239, 240, 292, 305, 309, 313, 314,
 338, 403, 523, 527, 530, 534, 542,
 546, 551
 Psychosocial stress, 230, 237
 Psychotropic side effects, 289–290, 524

Q

Quercetin, 12, 15, 16, 19, 50, 83

R

Radiation therapy, 503
 Recreational use, 101, 157, 200, 217, 286,
 326, 338, 400
 Relapse/reinstatement, 132–133
 Residual impact, 112, 170–171
 Resins, 2–4, 7–9, 13, 23, 27–28, 40, 41,
 43, 51, 595
 Reward processing, 104–106, 144, 197, 311
 Rheumatologic pain, 274–275
 Risk factors, 113, 114, 133, 138, 148, 161,
 185, 190, 193, 206, 216, 217, 221, 235,
 239, 309, 312, 333–334, 349, 416–417,
 429, 528, 540–542, 555, 575
 Rutin, 17

S

Saponins, 21, 28–29
 Schizophrenia, 61, 63, 80, 112, 113, 115, 120,
 129, 132, 145–150, 153, 188, 190–203,
 205, 209, 212, 214–225, 227–231, 234,
 236, 238, 287, 292, 293, 295, 315, 329,
 330, 355, 372, 528, 530, 534, 535,
 538, 542

- Schizophrenia-like psychoses, 200,
219, 236–237
- Schizotypal personality disorder, 211–212
- Seborrhea, 587–589
- Serotonin 5-hydroxytryptamine receptor 1A
(5-HT_{1A}R), 202, 240, 270, 600
- Serum cytokines, 395
- The skin, 22, 569, 570, 572, 575, 583–585,
600, 601, 605–607, 609, 619, 644
- Skin aging, 619–621
- Social endpoints, 328
- Somatometric alterations, 333–334
- Spasticity, 59, 62, 63, 294, 304, 363–365,
369–383, 396–404, 412, 528, 533, 630
- Spatial working memory, 114, 117, 121–123,
130, 135, 136, 160, 234, 343
- Spermatogenesis, 429, 430, 646
- Stress, 60, 109, 197, 288, 305, 366, 427, 515,
575, 649
- Stressful life, 232, 346
- Stress-induced ulcerogenesis, 288
- Structural abnormalities, 309, 394–395
- Sublesional bone loss, 648
- Synthetic cannabinoids, 85, 101, 113, 132,
189, 204, 236–238, 253, 256, 273, 276,
282, 283, 288, 290, 293, 310, 313, 314,
321, 328, 356, 370, 372, 408, 409, 431,
433, 443, 445, 446, 462, 465, 466, 476,
481, 491, 497, 502, 551–552, 582, 606,
611, 616
- Systemic sclerosis, 606–610, 614
- T**
- Tactile responses, 293
- Takotsubo cardiomyopathy (rTC), 295–296
- Terpenes, 13, 27, 28, 62, 81–84, 101, 185,
258, 259, 262–263, 270, 277, 456, 457,
621, 635
- The tetrad effects, 285–288
- Therapeutic perspectives, 171–172
- Therapeutic potential, 20, 206, 249, 259,
270–271, 274, 279, 282, 291, 321, 363,
365, 381, 414, 415, 428, 435, 438, 495,
525, 530, 541, 560, 569, 591, 632
- Tobacco, 8, 84, 113, 145, 157–158, 232,
237, 307, 311, 315, 320–332, 334–339,
341, 344–345, 349, 351–354, 356,
407, 435, 473, 499–501, 547, 575,
642
- Transient receptor potential vanilloid type 1
(TRPV1), 64, 80, 110, 172, 206,
270–271, 368, 369, 411, 412, 428, 435,
436, 442, 482, 489, 490, 492, 498, 517,
518, 529, 530, 533, 534, 573, 574, 588,
589, 600, 602, 604, 616
- Transport-related injuries, 335
- Traumatic brain injury (TBI), 130–131,
215, 519
- Tremors, 363, 372, 379, 381–382, 399, 400,
402, 513, 514, 522, 523, 525–528, 530,
533, 535, 536, 543, 549, 554, 559
- Trichomes, 59, 82, 84–85, 367
- U**
- Urinary functions, 396–397
- Urogenital cancer, 473–475
- V**
- Varieties, 1, 2, 4–6, 23, 59, 60, 84, 101, 287,
305, 429, 436, 437, 498, 523, 579,
586, 648
- Visual response, 293
- Vomiting during pregnancy, 320
- Vulnerable group, 228
- W**
- Withdrawal syndrome, 252, 329, 342, 393,
394, 475
- Working memory, 103, 112, 115–122,
125–127, 130–133, 135–144,
146–151, 153, 155, 157–161, 164,
168, 169, 186, 198, 201, 213, 214,
312, 313, 343, 345, 382, 385–387,
390, 393, 399
- Wound healing, 27, 277, 570, 583, 616,
617, 619–621