MESTRADO INTEGRADO EM MEDICINA

The impact of natural cannabinoids on neuroinflammation

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M

2021

The impact of natural cannabinoids on neuroinflammation

Dissertação de candidatura ao grau de Mestre em Medicina, submetida ao Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

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Agradecimentos | Acknowledgements

Esta dissertação representa um culminar de 6 anos de trabalho, percurso este que não teria sido possível sem a presença de várias pessoas importantes, as quais passo a agradecer:

Quero começar por agradecer à minha orientadora, Doutora Teresa Summavielle, por todo o apoio na concretização deste projeto, sempre marcado por um elevado rigor científico. Agradeço também a sua grande disponibilidade.

Gostaria também de agradecer à Joana Bravo pelo seu contributo neste trabalho. Espero que o teu percurso reflita o teu valor e a paixão que apresentas por este ramo.

À minha mãe, obrigada por tudo. Sabes que estes agradecimentos não são o meu ponto forte, mas também sabes tudo o que significas para mim. És a melhor mãe do mundo e ainda vamos ter muitas aventuras juntas. Eu e tu somos uma só.

Quero também agradecer ao Quim por todos estes anos. Por todas as tardes de estudo e tostas-mistas.

Estes seis anos não teriam definitivamente deixado tantas memórias se não fosse o nosso grupinho, Ana, Filipa, Maria e Susana. Vocês tornaram o estudo tolerável com todas as conversas e memes.

Quero ainda agradecer a toda a minha família, em particular aos meus avós. Obrigada por acreditarem em mim e espero ter-vos orgulhado.

Resumo

Introdução: A canábis persiste como substância ilícita mais consumida, com mais de 190 milhões de utilizadores a nível mundial. No entanto, o uso médico de extractos de canábis ou de diferentes formulações de canabinóides tem vindo a ser aprovado em vários países. A neuroinflamação consiste numa resposta inflamatória no sistema nervoso central que aparenta mediar várias das patologias para as quais os tratamentos baseados em canabinóides foram aprovados ou mostraram eficácia. A legislação relativa à legalização do uso médico e recreativo da canábis tem sido objecto de constante debate; no entanto, o enorme número de publicações obre os efeitos desta substância apresenta resultados inconsistentes.

Objetivos: Sistematizar a informação disponível acerca das consequências do efeito de diferentes canabinóides na inflamação no cérebro e medula espinal.

Metodologia: Recorrendo a uma análise estruturada da literatura, e abrangendo o período entre 2010 e 2020, foi executada na base de dados PubMed® uma pesquisa, com o uso de operadores booleanos e palavras-chave específicas. Um total de sessenta e três artigos, avaliando os efeitos de diferentes canabinóides no SNC, foi incluído nesta revisão.

Desenvolvimento: Esta revisão revelou uma função consistentemente anti-inflamatória para o canabidiol (CBD) e para combinações de CBD e tetrahidrocannabinol (THC). O uso de THC, pelo contrário, revelou efeitos marcadamente pró-inflamatórios.

Conclusão: Esta revisão vem clarificar a robustez e deficiências da literatura existente, permitindo uma melhor avaliação do potencial terapêutico de diferentes canabinóides.

Palavras-chave: Canábis, Canabidiol, Sistema Nervoso Central, Δ(9)-Tetrahidrocanabinol, Neuroinflamação.

Abstract

Introduction: Cannabis remains the most used psychoactive illicit substance, with over 190 million people consuming it worldwide. However, recently, cannabis extracts and/or specific cannabinoids were approved in several countries for medical use. Neuroinflammation consists of an inflammatory response within the CNS that appears to mediate several of the conditions for which cannabinoid-based treatments have been approved or demonstrated efficacy. Policies towards cannabis legalization are under debate all over the world; however, the enormous number of publications addressing the effects of different cannabinoids remains largely inconsistent. The present work, aimed at addressing this issue, focused specifically on neuroinflammation related effects.

Objectives: To systemize the available data on the consequences of cannabinoid treatment in inflammation in the brain and spinal cord.

Methods: A keyword literature search of articles from 2010 to 2020 was performed in the PubMed® database, using Boolean operators. A total of sixty three articles, assessing the effects of different cannabinoids on CNS, were included in this review.

Results: This work revealed a consistent anti-inflammatory function for cannabidiol (CBD) and combinations of CBD and tetrahydrocannabinol (THC), but not for THC alone, which displayed mainly proinflammatory features in animal models.

Conclusion: Because cannabinoids are often foreseen as promising therapeutic approaches for several conditions, including addiction and neurodegenerative disorders, the present scoping review allows a better interpretation of the available data and clarifies its strengths and weaknesses.

Keywords: Cannabis, Cannabidiol, Central Nervous System, Δ(9)-Tetrahydrocannabinol, Neuroinflammation.

List of Abbreviations

^{1}H-MRS: Proton magnetic resonance spectroscopy
3-NP: 3-Nitropropionic acid
AD: Alzheimer's disease
AIDS: Acquired immunodeficiency syndrome
Arg-1: Arginase-1
Aβ: Amyloid-beta
BBB: Blood brain barrier
BCAO: Bilateral carotid artery occlusion
BCP: β-caryophyllene
BDL: Bile duct ligation
BDNF: Brain-derived neurotrophic factor
bFGF: Basic fibroblast growth factor
BM-MSCs: Bone marrow mesenchymal stem cells
CB1 receptor: Cannabinoid receptor 1
CB2 receptor: Cannabinoid receptor 2
CBC: Cannabichromene
CBD: Cannabidiol
CBDA: Cannabidiolic acid
CBDV: Cannabidivarin
CBE: Cannabielsoin
CBG: Cannabigerol
CBL: Cannabicyclol
CBN: Cannabinol
CBND: Cannabinodiol
CBT: Cannabitriol
CCL: Chemokine ligands
CCL2: Chemokine ligand 2
CLG: Coumaroylaminobutanol
CNS: Central nervous system
COX-2: Cyclooxygenase-2
CPu: Caudate-putamen
CRP: C-reactive protein
CSF: Cerebrospinal fluid
CSPGs: Chondroitin sulfate proteoglycans
CU: Cannabis users
CUPID: Cannabinoid Use in Progressive Inflammatory brain Disease
CXCL10: C-X-C motif chemokine ligand 10
DA: Dopamine
DAM: Disease associated microglia
DM: Diabetes mellitus
DSC: Dorsal spinal cord
EAE: Experimental autoimune encephalomyelitis
EFC: Extract of Fructus Cannabis
EMCDDA: European Monitoring Centre for Drugs and Drug Addiction
FGF: Fibroblast growth factor
GFAP: Glial fibrillary acidic protein
HA: Human astrocytes
HAPI: Highly agressively proliferating immortalized
HBMECs: Human brain microvascular endothelial cells

HI: Hypoxia-ischaemia
HIV: Human immunodeficiency vírus
HPH: Hemp protein hydrolysates
HPI: Hemp protein isolates
i.h.: Intrahippocampal
i.m.: Intramuscular
i.p.: Intraperitoneal
i.v.: Intravenous
Iba-1: Ionized calcium binding adaptor molecule **1**
ICAM-1: Intercellular adhesion molecule 1
IFN: Interferon
IL: Interleukin
IL-1R: Interleukin-1 receptor
iNOS: Inducible nitric oxide synthase
ISAJE: International Society of Addiction Journal Editors
KA: Kainic acid
LDH: Lactate dehydrogenase
LPS: Lipopolysaccharides
MCAO: Middle cerebral artery occlusion
MCAO-C: MCAO animals treated with CBD
MCAO-V: MCAO animals treated with vehicle
MDMA: 3,4-Methylenedioxymethamphetamine
METH: Methamphetamine
miRNA: microRNA
MRI: Magnetic resonance imaging
mRNA: Messenger ribonucleic acid
MS: Multiple sclerosis
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
nNOS: Neuronal nitric oxide synthase
NO: Nitric oxide
NU: Non-users (of cannabis)
OPC: Oligodendrocyte progenitor cell
PFC: Prefrontal córtex
Pnd: Postnatal day
REM: Rapid eye movement
RmTBI: Repeated mild traumatic brain injury
ROS: Reactive oxygen species
RSD: REM-sleep deprivation
S.C.: Subcutaneous
SERT: Serotonin transporter protein
SOD1: Superoxide dismutase 1
TBI: Traumatic brain injury
TGF-β: Transforming growth factor beta
THCV: Tetrahydrocannabivarin
TLR4: Toll-like receptor 4
TNF: Tumor necrosis factor
TPA: Total phenylpropionamides
VCAM-1: Vascular cell adhesion molecule 1
VEGF: Vascular endothelial growth factor
Veh: Vehicle
WT: Wild-type
Δ8-THC: (-)-delta-8-*trans*-tetrahydrocannabinol

Δ9-THC/THC: (-)-delta-9-*trans*-tetrahydrocannabinol:
Δ9-THC/THC: Delta-9-tetrahydrocannabinol
Δ9-THCA: Δ9-tetrahydrocannabinolic acid

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1. Introduction

According to the 2020 World Drug Report, cannabis remains the most used substance worldwide, with an estimated 192 million users[1]. The use of this psychoactive substance tends to start during adolescence, with a prevalence of use of 4.7% between the ages of 12 to 17[2]. Cannabis use, particularly regular and frequent use, is on the rise independently of its legitimacy for non-medical purposes[3]. In 2012, Colorado and Washington became the first two US states to approve non-medical use of marijuana for adults over 21 years of age[3]. A year later, Uruguay legalized recreational cannabis use[4]. Since then, twelve more US states and the District of Columbia have legalized the recreational use of this substance[3,5]. Canada first legalized cannabis for medical purposes in 1999 and proceeded to establish a nationwide permission for recreational use of this substance by 2018[3]. According to several reports, In the US state jurisdictions where non-medical adult use of cannabis has been approved, there has been seen an increase in the prevalence and frequency of use[3,6,7]; in Colorado, for example, marijuana use increased in 2017 despite being stable from 2014 to 2016[8]. The opposite trend was observed in Canada, where cannabis use increased after legalization but later stabilized[3]. It is interesting to note, however, that the legalization of cannabis use had no effect on traffic fatalities, one of the public health concerns related to drug use, despite an initial rise in fatalities[9,10].

The composition of the plant Cannabis sativa includes more than 100 different cannabinoids, also named phytocannabinoids, and other components, such as terpenes[11]. Phytocannabinoids can be classified into 11 different types, namely: (-)-delta-9-*trans*-tetrahydrocannabinol (Δ9-THC), (-)-delta-8-*trans*-tetrahydrocannabinol (Δ8-THC), cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), cannabitriol (CBT), and miscellaneous-type cannabinoids[12]. Delta-9-tetrahydrocannabinol (Δ^9-THC) is the main psychoactive substance of cannabis and its concentration in plants for recreational use has been increasing[11,13]. CBD is the major non-psychotropic cannabinoid present in Cannabis *sativa*[12]. Interestingly, CBD has been proposed as to counteract some of THC's effects, including tachycardia, intoxication, and sedation[14]. Terpenes are aromatic compounds present not only in the cannabis plant, being responsible for its characteristic smell, but also in other non-cannabinoid plants[15]. Moreover, these constituents are also insect repellents while at the same time they attract pollinators[15]. There are four main types of terpenes: monoterpenes, sesquiterpenes, diterpenes, and triterpenes[16]. Monoterpenes have been found to have antiseptic and analgesic effects[17]. β-caryophyllene (BCP) is a major sesquiterpenoid found in cannabis plants and extracts[17]. This substance has multiple effects in various areas: gastro and neuroprotection,

anti-inflammation, analgesia, antioxidant, antibacterial, among others[17,18]. One interesting term that is currently used regarding these substances is the "entourage effect"[17], translating the contribution of these compounds to the beneficial effects related to cannabis *sativa*[15]. However, the true nature of such effect is still unclear[15,19,20].

Cannabinoids bind primarily to cannabinoid receptors, also known as cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). CB1 receptors are predominantly expressed in the brain, in particular in the cerebral cortex, cerebellum, basal ganglia, and hippocampus, and are responsible for the behavioral and psychotropic effects of cannabis consumption[11]. These receptors play a number of key roles in the central nervous system (brain and spinal cord; CNS), ranging from modulating early neurodevelopment, regulation of neurogenesis, and modulation of neurotransmission[21,22]. Activation of CB1 receptors has been shown to have neuroprotective effects by decreasing glutamate release by pre-synaptic neurons and nitric oxide (NO) production, as well as increasing brain derived neurotrophic factor (BDNF) expression[22]. Beneficial effects of the activation of these receptors in models of neurodegenerative conditions, such as Parkinson's, Alzheimer's (AD), and Huntington's disease have arisen[22].

CB2 receptors are mainly expressed in tissues involved in immune regulation, such as thymus, spleen, tonsils, as well as in the gastrointestinal tract, bone marrow, and peripheral blood mononuclear cells[11,21]. Despite former conflicting evidence of its presence in the CNS, recent studies report the presence of CB2 receptors in astroglial and microglial cells[21,23], cerebral cortex[23], hippocampus[23], and cerebellum[23]. Rodent studies have demonstrated that CB2 receptors modulate neuronal activity, particularly through suppression mechanisms[23]. Excitatory synaptic transmission was enhanced in situations where CB2 receptors were activated, while GABA and glutamate release was reduced[23]. These receptors also appear to be up-regulated in response to noxious stimuli, including ischaemic-induced hypoxia, AD, and multiple sclerosis (MS)[23].

Both CB1 and CB2 receptors are involved in oligodendrocyte progenitor cells (OPCs) regulation, while CB2 receptors are possibly implicated in neural progenitor cell proliferation, neurogenesis, and regulation of dopaminergic activity[21]. Regarding cannabis use, CB1 receptors' activation produces reinforcing effects and controls the rewarding consequences of substance use[23]. Cannabis' aversive effects, on the other hand, seem to be regulated through CB2 receptor activation[23].

THC is a partial agonist of CB1 and CB2 receptors; on the other hand, CBD shows little affinity for the orthostatic sites of these receptors[24]. Nonetheless, CBD exerts its functions through other

non-cannabinoid receptors, including adenosine A2A, TRPV1, GPR55, PPARγ and PPARα receptors, among others[25,26].

Investigation towards cannabis has intensified within the past decades, particularly related to the therapeutic benefits of natural cannabinoids in numerous diseases, such as Parkinson's disease and other dementias[27-29], multiple sclerosis[30,31], Huntington's disease[32], neuropathic pain[33,34], among others. A few cannabinoid-based treatments have also been approved for the treatment of intractable nausea and vomiting[35,36], anorexia associated with weight loss in acquired immunodeficiency syndrome (AIDS) patients[36], spasticity in multiple sclerosis[35], and severe treatment-resistant epilepsy[35,36]. Interestingly, inflammation appears to underlie the pathogenesis of several of the previously mentioned conditions, which may indicate that cannabinoids may exert its beneficial effects in those pathologies through modulation of the inflammatory pathways[37-39] .

Neuroinflammation can be described as an inflammatory response within the CNS, which is mediated through several cytokines, reactive oxygen species (ROS), chemokines, and other inflammatory markers. Different cells, such as glial cells, which comprehend microglia, astrocytes, oligodendrocytes, and OPCs, in addition to endothelial cells and peripheral immune cells, produce and release these inflammatory mediators[40,41]. These inflammatory players, in particular cytokines, play important roles in various nervous system functions and pathologies, such as major depression, bipolar disease, and schizophrenia[41].

Of note, several psychoactive substances, including cocaine, opioids, and alcohol, have showed to be linked to inflammatory processes of the CNS, making it plausible that these processes play a role in addiction development and maintenance[42]. However, cannabinoids may be playing a different role.

In that sense, the interplay between cannabis and neuroinflammation in several neuropsychiatric and/or neurodegenerative diseases, through the modulation of glial cells, has gained significant interest recently. In this context, our main focus was to understand how cannabinoids affect inflammatory processes in the brain and spinal cord. Although both THC and CBD have been described as being anti-inflammatory substances, most of these studies focused largely on their peripheral effects. Nevertheless, there is evidence showing that these compounds are capable of opposing inflammation in the CNS[24,25]. On the other hand, other authors reported proinflammatory effects for both THC and CBD [43,44]. The systematization of published evidence is,

therefore, well justified. Thus, this systematic review aims to provide an overview on the role of cannabis extracts and isolated natural cannabinoids in counterbalancing neuroinflammation.

2. Methods

2.1 Research Question

The present study was conceived to respond the following research question: What is the current knowledge concerning cannabis extracts and isolated natural cannabinoids' impact on neuroinflammation? For this matter, our aim was to provide a synthesis of the findings encountered, considering also the different research models frequently used to address this topic (human studies, animals models, *in vitro* models).

2.2 Literature Search

The search strategy was initially drafted by two of the authors and further refined through team discussion. The final literature search for relevant articles was conducted on PubMed® using the following search equation: (Neuroinflammat* OR Neuroimmune OR Microglia OR Astrocytes OR Glia) AND (Cannabis OR Marijuana OR THC OR Tetrahydrocannabinol OR CBD OR Cannabidiol OR CNB OR Cannabinol) NOT (Collagen-binding domain OR Corticobasal degeneration). To be included in this review, peer-reviewed journal articles had to be published between 2010 and 2020, written in English, and available in full-text in the database searched. The PubMed® database facilitated searching across MEDLINE and PubMed Central, and allowed the implementation of the research equation already described, returning an agile and organized metadata visualization, exportable in several formats. It also provides direct connections for full-text extraction. Because cannabis is a scheduled drug, we also verified that PubMed® search included the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) directory of drug research peer-reviewed journals in Europe, as well as those available at the directory of International Society of Addiction Journal Editors (ISAJE).

2.3 Relevant Studies

Articles were considered to be of relevance for this review if the effects of substances compatible with the research question were analyzed in the brain and/or spinal cord of animal and human studies or in cellular models pertinent to this review. An initial time period englobing the last ten years was defined.

The following publication types were excluded: reviews, systematic reviews, meta-analysis, commentaries, retracted articles, and papers without an available abstract. Afterwards, only papers from quartile 1 peer-reviewed journals (according to the Scimago Journal & Country Rank [SJR]) were included.

2.4 Study Selection

A total of one hundred and seventy (170) papers remained eligible for analysis. A preliminary analysis based on titles and abstracts was independently performed by two reviewers. Both reviewers proceeded to exclude articles that were clearly irrelevant to the research question. Peer agreement was obtained with Cohen's kappa test (k = .937). Discrepancies were approached by the reviewers through discussion and, when necessary, conversation with a third author. One hundred and one (101) papers were excluded as a result of this process. After full-text analysis, performed by the first author, a total of sixty three (63) papers were included in this scoping review (Figure 1). The final list was approved by the remaining authors.

2.5 Chart the Data

The data was later organized employing Microsoft Excel for this purpose. Characteristics of each study were extracted, depending on the population investigated, which included names of authors, title, year of publication, study design, experimental procedure, type of *in vivo* or *in vitro* model used, animal strain (if justified), CNS region studied, and key findings.

2.6 Collate, Summarize, and Report Results

The final stage consisted in organizing all relevant findings based on the importance to the research questions. Studies were grouped in different tables, focusing on the type of model used and the substance(s) analyzed. All data was reported in the results section.

3. Results

The preliminary PubMed® search identified 291 articles. After exclusion of several publication types, as described in the methodology section, 170 papers remained eligible for screening. Through screening based on title and abstract, a further 101 articles were excluded for not meeting our inclusion criteria. Following this initial screening, 6 more articles were excluded for not being related to the theme. Therefore, 63 articles were included in the final review.

The results were analyzed in separate categories: (i) effects of cannabis (whole plant extracts), (ii) effects of THC, (iii) effects of CBD, (iv) effects of combinations of THC and CBD; (v) effects of non-THC/CBD cannabinoids; and (vi) effects of other substances (non-cannabinoids). Within each category, results were further separated by type of study model used (studies in humans, studies in animal models, and studies *in vitro*).

3.1 Studies addressing the effects of cannabis (whole plant extracts) on inflammation

Based on the inclusion criteria described in the methodology, only two studies evaluating the effect of cannabis could be included in this section, both of them addressing effects in human users (see Table I). These studies were conducted in cannabis users and neuroinflammatory markers were assessed indirectly, either through cerebrospinal fluid (CSF) evaluation or magnetic resonance imaging (MRI). Ellis and colleagues (2020)[45] assessed human immunodeficiency virus (HIV) positive or negative individuals either using or not cannabis (at various levels of exposure), and measured the levels of several proinflammatory cytokines and chemokines (including interleukin (IL)-16, C-reactive protein (CRP), IL-6, interferon (IFN)-gamma-induced protein [IP]-10, soluble CD14, and soluble tumor necrosis factor (TNF) receptor type II) in both CSF and plasma samples. Cannabis use was determined through self-reporting. The authors reported that recent

cannabis use was associated with lower levels of IL-16, CRP, and soluble TNF receptor type II, which reflects reduced neuroinflammation. According to the authors, study limitations included: reduced sample possibly associated with selection bias; the risk of relying only on self-reported use of cannabis; and the fact the composition of the cannabis used was not characterized in terms of CBD and THC. One could also add that no anti-inflammatory markers were evaluated.

In the other study, Blest-Hopley *et al*. (2019)[46] employed proton magnetic resonance spectroscopy (^{1}H-MRS) to evaluate different metabolite (glutamate, myoinositol, and *N*-acetyl aspartate) concentrations in the hippocampus of both cannabis users (CU) and non-users (NU). To be characterized as cannabis users, individuals had to have started regular use of this drug before the age of 18 and to be using it a minimum of 4 days per week for the previous 2 years. On average, those included in this study had been using cannabis approximately 6 days per week for the last 10 years. Again, cannabis use was self-reported. Despite the moderate sample size (21 NU and 22 CU participants), the authors found reduced myoinositol hippocampal concentrations in cannabis users. Myoinositol may function as a marker of glial dysfunction, since activated glia tend to have higher levels of this metabolite[47]. On the other hand, no significant effects of cannabis on glutamate levels, a presumed mediator of neuroinflammation, were detected[46]. It is important to note, however, that the methodology used may have low resolution and poor signal quality, and that the signal-to-noise ratio was significantly different between the two groups studied[46].

Even though the number of papers included was very low, both studies revealed potential anti-inflammatory effects of cannabis in the central nervous systems of healthy and HIV-positive individuals.

3.2 Studies addressing the effects of THC on inflammation

A total of twenty two publications addressing the effects of THC in inflammation were included. Of those, ten examined the effects of THC on inflammation in animal models (see Table II) and twelve examined these effects using *in vitro* models (see Table III).

3.2.1 Studies addressing THC effects using animal models

A total of ten publications addressing the effects of THC *in vivo* were included in this subsection (see Table II). In the majority of the studies using animal models, THC treatment resulted in a proinflammatory status, by increasing the expression of inflammatory cytokines and other mediators (e.g. glutamate and oxidative stress' markers), microglial and astrocytic reactivity, and activation of proinflammatory pathways[48-54].

Simultaneously, THC was reported as having anti-inflammatory effects in four publications[49,53,55,56]. Importantly, in two publications, THC is described as anti-inflammatory in acute exposure but pro-inflammatory later on[49], or pro-inflammatory *per se*, but of reducing MDMA-induced release of proinflammatory factors, while increasing IL-10 levels[55].

The variability in the results may reflect the diversified experiments operated, especially in terms of THC dosages and treatment duration. Despite the highly variable THC doses used in these publications, it is not possible to evaluate an eventual correlation between THC dosage and reported effects. However, in most studies where THC exhibited proinflammatory effects, the treatment duration was considerably longer[48-51,53,54]. It is important to note, on the other hand, that administration of THC for 10[49], 18 days[53] or 12.5 weeks[57] also led to anti-inflammatory responses. Another important factor seems to be the brain region evaluated. Zamberletti *et al*. (2015)[50] treated Sprague-Dawley rats for 11 days, showing increased microglial reactivity and higher levels of proinflammatory markers, including TNFα, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), in the prefrontal cortex (PFC). In this same work, when looking into other regions, such as the nucleus accumbens, hippocampus, or amygdala, no significant changes were observed. Of note in a similar experience[51], THC provoked astrocytic reactivity and increased iNOS and TNFα levels in the hippocampus, but not in the PFC, where only COX-2 levels were elevated. In addition, sex differences can also account for these discrepancies as, for example, Lopez-Rodriguez and colleagues (2014)[53] showed that, while THC reduced microglial activation in the hippocampus of female rats, it had the opposite effect in males.

The studies included pose another interesting question. Moretti *et al*. (2015)[49] also compared acute versus chronic THC treatment in both adolescent and adult mice. The authors treated adolescent and adult mice with a single administration of THC or 10 daily doses, showing that both treatments resulted in general anti-inflammatory outcomes. Nevertheless, these effects were apparently more pronounced in adolescent mice as, for example, adult mice treated with a single subcutaneous (s.c.) dose of THC maintained their cytokines' levels, while the same

treatment in adolescent mice resulted in a decrease in both TNFα and IL-1β levels. These differences were also present when mice were subjected to the chronic treatment. When evaluated right after the last dosing of chronic THC, both adult and adolescent mice presented an anti-inflammatory pattern. However, when evaluated six weeks after the last administration of THC, adult mice had returned to their normal; while mice treated during adolescence, revealed an increase in the levels of TNFα and IL-1β and a reduction in IL-10 release, suggesting a shift towards neuroinflammation.

In summary, in animal models, THC effects are not sufficiently coherent. Nevertheless, and despite confounding factors, a majority of studies seems to favor the existence of pro-inflammatory effects.

3.2.2 Studies addressing THC effects using in vitro models

A total of twelve papers examining the effects of THC using *in vitro* approaches were included in this review (see Table III).

Contrary to the results presented on the previous section, THC, *in vitro*, was able to decrease the production of proinflammatory cytokines and chemokines as well as glial cells' migration, increasing the production of anti-inflammatory cytokines, and inhibiting proinflammatory and metabolic pathways in most selected publications[58-67]. THC also exhibited antioxidant effects, displayed as a decrease in both iNOS transcript levels and ROS production[61]. Globally, three cytokines were consistently seen reduced by this cannabinoid: TNF-α, IL-6 and IL-1β[58,62,63,66,67]. Importantly, while these results seem consistent among different studies, they do not compatible with *in vivo* data, which reported mainly pro-inflammatory outcomes, highlighting the relevance of using co-cultures of primary cells to better mimic the brain tissue.

THC influence in chemokine's regulation was slightly less consistent. Focusing on messenger ribonucleic acid (mRNA)'s regulation, Kozela *et al*. (2010)[58] failed to demonstrate any effect of THC on *chemokine ligand 2* (CCL2) mRNA levels, while two other studies, also conducted in BV-2 cell cultures, revealed a reduction in different chemokine's mRNA levels, including *CCL2*, using the same doses. Confirming the relevance of co-culturing adequate cell types, Rizzo and colleagues (2019)[66] showed opposite effects of THC on chemokine production depending on the experiment. In a monoculture of astrocytes, THC was able to reduce the production of CCL2; while THC

reduced the percentage of astrocytes which expressed this chemokine, without changes in its levels in an astrocytes-monocytes co-culture. Henriquez *et al.* (2020)[63] also failed to demonstrate a reduction in CCL2 levels, when using a co-culture of $CD8^{+}$T and U251 cells.

In addition, two publications[58,65] using a similar experimental procedure reported different effects on a specific pathway in BV-2 cells. In one of the studies[58], THC was not able to influence the lipopolysaccharides (LPS)-induced activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway while, in another experiment[65], THC attenuated the activation of this pathway. Furthermore, Henriquez and colleagues (2020)[63] raised another interesting point, showing that THC exhibited different results depending on the type of cellular stimulation (before or after the insult). This cannabinoid inhibited IFNγ-induced production of C-X-C motif chemokine ligand 10 (CXCL10) but when the cell cultures were stimulated with TNFα and IFNγ, THC was not able to affect this chemokine's levels.

On the other hand, one study[65] displayed proinflammatory effects of THC, in particular an increase in mRNA expression of IL-15. Of note, THC treatment did not modify inflammatory parameters on unstimulated (control) cells[58,60,62,63,65,67,68].

Overall, despite the reductions of the cytokines already discussed, it is important to note that, in several papers, THC did not modify proinflammatory pathways' activation, inflammatory mRNA and microRNAs (miRNA) expression, glial cell's phagocytosis, and proinflammatory chemokines' production[58,61,63,66,68,69]. Also, most studies were conducted in cell lines or mono-cultures of primary cells, which does not seem to be adequate models to reproduce in vivo results.

3.3 Studies addressing the effects of CBD in inflammation

A total of twenty eight publications addressed the effects of CBD in inflammation, twenty one using animal models (mouse and rat; see Table IV), and seventeen addressing inflammation in *in vitro* models (see Table V).

3.3.1 Studies addressing CBD effects using animal models

A total of twenty one papers examining the effects of CBD using *in vivo* approaches were included in this review (see Table IV). The high number of publications found for this cannabinoid reflects the interest in its putative medical use, which has already been approved for several conditions in a few countries.

The majority of these publications[70-88] exhibited CBD-driven anti-inflammatory responses in diverse CNS regions, including the spinal cord. This cannabinoid displayed the ability to reduce proinflammatory cytokines and chemokines levels, microglial activation, and oxidative stress markers as well as to reverse microglial reactive phenotypes induced by other agents.

In what concerns cytokines, studies were mostly focused on evaluating CBD's ability to decrease the classical proinflammatory cytokines TNFα[70,74,76,77,79,80,86,89], IL-1β[77,80,86], and IL-6[75,79,80]. These effects were observed in different CNS regions, such as brain cortex, hippocampus, striatum, and spinal cord. A number of publications, examining different conditions, also reported that CBD exerted no significant changes in their mRNA or protein levels of such cytokines[75,80,85,86,89,90]. Karimi-Haghighi and colleagues (2020)[80] divided Wistar rats into two different groups: i) rats undergoing methamphetamine (METH) extinction received a ventricular administration of CBD (5µg) or vehicle (veh), followed by the administration of an effective priming dose of METH (drug-reinstatement); and ii) after METH extinction was established, rats were injected with CBD or veh 60min prior to rapid eye movement (REM)-sleep deprivation (RSD), followed by the administration of an ineffective priming dose of METH. Afterwards, both the hippocampus and the PFC of these animals were evaluated. In the PFC, CBD was able to revert the increase in IL-1β and IL-6 transcription induced by either METH or RSD (but not TNFα). In the hippocampus, however, this cannabinoid reduced METH-increased mRNA levels of TNFα, IL-1β, and IL-6. However, the opposite effect was observed in the hippocampus of rats subjected to RSD, in which CBD intensified the increase in these cytokines. Another inconsistency is observed, in the same paper[80], when analyzing the prominent anti-inflammatory cytokine, IL-10. In the hippocampus of METH-treated rats not subjected to RSD, METH resulted in an increase in IL-10 levels, a consequence that was not altered by CBD treatment. While in RSD rats, METH increased the mRNA transcription of this anti-inflammatory cytokine, which was then exacerbated by CBD in the hippocampus but reduced in the PFC. This publication was the only one conducted in animals that revealed proinflammatory effects for CBD.

Common methods of evaluating glial responses include the assessment of the number of cells and immunohistochemistry processes using, in particular, ionized calcium binding adaptor molecule 1 (Iba-1) and glial fibrillary acidic protein (GFAP) antibodies to determine microglia and astrocytes reactivity, respectively. Twelve studies assessed glial cells density and/or activation in the CNS in reaction to CBD. The majority of these papers revealed a decrease in these responses, caused by CBD, affecting both microglia[71,77,78,81,82] and astrocytes[72,74,78,81,82,84]. One aspect frequently analyzed in these publications, contrarily to the ones included in the previous sub-sections, was the phenotype of glial cells. Resting astrocytes have a typical star-like appearance with thin fibers, on opposition, to activated astrocytes, which are less star-like and develop thick projections. LaFuente *et al.* (2011)[74] induced HI in piglets, and then administered CBD or veh, intravenously (i.v.). Seventy-two hours later, this substance resulted in a switch of astrocytes' morphology back to a non-reactive appearance similar to that displayed by sham animals. The same results were reported by Friedman and colleagues (2018)[84], who showed that intra-hippocampal (i.h.) treatment with CBD, in Sprague-Dawley rats, reverted the activated morphology aspects of astrocytes induced by kainic acid (KA), and also supported by Gomes *et al.* (2015)[81] in C56BL/6J mice. Microglia, on the other hand, can be classified into different types according to their morphological characteristics[79]. The classic classification in resting or ameboid microglia was progressively replaced by a larger number of different states that range from homeostatic microglia to dystrophic microglia, that present disease associated characteristics (also known as DAM, disease associated microglia). Six studies revealed that CBD was able to transform these cells into less activated phenotypes by reducing mean soma size and increasing ramifications[77-79,82,85,86].

Four papers evaluated the effect of CBD on cellular infiltration in murine experimental models of multiple sclerosis[73,77,83,87]. These studies revealed that this substance is capable of reducing cellular infiltration, including that of T-cells and other mononuclear cells, in both the brain and spinal cord. This is consistent with the results of two other studies[76,77], where mice treated with CBD (3 or 5mg/kg) presented decreased cell margination processes and vascular cell adhesion molecule 1 (VCAM-1) expression, which translated in reduced adhesion of lymphocytes to endothelial cells. Moreover, CBD prevented the disruption of the blood brain barrier (BBB) integrity caused by LPS[76].

The experiments conducted in the papers included in this sub-section were highly variable, either in terms of CBD concentration or duration of treatment. Interestingly, eight studies[72,73,75,77,83,87,89,90] used experimental models of neurodegenerative diseases, in particular of

Alzheimer's disease and multiple sclerosis. The majority of these papers revealed anti-inflammatory properties of CBD. Models of brain ischaemia were also practiced upon, either by inducing hypoxic-ischemic insults[74,85], bilateral carotid artery occlusion (BCAO)[82] or middle cerebral artery occlusion (MCAO)[78]. Additionally, CBD revealed to be anti-inflammatory in these conditions days after the lesions were established. Céprian *et al.* (2017)[78] treated with CBD (5mg/kg) rats that underwent MCAO. Seven days after, while the number of microglial cells was reduced in the peri-infarct area, the number of astrocytes was not altered. However, thirty eight days after the procedure, the number of both astrocytes and microglial cells was reduced, although no differences in microglial phenotypes were observed.

In summary, CBD exerted predominantly anti-inflammatory responses in the animal studies included. Of note, this substance was also relevant when used in models of neurodegenerative diseases, brain ischaemia, and also other conditions, such as diabetes mellitus (DM), seizure activity, and nicotine dependence.

3.3.2 Studies addressing CBD effects using in vitro models

The research conducted following the inclusion criteria exposed in the methods section resulted in a total of seventeen papers on the effects of CBD on various cell models (see Table V).

In the majority of the experiments conducted, CBD was able to reduce inflammation and/or inflammatory markers, such as cytokines and chemokines' protein and mRNA levels, oxidative stress markers, and reactive gliosis[58,60,64,65,68,72,75,77,79,88,91-93]. This cannabinoid consistently reduced the release of IL-1β[58,72,91,93] and IL-6[58,92]. Oxidative stress was also a crucial player in the CBD's anti-inflammatory outcomes, as seen by the reduction in NO[75] and ROS[79,88,93] production. Juknat and colleagues (2012)[64], however, demonstrated an increase in ROS formation in BV-2 cells treated with 10μM of CBD for 4h.

Despite a major bulk of reports, in a few publications, CBD was shown to also exhibit proinflammatory outcomes, increasing mRNA expression of proinflammatory cytokines, including IFN-β and IL-15[64,65,94,95] and oxidative stress[64,94]. Interestingly, two of the studies that showed marked proinflammatory consequences of CBD treatment were conducted in glioblastoma cell lines[94,95]. In these papers, three different glioblastoma cell lines (U87MG, U118MG, and T98G) were exposed to varied concentrations of CBD (5-40μM). There was evidence of increased IL-1β,

IL-6, and IL-8 mRNA levels as well as an elevation in COX-2, TNFα, and IL-6 levels. Additionally, in both cases, CBD was responsible for an increase in cell death.

In addition to the data reported for glioblastoma cells[94,95], CBD was also seen to provoke apoptosis of murine primary microglia[96], rat astrocytes[88], and BV-2 cells[91]. Nevertheless, these results were not consistent among studies, as Kozela *et al*. (2010)[58] failed to show differences in cell viability of BV-2 cells exposed to CBD.

Two other parameters were also evaluated in the studies included: phagocytosis and cell migration. Phagocytic capacity is a marker of microglia reactivity. Phagocytosis is essential to remove cell debris and prune synaptic spines, however, excessive or unbalanced phagocytosis can be a cause of disease[97]. Hassan *et al*. (2014)[69] exposed different cell lines (murine primary microglia, BV-2 cells, highly aggressively proliferating immortalized (HAPI) cells, and RAW-264.7 cells [monocyte/macrophage-like]) to CBD (10μM) for a total of 24h, and verified that CBD led to increased phagocytosis in all cell types. Regarding microglia motility, Martin-Moreno and colleagues (2012)[75] stimulated rat primary microglial cells with LPS and added CBD to the medium, observing enhanced microglial cell migration.

In summary, and coherently with what was observed *in vivo*, *in vitro* studies evaluating CBD reported mainly anti-inflammatory effects. Of note, we could not find any clinical studies addressing the role of CBD in inflammation in humans.

3.4 Studies addressing the effects of combinations of THC and CBD in inflammation

Papers on the effects of Sativex®-like combinations of THC and CBD were also included in this study, comprising a total of four publications using animal models (see Table VI) and one publication using in vitro approaches (see Table VII).

3.4.1 Studies addressing the effects of combinations of THC and CBD using animal models

All animal studies included reveal that a 1:1 combination of THC and CBD has anti-inflammatory properties, mostly translated in reduced proinflammatory cytokines and cellular infiltration. Al-Ghezi and colleagues (2019)[98] was the only publication that evaluated cytokines levels in response to CBD in the CNS of a mice model for experimental autoimmune encephalomyelitis (EAE). In this study, CBD efficiently opposed the proinflammatory profile caused by EAE, by decreasing the release of IL-1β, IL6, IL-17A, TNFα, and IFNγ, while promoting the levels of the anti-inflammatory cytokines IL-10 and transforming growth factor beta (TGF-β). These results were further supported by data showing that CBD influenced microRNAs whose targets include cytokines and molecules involved in EAE progression. In other articles, CBD also reduced IL-1b[99], IFNγ[99], TNFα[99], iNOS[100], IGF1[100], VCAM-1[99], and intercellular adhesion molecule 1 (ICAM-1)[99] mRNA expression. Additionally, a Sativex®-like combination used in mice also revealed a reduction in chondroitin sulfate proteoglycans (CSPGs) release. Through these regulatory mechanisms and also by counteracting microglia and astrocytes activation[99,100] and hindering cellular infiltration[98,99,101], a Sativex®-like combination appears to be effective in reducing inflammation. In summary, a 1:1 combination of THC and CBD treatment seems sufficient to afford neuroprotection for the tested conditions.

3.4.2 Studies addressing the effects of combinations of THC and CBD using in vitro models

In vitro, a single publication using a Sativex®-like combination was included (see Table VII). In this study[99], rat primary astrocytes were treated with TGF-β1 and basic fibroblast growth factor (bFGF), which resulted in decreased synthesis and release of CSPGs. CSPGs are upregulated by activated glia, and play a key role in glial scar formation[102]. Besides, these components of the extracellular matrix exhibit proinflammatory functions, including leukocytes' activation and migration, and seem to be increased in MS and EAE's lesions[102].

Therefore, despite the low number of articles included in this sub-section, the results are consistent with those presented in studies using animal models, and markedly anti-inflammatory.

3.5 Studies addressing the effects of non-THC/CBD cannabinoids in inflammation

This section examined articles evaluating other cannabinoids, namely Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), tetrahydrocannabivarin (THCV), cannabidiolic acid (CBDA), cannabidivarin (CBVD), cannabigerol (CBG), cannabisin F, and extracts of Fructus Cannabis (EFC), comprising a total of three articles in animal models (see Table VIII), and four articles using *in vitro* studies (see Table IX).

3.5.1 Studies addressing the effects of non-THC/CBD cannabinoids using animal models

On animal models, EFC[103] and THCA[104] revealed anti-inflammatory responses. Sprague-Dawley rats underwent D-gal-induced aging and then were treated for 14 weeks with EFC[103]. This extract was able to reduce the number of activated astrocytes as well as revert the oxidative stress provoked by D-gal treatment in the hippocampus. THCA treatment of C57BL/6 mice with striatal neurodegeneration induced by 3-Nitropropionic acid (3-NP) reduced both astrogliosis and microgliosis responses to neurodegeneration, as well as the the expression of proinflammatory markers[104].

3.5.2 Studies addressing the effects of non-THC/CBD cannabinoids using in vitro models

Using *in vitro* models, various kinds of cannabinoids, such as CBDA[69], CBDV[69], CBG[88,105], THCV[69], and cannabisin F[106] were evaluated. Wang and colleagues (2019)[106] treated BV-2 cells with different concentrations of cannabisin F for 1h and then stimulated them with LPS for a period of 24h. The authors showed that this cannabinoid was able to counteract the inflammatory properties of LPS, by reducing IL-6 and TNFα protein levels and preventing oxidative stress. CBG was studied in two other papers[88,105] and also revealed anti-oxidative stress properties. In a co-culture of RAW 264.7 cells and NSC-34 (motor neuron-like hybrid) cells, this substance reduced nitrotyrosine levels as well as the genetic expression of superoxide dismutase 1 (SOD1) and iNOS[105]. This cannabinoid also protected rat astrocytes from H_2O_2 damage while decreasing ROS production by these cells[88]. On the other hand, CBG, not only reduced apoptosis of rat astrocytes, but also increased proliferation of these cells[88]. Hassan *et al*. (2014)[69] studied the effect of 10µM

of CBDA, CBDV or THCV on phagocytosis in a BV-2 cell culture, but failed to demonstrate any alteration caused by these cannabinoids on the phagocytic function.

Of all the cannabinoids included in this section, CBDA was the only one to induce a proinflammatory response[107]. Interestingly, mice subjected to a 10-day treatment with CBDA, but not those who only received a single intraperitoneal (i.p.) injection, displayed higher levels of IL-6[107].

Unfortunately, the number of papers included in this sub-section was low, making it difficult to draw robust conclusions. However, all cannabinoids studied demonstrated anti-inflammatory properties, as also observed in animal models, with the exception of CBDA.

3.6 Studies addressing the effects of non-cannabinoid components of cannabis in inflammation

We also included four studies addressing the effects of different substances, such as phenylpropanamides, BCP and hemp proteins. We found one study in mice (see Table X) and a total of three papers *in vitro* (see Table XI).

The only experiment on animals included in this sub-section studied the effects of total phenylpropionamides (TPA) on Kunming mice[108]. A higher concentration (2g/kg) of TPA had no effect on cytokines' levels. However, when the concentration of TPA was lower (1g/kg), treatment with this substance resulted in a decrease of IL-1β, IL-6, and TNFα levels induced by LPS.

Hemp protein isolates or hydrolysates (HPI/HPH)[109] and coumaroylaminobutanol glucopyranoside (CLG)[106] were studied in BV-2 cells. Neither of these substances had any effect on cell viability. CLG, HPI, and HPH produced anti-inflammatory responses: CLG treatment for 1h reduced the genetic expression of *IL1b* and *IL6* and protected cells against oxidative stress[106]; HPI and HPH also decreased *IL6*, *TNFα*, and *IL1b* mRNA levels, while upregulating *IL10* expression[109]. Askari *et al*. (2019)[110] incubated OLN-93 cells (deriving from a permanent oligodendroglia cell line) with BCP for 24h, and afterwards exposed them to BCP and LPS for another 24h. BCP did not alter cell viability, but switched to an anti-inflammatory state, by decreasing the LPS-induced levels of TNFα, NO, and ROS.

Again, this sub-section is supported by a reduced number of studies, which do not allow reaching robust conclusions. Nevertheless, all substances evaluated demonstrated anti-inflammatory properties.

4. Discussion

This review is, to our knowledge, the first systematic review examining the effects of natural cannabinoids on neuroinflammation in both cellular, animal models and humans. Even though there is abundant literature addressing cannabinoids effects, there are few reviews on the specific inflammatory effects of these substances on the CNS, and the vast majority of those examined their effects in CNS disorders and their neuroprotective potential[111-117], focusing on the pharmacological potential of cannabinoids on neuroinflammation[118]. Other aspects commonly analyzed consist of cannabinoid's functions and role in peripheral inflammation[25,119-125] and cannabis' effects on brain structure and function in humans[126,127].

In the present review, the results suggest that cannabinoids, in particular CBD and THC-CBD combinations, potentiate important anti-inflammatory responses in the CNS. These findings are consistent with the anti-inflammatory properties of these substances on peripheral inflammation processes *in vivo*[128-131] and *in vitro*[131,132] examined in several studies.

The current review granted three key findings: (1) THC displayed considerably anti-inflammatory properties in *in vitro* models, however, the results were inconsistent when compared with those obtained in animal models; (2) CBD was predominantly an anti-inflammatory substance in all models studied; and (3) combinations of THC and CBD were consistently anti-inflammatory.

The studies addressing the effects of THC in animal models were not consistent. More than half of these publications consisted of non-randomized experiments[49-51,54,55,57], with only one double-blind randomized experiment out of the eleven articles included[53]. Also, the experiment planning was highly variable, as can be observed in Table II. In rodent models, treatment dosages of THC varied from 1mg/kg[48,56] to 20mg/kg[48,55], administered in a single dose[49,56] or repeated administrations[50,54]. The longest experiment of all the animal studies included was conducted with twice-daily THC administrations of 0.18-0.32mg/kg in rhesus macaques for a total of approximately 12.5 weeks[57]. One of the factors that may contribute to the discrepancies in neuroinflammatory responses is the age at initiation of use. Zamberletti *et al*. (2015)[50] treated

adolescent female Sprague-Dawley rats with THC and revealed an induction of signs of neuroinflammation, particularly in the PFC, that persisted until adulthood. This outcome was also suggested by one other 2015 publication[49], which exhibited evidence that chronic THC treatment during adolescence resulted in long-lasting proinflammatory changes in murine hypothalamus and hippocampus. This may contribute to explain why morphological brain alterations due to chronic cannabis are much more severe during adolescence, possibly resulting in long-lasting changes in the CNS.

Of note, in *in vitro* models, THC displayed robust anti-inflammatory properties. The majority of the articles reviewed revealed a reduction of the production and release of proinflammatory molecules, accompanied by a decrease in ROS production and an increase of anti-inflammatory cytokines levels. Half of these studies was conducted using the BV-2 cell line, an extensively used model for microglia, which has been supported as a suitable alternative for primary microglia and animal models in neuroinflammation studies[133]. However, isolated cells can hardly recapitulate what happens in the brain tissue, where several different cell types contribute to an emergent outcome.

CBD had a more consistent anti-inflammatory response in both *in vitro* and *in vivo* models. With regard to animal models, CBD showed a coherent protective function against inflammation. Of the four articles[72,75,89,90] analyzing the effects of CBD on Alzheimer's disease, only two used transgenic mouse models of AD[89,90]. Despite the differences in experimental design employed in these articles, Cheng *et al*. (2014)[89] treated mice with gel pellets of CBD (20mg/kg) for 8 months, while Watt and colleagues (2020)'[90] mice were administered intraperitoneally CBD (50mg/kg) for 7 weeks, both resulted in anti-inflammatory effects, confirming a robust effect. When AD mice were treated with higher doses of CBD[90], the levels of proinflammatory cytokines and microglia reactivity were not altered. Lower doses of these substance[89], however, resulted in a reduction of cytokine levels, in AD mice, to values similar to those of wild-type (WT) mice, despite not changing Il-1β and TNFα mRNA levels. For the study of multiple sclerosis, two different rodent models were used: experimental autoimmune encephalomyelitis[73,83,87] and virally-induced demyelination[77]. The results were consistent among these four articles, with CBD acting as an anti-inflammatory agent in both the brain and the spinal cord.

Different cell lines were also used across these articles to evaluate CBD in neuroinflammation, including two publications[94,95] which studied the effect of CBD in three different glioblastoma cell lines: U87MG, U118MG, and T98G. Glioblastoma is the most prevalent malignant tumor in adult patients[134] and it communicates with other cells, including microglia, astrocytes and infiltrating

macrophages, through the production and release of inflammatory mediators[135]. In both studies[94,95], CBD produced a proinflammatory response in the glioblastoma cell lines, as seen by the increase in inflammatory cytokines' mRNA transcripts and protein levels and oxidative stress.

Although the number of publications addressing Sativex®-like combinations on neuroinflammation is low, this combinatory effect demonstrated anti-inflammatory properties in both *in vitro*[99] and *in vivo*[98-101]. All these combinations were used in animal models of neurodegenerative diseases, such as multiple sclerosis and Huntington's disease. Sativex® is already approved in several countries for the treatment of MS-related spasticity and pain[136]. Despite its encouraging results in symptomatic treatment of MS and inflammation control in animal models, the literature is at fault when it comes to the study of the anti-inflammatory effects of this compound in the CNS of MS patients. The Cannabinoid Use in Progressive Inflammatory brain Disease (CUPID) trial[137], however, attempted to analyze if oral THC treatment of MS patients could alter disease progression. Unfortunately, this 3-year, phase III clinical trial failed to demonstrate a significant and beneficial effect of THC in MS progression. Nevertheless, in a subgroup analysis of less disabled patients, oral THC treatment appeared to significantly slow progression.

It should be emphasized that in the majority of studies on cellular models, cells were stimulated with different substances (insults), with particular emphasis on LPS, a classic stimulus to induce inflammatory responses. LPS-induced inflammation is one of the most common models used for the study of neuroinflammation. LPS is present in the outer membrane of Gram-negative bacteria, and when it activates its main target, the toll-like receptor 4 (TLR4), a plethora of pro-inflammatory responses are induced[138]. LPS has proved to produce neuroinflammatory outcomes in various *in vitro*[139,140] and *in vivo*[141,142] models, even maintaining this ability when given systemically[141]. While most cannabinoids were effective in counteracting the effect of LPS, these cannabinoids did not exhibit a response when administered to healthy (control) non-stimulated animals/cells[50,51,55,70,76,79,85]. An exception can be made for THC, which demonstrated proinflammatory properties[48-51,53,54]. Sonego *et al*. (2018)[79] was also able to induce an anti-inflammatory response when treating non-stimulated mice with CBD, although these results may reflect the much higher cannabinoid dose used in this experiment. As previously mentioned, cannabinoids are extensively studied in diseased patients, but less so on healthy individuals. These contradicting results should then be assessed in both models. An opposite response in terms of peripheral inflammation has been noted in other studies, as demonstrated by a 2021 systematic review [143], in which healthy cannabis users volunteers had a diminished inflammatory

response, while cannabinoid based treatment of patients with MS showed little impact on their peripheral inflammatory markers.

Lastly, the inflammatory mechanisms presented in the articles reviewed here focused on three key areas: glial reactivity, production of inflammatory mediators, and oxidative stress. One of the inflammatory markers analyzed was glutamate, the main excitatory neurotransmitter in the CNS[144]. Glutamate-induced excitotoxicity and neuroinflammation are involved in the pathogenesis of several neurodegenerative diseases[144]. Glia cells, especially astrocytes and microglia, participate in regulating glutamate levels and glutamatergic transmission in the CNS and glia dysfunction contributes to glutamate-mediated toxicity[144]. The results on cannabinoids' influence on glutamate levels were contradicting. CBD reduced glutamate's levels on a cellular model[93], but THC was responsible for the increase secretion of this neurotransmitter in an animal model[54]. These findings are compatible with a previous human study which has found that THC increased glutamate concentrations in the striatum, although these concentrations were not altered in the anterior cingulate cortex, suggesting that these effects may be region-dependent[145]. On the contrary, a recent systematic review suggests that chronic cannabis use reduces glutamate levels in the human brain[119].

Oxidative stress has demonstrated to contribute to neuroinflammation[146] and to be involved in the pathogenesis of several conditions[147-149]. Similar to previous results, CBD is consistently more effective in reducing oxidative stress[72,75,76,79,88,93], what could partially explain its anti-inflammatory mechanisms. This is consistent with a recent systematic review[120], which revealed that CBD down-regulated ROS production on normal human healthy cells. In addition, oxidative stress is one of the regulators of glutamate levels in the CNS[150], thus its control could perhaps explain in part the decrease in glutamate levels by cannabinoids.

The first key limitation in this review was the exclusive use of PubMed® for the search, which may have hindered the identification of other relevant studies and increased bias. Also, there were both date (2010-2020) and language (English) restrictions. In this review, the investigation was extended to include *in vitro* cell populations and *in vivo* animal and human studies. Other weaknesses encountered in the included studies refer to the lack of blinding and randomization in animal models. However, it is important to note that most publications presented a placebo group. The experimental procedures, in particular, dosages and treatment period, were varied, yielding diverse results, and decreasing the robustness of effects across studies.

5. Conclusion

Together, the sixty three articles discussed in this review suggest that cannabinoids exert anti-inflammatory responses in the central nervous system in preclinical models of health and disease. More than half of the articles included corresponded to experiments conducted *in vitro*. Also, only two studies included presented the effects of cannabinoids on humans. It is important to recall that the cannabinoids studied, in particular THC, exhibited proinflammatory properties in the CNS, which is in line with other publications revealing higher levels of proinflammatory cytokines in individuals with cannabis use disorder. The magnitude and extension of these effects, however, may depend on several factors, such as dosage, duration of treatment and age at onset of use. Although some of these substances are currently used in clinical practice for symptomatic treatment of certain conditions, it remains to be clarified how impactful these responses may be for the treatment of neuroinflammation and even on addiction development and treatment.

Appendix

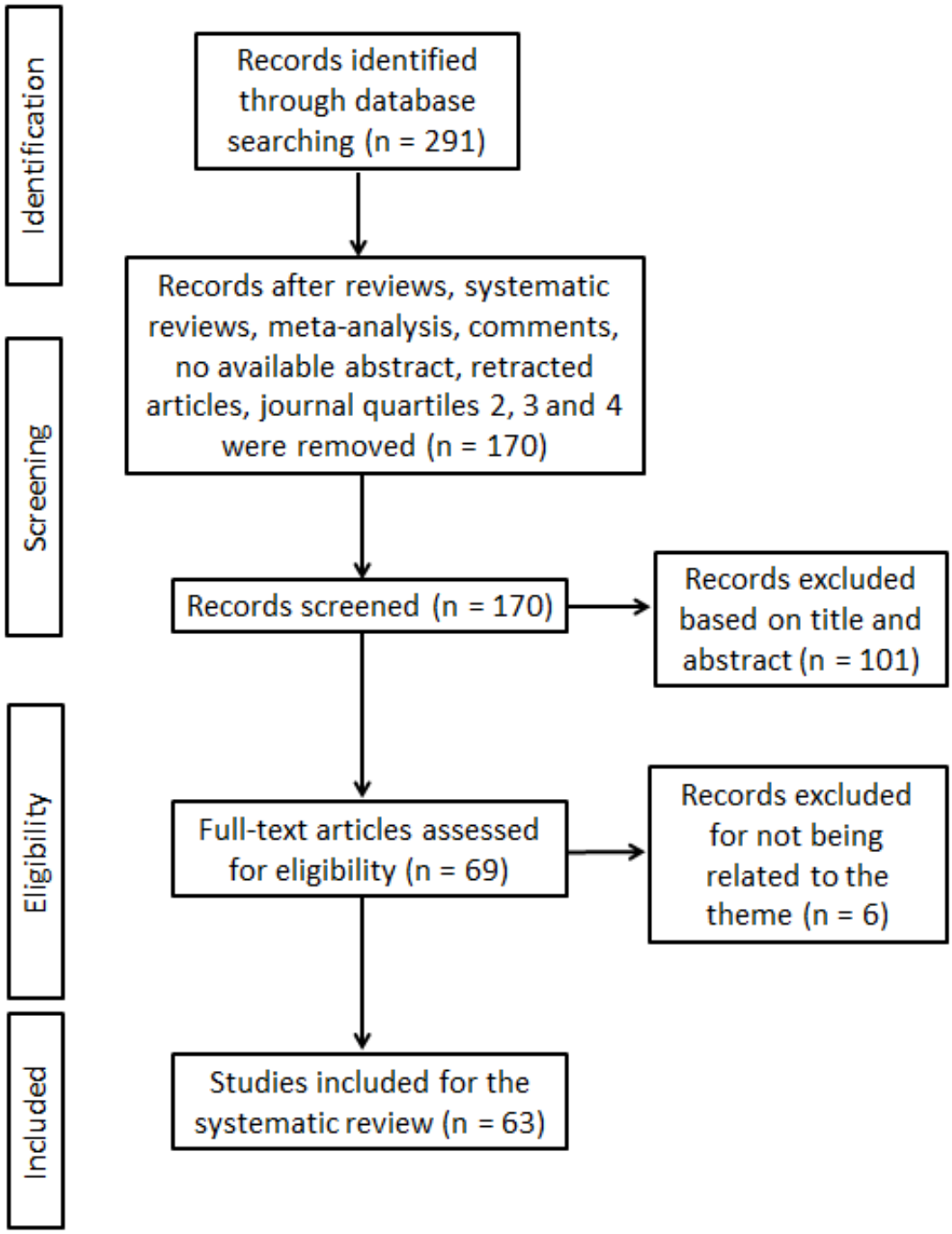


Figure 1: Flow diagram of study selection process

Table I: Studies addressing the impact of cannabis use in inflammatory markers in humans

Author/Year	Country	Study Design	Sample Details	Age, years (mean ± SD)	Co-Variates	Protocol	Evaluations Conducted	Results
Ellis, 2020[45]	United States of America	Cross-sectional prospective observational cohort study	35 HIV(+) and 21 HIV(-) individuals with (n=41) or without (n=15) cannabis use	HIV(+): 47±14.7 HIV(-): 42.27±14.2	HIV status, age, gender, CD4 count	Participants underwent neuromedical assessment, blood draw, and lumbar puncture	IL-16, CRP, IL-6, IFN-gamma-induced protein [IP]-10, soluble CD14, and soluble TNF receptor type II in cerebrospinal fluid and blood.	Recent cannabis use was associated with lower CSF levels of IL-16, CRP, and soluble TNF receptor type II, reflecting reduced inflammation.
Blest-Hopley, 2019[46]	United Kingdom	Cross-sectional prospective observational cohort study	Individuals with our without cannabis use	CU: 25.05±3.5 NU: 24.24±4.11	Cocaine use, nicotine use, alcohol use, age, gender	^{1}H-MRS was performed on all participants	Glutamate, myoinositol and N-acetyl aspartate concentrations in the hippocampus.	Cannabis use was not related to hippocampal glutamate levels but reduced myoinositol levels.

Table II - Characteristics of studies addressing THC effects in animal models

Author/Year	Study Design	Subjects	Experimental Procedure	CNS Region	Results
Touriño, 2010[55]	Non-randomized, placebo-controlled	C57BL/6 mice *Cb1*$^{-/-}$ mice *Cb2*$^{-/-}$ mice *Cb1*$^{-/-}$ + *Cb2*$^{-/-}$ mice	Binge 3,4-Methylenedioxymethamphetamine (4x20mg/kg, i.p.; MDMA) or saline; 1h before each MDMA/saline administration, THC (3mg/kg, i.p.) or veh were administered to WT or CB1 and CB2 deficient mice.	Striatum	THC prevented microglia and astrocytes reactivity induced by MDMA administration, as well as the loss of dopaminergic terminals and the hyperthermic effect (at 21˚C, but not at 26˚C); THC alone did affect microglia and/or astrocytes reactivity (evaluated by CB11b and GFAP). Of note, THC did not prevent MDMA-induced hyperthermia, glial activation and dopamine (DA) terminal loss when the CB1 receptor antagonist AM251 was used, neither in CB_1 and CB1/CB2 knockout mice, indicating a role for CB1 receptors in mediating the protective effect.
Cutando, 2013[48]	Single-blind, randomized, placebo-controlled	WT Swiss albino mice *Cb1*$^{-/-}$ mice (on a CD-1 background) *Cb1a6*- mice (on a C57BL/6 background); Gabra6cre; Cb1 f/f mice	Mice were administered THC (1mg/kg, 2.5mg/kg, 5mg/kg, or 20mg/kg, i.p.) or veh twice daily for 5 consecutive days; on the sixth day, mice received one injection of THC or veh	Cerebellum	The study describes cerebellar activation of microglia as a result of dysregulated CB1R activity produced either by subchronic THC exposure or CB1R genetic disruption. Subchronic THC dose-dependently increased microglial activation changing morphology, mainly in the molecular layer, and increasing expression of proinflammatory genes (IL-1b). Pharmacological blockade of microglial activation and IL-1 receptor (IL-1R) signaling prevented the behavioral and motor deficits associated with the cerebellum.
Castelli, 2014[56]	Single-blind, randomized, placebo-controlled	Adult male Sprague-Dawley rats	Rats were divided into two groups: METH (10mg/kg, s.c.) or saline; both groups were treated with THC (1mg/kg or 3mg/kg, i.p.) or veh 30min before or 0.5, 12, 24, 36 and 48h after the last METH or saline administration	Caudate-putamen (CPu); PFC	Either pre- and posttreatment with THC attenuated the neurotoxicity of METH, reducing METH-induced nNOS (neuronal NOS) overexpression and astrogliosis (measured by GFAP). Evaluation was conducted in the CPu and PFC.
Lopez-Rodriguez, 2014[53]	Double-blind, randomized, placebo-controlled	Male and female adolescent Wistar rats	From postnatal day (pnd) 28 to 45, rats were given THC (2.5mg/kg, from pnd 28 to 34; 5mg/kg from pnd 35 to 40; 10mg/kg from day 41 to 45; i.p.) or veh; every 5 days, from pnd 30, rats received 2 daily administrations of MDMA (10mg/kg, s.c.) or saline	Hippocampus	THC alone increased astrocytes reactivity; in males, THC alone (or with MDMA) increased microglial reactivity; in females, THC or MDMA, alone, reduced microglial reactivity (THC and MDMA together resulted in return to control values). THC counteracted METH-induced serotonin transporter protein (SERT) reduction in both sexes; while reducing CB1 receptors in females.

Moretti, 2015[49]	Non-randomized, placebo-controlled	BALB/cJ mice (adolescents [1] – pnd 33; adults [2] – pdn 80)	Acute treatment: mice were administered with THC (15mg/kg, s.c.) or veh Chronic treatment: [1] THC or veh were administered from pnd 33 to pnd 42 (5mg/kg pnd 33-35; 10mg/kg pnd 36-38; 15mg/kg from pnd 39-42; s.c.) [2] THC or veh were administered from pnd 80 to pnd 89 (5mg/kg pnd 80-82; 10mg/kg pnd 83-85; 15mg/kg pnd 86-89; s.c.)	Hypothalamus; Hippocampus	Adolescents: THC reduced TNFα and IL-1β levels; IL-10 levels were not affected. Adults: THC had no effect on levels of these cytokines. Adolescents: THC reduced TNFα levels after treatment but these were increased 6 weeks later; IL-1β levels were increased 6 weeks after the last THC injection; IL-10 levels were increased after THC treatment but reduced at pnd 90 Adults: TNFα and IL-1β levels were decreased after THC treatment but this effect was not present after 6 weeks; THC treatment increased IL-10 levels, an effect also not present 6 weeks later.
Zamberletti, 2015[50]	Non-randomized, placebo-controlled	Adolescent female Sprague Dawley rats	Rats were injected twice a day with THC or veh from pnd 35 to pnd 45 (2.5mg/kg pnd 35-37; 5mg/kg pnd 38-41; 10mg/kg pnd 42-45; i.p.)	PFC; Nucleus accumbens; Hippocampus; Amygdala	PFC: THC increased microglial reactivity (↑ Iba1 expression and activated-state morphology of microglial cells); THC also increased the levels of TNFα, COX-2, and iNOS, while reducing IL-10 levels. Other regions: these effects were not observed in the other brain regions evaluated. Chronic treatment with an anti-inflammatory agent (Ibudilast) concomitantly to THC reduced Iba1 expression and the previously mentioned proinflammatory mediators levels.
Simon, 2016[57]	Non-randomized, placebo-controlled	SIV Rhesus macaques	Animals were injected intramuscular (i.m.) twice a day with veh or THC – these injections were started 4 weeks before SIV infection (0,18mg/kg) then the dose was increased (0,32mg/kg) over 2 weeks and continued for the duration of the study 4 groups were created: veh + no infection; THC + no infection; veh + infection; THC + infection	Striatum	THC alone decreased miRs expression that regulate mRNAs of proteins involved in many biological processes: regulation of transcription and cell migration, fibroblast growth factor (FGF) signaling pathway, immune response, toll like receptor pathway, negative regulation of apoptotic processes, cell proliferation. In the THC/SIV group, there were increased miRs regulating proteins involved in DNA repair, negative regulation of apoptosis and viral reproduction, and response to stress; while, miRs mediating processes such as negative regulation of apoptosis and cell proliferation, regulation of transcription, and insulin receptor signaling pathway, were decreased. mRNA expression of *TNFa* was significantly diminished in the THC/SIV group compared to VEH/SIV, as well as the expression of *IL6*, *IL1b* and *Ccl2* (but not of significant difference).

Zamberletti, 2016[51]	Non-randomized, placebo-controlled	Male and female Adolescent Sprague Dawley rats	Rats were injected with THC (2.5mg/kg pnd 35-37; 5mg/kg pnd 38-41; 10mg/kg pnd 42-45) or veh twice a day from pnd 35 to pnd 45; i.p.). Sensitization was tested using phencyclidine (PCP).	Hippocampus; PFC	THC led to poor memory performance and psychotic-like behaviors after PCP. Hippocampus: THC increased astrocytes reactivity and expression of iNOS and TNFα, but did not affect COX-2 levels; while decreasing IL-10 expression. PFC: THC had no effect on TNFα, iNOS and IL-10 expression, but led to increased COX-2 levels. No changes in microglial activation were observed (as evaluated by iba1 and cd11b).
Jouroukhin, 2019[54]	Non-randomized, placebo-controlled	Adolescent mice with inducible expression of DN-DISC1 selectively in astrocytes	Mice were treated from pnd 30 for 3 weeks with daily injections of THC (8mg/kg, s.c.)	Hippocampus	DN-DISC1 adult mice treated with THC in the adolescence showed an upregulation of genes involved in inflammatory pathways (including NF-κB signaling) and of *Ptgs2* (COX-2); there was also an increase in COX-2 levels, of phosphorylation of NF-κB p65 and IκBα and of glutamate secretion. The cyclooxygenase-2 inhibitor, NS398, prevented THC-induced cognitive changes.
Bhatt, 2020[52]	Randomized, placebo-controlled	Sprague Dawley rats	Rats were assigned to 4 groups: a) post-THC + repeated mild traumatic brain injury (RmTBI); b) post-THC + sham; c) placebo + RmTBI; d) placebo + sham. 12 daily injections of THC were given between pnd 44 and pnd 54 (1.25mg/kg, i.p.)	PFC; Hippocampus; Nucleus accumbens	THC affected anxiety and cognitive performance. THC increased IL-1b serum levels, THC increased *Iba-1* mRNA expression in the PFC, suggesting microglial activation, but not in the hippocampus nor the nucleus accumbens; THC did not confer therapeutic benefits in rats exposed to TBI

Table III - Characteristics of studies addressing THC effects in *in vitro* models

Author/Year	Cell Culture	Experimental Procedure	Results
Kozela, 2010[58]	BV-2 cell line	Cells were treated with THC (1, 5, or 10μM); 2h later, cells were stimulated with LPS	THC reduced the release of IL-1β, IL-6 and IFN-β dose dependently from LPS-stimulated cells and also presented lower levels of mRNA of Il1-β and IFN-β ; THC decrease the activation of proinflammatory signaling by interfering with the TRIF/IFNβ/STAT pathway, but did not reduce the activity of the NF-κB pathway.
Fraga, 2011[59]	BV-2 cell line	Cells treated with THC (1 μM and 10 μM) or veh, were assessed for migration in response to HIV-Tat	THC inhibited microglial cell migratory response to HIV-Tat protein.
Juknat, 2012 [64]	BV-2 cell line	Cells were treated with THC (10μM) or veh for 6h	THC inhibited the expression of a subset of proinflammatory genes (particularly, chemokine ligands (CCL) – *Ccl7*, *Ccl9*, *Cxcl14*, *Cx3cr1*, *IL1*b and *IFN1b*), induced a cellular stress response, activated the GCN2/eIF2α/p8/ATF4/CHOP-TRIB3 pathway, which leads to autophagy and apoptotic cell death.
Juknat, 2013[65]	BV-2 cell line	Cells were treated with THC (10μM) for 2h; LPS was added for another 4h	THC inhibited the LPS-induction of proinflammatory genes, including *IL1b, IL14, IL27, IL4i1, Ccl2, Ccl7, Ccl9,* and *IFN1b*, but enhanced the LPS-upregulation of *IL15* and *Gdf15* mRNA expression; THC attenuated LPS-stimulated activation of NF-κB.
Hassan, 2014[69]	BV-2 cell line	Cells were treated with THC (10μM) for 24h	THC did not affect phagocytosis.
Janefjord, 2014[60]	BV-2 and SH-SY5Y cell lines	Microglial cells were treated with LPS with or without THC (10μM); SH-SY5Y cells were then cultured in microglial-conditioned media	THC protected SH-SY5Y cells from BV-2 conditioned media activated via LPS.
McCarthy, 2016[61]	Murine immortalized microglial cells (adult C57BL/6J mice, infected with v-raf/v-myc retrovirus)	Cells were treated with THC (10μM) for 24h	THC inhibited the amyloid-β (Aβ) -induced increase in iNOS transcript levels and didn't alter the expression of Arginase-1 (M2 marker; Arg-1) in Aβ-treated IMG cells.
Xie, 2016[67]	Murine bone marrow mesenchymal stem cells (BM-MSCs) Murine primary microglia	MSCs were treated for 24h with THC (1μM); primary microglia was cultured in BM-MSCs conditioned medium and then stimulated for 24h with LPS	Pre-treatment with THC improved the immunomodulatory properties of BM-MSCs in microglia LPS-stimulated, reducing the secretion of TNFα, IL-6, IL-8, and IL-1β, and increasing IL-10.
Xie, 2016[62]	Murine primary microglia	Cells were treated with nanoparticles with or without THC (10μM), and then treated with LPS for 24h	THC, either directly or to a greater extent in nanoparticles containing THC-MSN-ARA290, increased IL-10 secretion and reduced TNF-α, IL-6 and IL-1β levels (induced by LPS).

Juknat, 2019[68]	BV-2 cell line	Cells were treated with THC (10μM) for 2h; LPS was added for another 4h	THC decreased the LPS-upregulation of miR-146a, but had no effect on miR-155-5p and -34a(miRNAs associated to Toll-like receptor and NF-κB signaling).
Rizzo, 2019[66]	Fetal-derived human astrocytes Human monocytes	Astrocytes were treated with THC (1, 5 and 10μM) and monocytes were posteriorly added; cocultures were activated with R837 (TLR7 agonist)	THC reduced astroglial production of CCL2 and IL-6.
		Astrocytes were treated with THC and activated using recombinant IL-1β plus R837 for 20h	THC reduced the percentage of astrocytes expressing CCL2 and IL-6, with no effects on the levels of CCL2 and a minor decrease in IL-6 levels.
Henriquez, 2020[63]	$CD8^+$ T cells U251 glioblastoma cell-line	$CD8^+$T cells were stimulated, using anti-CD28 and anti-CD3 antibodies, IFNγ, and IL-2 for 72h; these cells were then co-cultured with U251 cells for 24h; $CD8^+$T cells were treated with THC either at the time of initial stimulation or after 72h of stimulation	THC reduced $CD8^+$T cell-mediated induction of CXCL10 and IL-6 responses in astrocytes but had no effect on CCL2.
		U251 cells were treated with THC (1, 5 or 10μM) for 30min and then stimulated with TNFα, IFNγ or TNFα+IFNγ	THC reduced the TNFα-induced increase in IL-6 levels but did not suppress CCL2 production; THC suppressed CXCL10 induced by IFNγ; THC had no effect on CCL2 and CXCL10 but reduced IL-6 levels in cultures co-stimulated with TNFα+IFNγ.

Table IV - Characteristics of studies addressing CBD effects in animal models

Author/Year	Study Design	Subjects	Experimental Procedure	CNS Region	Results
Magen, 2010[70]	Randomized, placebo-controlled	Female Sabra mice	Mice were assigned to 5 groups: a) sham + veh; b) sham + CBD (5mg/kg; i.p.); c) bile duct ligation (BDL) + veh; d) BDL + CBD, and e) BDL + CBD + WAY-100635 Drugs were started 1 day after surgery and continued for 4 weeks	Hippocampus	CBD improved cognitive and motor function in a model of hepatic encephalopathy. CBD restored mRNA expression levels for *TNF receptor 1* and BDNF. These effects seem to be modulated through the 5-HT_{1A} receptor. CBD did not affect BDNF mRNA expression in sham animals.
Toth, 2010[71]	Single-blind, randomized, placebo-controlled	Streptozotocin (STZ)-diabetic CD1 mice	Mice were assigned to 4 groups: a) DM + CBD (onset), b) DM + CBD (late treatment), c) non-DM + placebo (onset) and d) non-DM + placebo (late treatment) CBD: 0,1mg/kg, 1mg/kg, 2mg/kg (intranasal or i.p.) - Onset: 3 months of treatment - Late: 1 month of treatment	Dorsal spinal cord (DSC); Thalamus	CBD reduced microglial density, Iba-1 and p-p38 MAPK protein expression in the DSC of mice at diabetes onset; no changes were observed in the thalamus or in mice with long standing diabetes. CBD also limited the development of a neuropathic pain state.
Esposito, 2011[72]	Non-randomized, Placebo-controlled	Sprague-Dawley rats	Rats underwent inoculation with human Aβ or placebo; then, rats were administered with CBD (10mg/kg; i.p.) or veh for 15 consecutive days	Hippocampus	CBD decreased Aβ-induced astrocytes activation. CBD seems to exert its protective action through a PPARγ dependent activation, resulting in reduce reactive gliosis and consequently lower neurodegeneration.
Kozela, 2011[73]	Non-randomized, Placebo-controlled	C57BL/6 mice	EAE was induced in mice (by injection of MOG); Control mice received veh; then CBD (5mg/kg; i.p.) or veh were injected for 3 consecutive days	Spinal cord	In EAE mice, CBD decreased inflammation – reducing CD3+ T cells infiltrates and microglial/macrophage activation, compared to EAE mice not treated with CBD, diminishing also axonal damage. CBD had no effects in control mice.
LaFuente, 2011[74]	Non-randomized, Placebo-controlled	Piglets	Hypoxia-ischaemia was induced in Newborn animals; then piglets were administered CBD (0,1mg/kg; i.v.) or veh	Whole brain	CBD led to a neuroprotective effect reflected in aEEG, NIRS, histological, biochemical, and neurobehavioral studies (returned TNFα(+) cells to sham levels as well as the number and morphology of astrocytes similar to sham 72h after HI, suggesting anti-inflammatory effects.

Martin-Moreno, 2011[75]	Non-randomized, Placebo-controlled	C57BL/6 mice	Mice were injected with fibrillary Aβ or saline; treatment with CBD (20mg/kg; i.p.) was initiated the next day and lasted for 3 weeks	Cerebral cortex	CBD reduced nitrite formation and IL-6 mRNA levels (but not *TNFα*) in Aβ-injected mice, improving performance in the water maze. CBD also promoted microglia migration (in primary cultures).
Ruiz-Valdepeñas, 2011[76]	Non-randomized, Placebo-controlled	C57BL/6J mice	A mouse model of sepsis-related encephalitis by i.v. LPS was used. Mice were assigned to 4 groups: a) veh, b) LPS, c) LPS + CBD (3mg/kg; i.v.) or d) CBD (3mg/kg; i.v.)	Whole brain	CBD weakened the vasodilator effect of LPS and decreased the LPS-induced cell margination and expression of *COX-2*, *TNFα* and *iNOS* mRNA CBD prevented the LPS-induced disruption of BBB integrity. CBD alone had no effects.
Mecha, 2013[77]	Non-randomized, placebo-controlled	SJL/J mice	CBD (5mg/kg, i.p.) or veh were administered in sham or TMEV-IDD mice once daily for 7 days (acute effects) or 10 days (long-term effects)	Cerebral cortex; Spinal cord	CBD decreased: VCAM-1 expression to control levels, and lymphocytes adhesion to endothelial cells. CBD also reduced the infiltrate score to sham animals levels, as well as microglial activation and IL-1β and TNFα mRNA expression. Microglia from CBD-treated mice showed a morphology similar to that of sham infected animals.
Cheng, 2014[89]	Quasi-randomized, placebo-controlled	AβPP×PS1 and WT mice	Mice were familiarized with gel pellets with CBD (20mg/kg) or veh for 7 days; then mice were isolated in their home cages and given veh or CBD daily for 8 months	Cerebral cortex	CBD rescued social recognition memory and improved object recognition in male APPxPS1 transgenic mice. CBD did not significantly change *IL1b* and *TNFα* mRNA levels, however the cytokine levels of CBD-treated AβPP×PS1 mice were more similar to WT levels than to those of veh-treated AD transgenic mice.
Gomes, 2015[81]	Non-randomized, placebo-controlled	C57BL/6J mice	Animals were treated with an NMDA-R antagonist (MK-801to induce a schizophrenia like phenotype; on the sixth day of therapy, animals started daily treatment with CBD (60mg/kg) or veh for 22 days.	mPFC; Dorsal striatum; Nucleus accumbens (core and shell); Dorsal hippocampus	CBD attenuated MK-801-induced impaired performance in the social interaction and NOR tests. CBD 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.
Ceprián, 2017[78]	Non-blind, Randomized, placebo-controlled	Wistar rats	Rats were assigned to temporary MCAO or control; 30min after MCAO, rats were treated with CBD (MCAO-C; 5mg/kg; i.p.) or veh (MCAO-V)	Cerebral cortex (ipsilateral to the infarct area)	7 days after MCAO: CBD did not alter the number of astrocytes in the peri-infarct area; CBD reduced the MCAO-induced increase in microglial cells (these cells showed a less activated phenotype).

					38 days after MCAO: CBD reduced MCAO-induced increase in astrocytes and in microglial cells (no differences in microglial phenotypes were observed).
Mori, 2017[82]	Non-blind, randomized, placebo-controlled	C57BL/6 mice	Rats were assigned to BCCAO or control; CBD (10mg/kg; i.p.) or veh was administered 30min before and 3, 24 and 48h after surgery	Hippocampus	21 days after BCCAO, CBD prevented cognitive and emotional impairment, neurodegeneration and white matter injury. CBD also reduced astrocytes and microglia reactivity, accompanied by a decrease of the reactive phenotype of the latter; CBD also increased BDNF levels.
Elliott, 2018[83]	Non-randomized, placebo-controlled	C57BL/6 mice	EAE was induced in mice; CBD (20mg/kg; i.p.) or veh was administered daily, starting at day 9 through day 25	Whole brain; Spinal cord	CBD caused a strong increase in myeloid-derived suppressor cells, attenuating the increase in mononuclear cell infiltrates in the brain and spinal cord of EAE mice and reduced the EAE-induced increase in $CD4^+$ and $CD8^+$ T lymphocytes.
Friedman, 2018[84]	Non-randomized, non-placebo-controlled	Sprague-Dawley rats	Seizure activity was induced with KA (i.p. or i.h.); rats were assigned to 7 groups: a) KAi.h., b) KA + CBDi.h. (3.18μM), c) KA/CBDi.h., d) KAip, e) KA + CBDip, f) KA/CBDip or g) CBD KA+CBD: Administration of KA and CBD in the same cocktail KA/CBD: KA administration followed by CBD injection	Hippocampus	CBD (intra-hippocampal treatment) resulted in a significant reduction in reactive astroglia and reversed the KA-induced changes in astrocyte morphology. KA-induced hyper-locomotor activity was also reduced.
Sonego, 2018[79]	Non-randomized, placebo-controlled	Swiss mice	Mice were injected for 21 days with CBD (60mg/kg¸i.p.) or veh; 30min later, haloperidol (2 or 3mg/kg) or veh was administered	Striatum	CBD reversed the haloperidol (3mg/kg)-induced increase in IL-1β and TNFα levels; CBD alone did not change significantly IL-10 levels but it increased IL-10 levels compared to animals that received only veh or haloperidol; CBD reduced the haloperidol-induced increase in *iNOS, TNFα* and *IL6* mRNA expression, but that of *COX2*. CBD alone increased the levels of catalase and attenuated the decrease in catalase activity induced by haloperidol. CBD alone resulted in an increase in the number of type I microglial cells (surveilling); CBD decreased the haloperidol-induced increase in the number of type IV (hypertrophic) and V (bushy) microglial cells.
Barata, 2019[85]	Non-blind, randomized, placebo-	Piglets	New-born piglets underwent cerebral hypoxic-ischemic (HI) insult; piglets	Whole brain	CBD had no effect on the increased oxidative stress induced by HI; CBD alone did not reduce the HI-induced increase of TNFα

	controlled		received CBD (1mg/kg, i.v.) or veh at 1, 24h and 48h		levels nor the increased levels of apoptosis (but did so when combined with hypothermia); CBD resulted in smaller and more ramified microglial cells, suggesting reduced activation.
Saravia, 2019[86]	Blind, randomized, placebo-controlled	C57BL/6J mice	Nicotine dependence was initially induced; afterwards, nicotine withdrawal was precipitated; 4 days afterwards, CBD (10mg/kg, i.p.) or veh was injected for 4 days	Hippocampus; PFC	CBD abolished memory impairment caused by nicotine withdrawal. CBD attenuated the microglial reactive phenotype (in the CA1, dentate gyrus and in the PFC). CBD reduced the nicotine abstinence-induced *IL1b* (hippocampus) and *TNFα* (PFC) mRNA levels but had no effect on *IFNγ*. CBD had no effect on the expression of COX-1, COX-2, IL-6 and BDNF in the hippocampus.
Karimi-Haghighi, 2020[80]	Non-randomized, placebo-controlled	Wistar rats	Rats were exposed to chronic METH, followed by extinction. On reinstatement day, CBD (5µg/5ml, intracerebroventricular microinjection) or veh were administered 60min before an effective priming dose of METH. On two other groups, CBD or veh were administered after extinction, 60min before REM sleep-deprivation and then an ineffective priming dose of METH was administered.	Hippocampus; PFC	PFC: CBD had no effect on the increased *TNFα* mRNA expression induced by METH and by RSD; CBD decreased METH-induced increase in *IL1b*, *IL6* and *IL10* mRNA expression. Hippocampus: CBD reduced the METH induced increase in *TNFα*, *IL1b* and *IL6* mRNA expression; CBD intensified the increase in *IL1b*, *IL6* and *IL10* mRNA expression in RSD rats; CBD had no effect in METH-induced increase in IL-10 levels in non-RSD rats.
Nichols, 2020[87]	Non-randomized, placebo-controlled	C57BL/6 mice	EAE and mild EAE were induced in mice; 24h after induction, mice were administered daily with CBD (75mg/kg, p.o.) or veh for 5 days	Whole brain; Spinal cord	CBD reduced cellular infiltration induced by EAE (in the white matter tracts of the cerebellum and in the spinal cord); CBD reduced the number of $CD3^+CD4^+$ T cells (but not of $CD3^+CD8^+$ and $CD3^+CD4^+CD8^+$ T cells) and the lesions size in the spinal cord.
Watt, 2020[90]	Non-randomized, placebo-controlled	AβPPxPS1 and WT mice	Mice were injected daily with CBD (50mg/kg, i.p.) or veh for 7 weeks	Brain cortex; Hippocampus	CBD reversed behavioral deficits in social recognition memory and reversal spatial learning. CBD treatment had a moderate effect on A40 levels in the hippocampus which exhibited increased cortical Iba1 levels. CBD had no effect on microglia reactivity and on the levels of BDNF, TNFα and IL-1β.
di Giacomo,	Non-randomized, non-	Sprague-	Prefrontal cortexes were exposed to	PFC	CBD inhibited the hydrogen-peroxide-induced increase in ROS

2020[88]	placebo-controlled	Dawley rats	H_2O_2 followed by exposure to CBD (0,001-1µM) for 1h	production.

Table V: Studies addressing CBD effects in *in vitro* models

Author/Year	Cell Culture	Experimental Procedure	Results
Kozela, 2010[58]	BV-2 cell line	Cells were treated with CBD (1, 5, or 10μM); 2h later, cells were stimulated with LPS	No effect on cell viability was seen with CBD, either alone or with addition of LPS; CBD reduced the release of proinflammatory cytokines from LPS-stimulated cells but had no effect on unstimulated cells; these effects do not seem to be mediated by CB1 and CB2 receptors; CBD decreased the LPS activation of the pathway leading to NF-κB phosphorylation; CBD reduced the LPS-induced increase in CCL2 and SOCS3 mRNA with no effect on CISH mRNA
Esposito, 2011[72]	Rat primary astrocytes	Cultures were treated with Aβ, in the presence or absence of CBD (10^{-9}-10^{-7}M)	CBD reduced the Aβ-induced increase of NO, IL-1β, TNFα and S100B; CBD decreased reactive gliosis
Martín-Moreno, 2011[75]	Rat primary microglia	Cells were treated with LPS, alone or with CBD (10–1000 nM), for 24h	CBD promoted microglial cell migration and reduced LPS-induced NO generation
	BV-2 cell line	Cells were treated with LPS, alone or with CBD (10–1000 nM), for 24h	CBD reduced NO generation induced by LPS
Rimmerman, 2011[91]	BV-2 cell line	Cells were treated with CBD or veh for 30min; then cells were treated with CBD (10μM) or veh for 2h	CBD resulted in increased cell death, in serum-free medium, and in increased anandamide levels
Juknat, 2012 [64]	BV-2 cell line	Cells were treated with CBD for 2h and then stimulated with LPS	CBD prevented LPS-induced increase in IL-1β levels
		Cells were treated with CBD (10μM) or veh for 6h	CBD inhibited the expression of a subset of proinflammatory genes (particularly, chemokines ligands and *IL-1β*), induced a cellular stress response, and activated the pathway leading to autophagy and apoptotic cell death CBD increased the expression of *IFN1b*
Wu, 2012[96]	Murine primary microglial cells	Cells were treated with CBD (1μM) for 4h	CBD increased ROS formation
		Cells were assigned to 3 main groups: a) untreated, b) CBD (1-24μM), and c) veh, for 24h	CBD increased apoptosis in a concentration- and time-dependent manner
Juknat, 2013[65]	BV-2 cell line	Cells were treated with CBD (10μM) for 2h; LPS was added for another 4h	CBD inhibited LPS-induction of proinflammatory genes (in particularly, cytokines and chemokines), of matrix metalloproteinases and of positive regulators of inflammatory pathways; CBD reduced basal expression of chemokines genes (*Ccl2, Ccl7, Ccl9*) and

			LPS-induced activation of NF-κB; CBD also increased LPS-induced expression of *Gdf15/MIC-1* and *IL-15* and of *Lcn2* (involved in cell migration/invasion and inflammation)
Mecha, 2013[77]	Rat primary astrocytes	Cells were incubated with IL-1β + TNFα for 6h; then cells were treated with CBD (1 or 5μM) or veh	CBD inhibited IL-1β+TNFα-induced release of CCL2 but had no effect on basal CCL2 release in unstimulated cells
	Murine brain endothelial cells	Cells were treated with CBD (1 or 5μM) and 1h after cells were infected with TMEV virus	CBD inhibited TMEV-induced release of sVCAM-1
Hassan, 2014[69]	Murine primary microglia	Cells were treated with CBD (10μM) for 24h	CBD increased phagocytosis
	BV-2 cell line	Cells were treated with CBD (10μM) for 24h	CBD increased phagocytosis
	HAPI cells	Cells were treated with CBD (10μM) for 24h	CBD increased phagocytosis
	RAW-264.7	Cells were treated with CBD (10μM) for 24h	CBD increased phagocytosis
Janefjord, 2014[60]	BV-2 cell line SH-SY5Y Cells	Microglial cells were treated with LPS or Aβ with or without CBD (10μM); SH-SY5Y cells were then cultured in microglial-conditioned media	CBD suppressed LPS-induced microglial activation
Hind, 2016[92]	Human brain microvascular endothelial cells (HBMECs) Human astrocytes (HA)	Co-culture was incubated in ischaemic conditions and then returned to the reperfusion period; CBD (100nM, 1μM or 10μM) was given before or immediately after the previous protocol (OGD)	CBD reduced the OGD-induced increase in BBB permeability; CBD reduced lactate dehydrogenase (LDH) levels and VCAM-1 induced by OGD + reperfusion; CBD reduced IL-6 and VEGF levels
		HBMECs monoculture was treated with CBD (100nM, 1μM or 10μM) for 28h in normal conditions	CBD increased LDH and vascular endothelial growth factor (VEGF) levels and decreased VCAM-1 levels; IL-6 levels were affected dose-dependently (1μM decreased levels while 10μM increased levels)
		HA monocultures were treated with CBD (10μM) for 28h	CBD increased LDH levels and decreased IL-6 and VCAM levels
Ivanov, 2017[94]	Glioblastoma cell lines:	Cells were exposed to CBD (5-10μM) or γ-	CBD induced apoptosis of glioblastoma cells; CBD increased active NF-κB and heme

	U87MG, U118MG, and T98G	irradiation, alone or in combination	oxygenase-1 levels; CBD increased *TNFα*, *IL1β*, *IL6*, *IL8* mRNA levels and COX-2, TNFα and IL-6 levels
Sonego, 2018[79]	Murine primary microglia	Cells were pretreated with a PPARγ antagonist (GW9662); CBD (10μM) was added to the culture 30min later; after 4h, cells were stimulated with LPS	CBD prevented LPS-induced microglial activation and reduced the increased ROS production induced by LPS; CBD attenuated the increased NFκB translocation into the nucleus induced by LPS
Ivanov, 2019[95]	Glioblastoma cell lines: U87MG, U118MG, and T98G	Cells were exposed to CBD (5-40μM) and γ-irradiation, alone or in combination	CBD decreased cell viability; CBD increased *IL1β*, *IL6*, *IL8* and *FAS-L* mRNA levels; NF-κB levels remained stable
Juknat, 2019[68]	BV-2 cell line	Cells were treated with CBD (10μM) for 2h; LPS was added for another 4h	CBD decreased miR-146a in homeostatic cells and decreased the LPS-induced increase in miR-146a and -155-5p; CBD reduced LPS-induced increase in *IL6* and *Ifnb* mRNA levels; CBD increased miR-34-a
di Giacomo, 2020[88]	CTX-TNA2 rat astrocytes	Cells were pretreated or not with H_2O_2 for 3h and then treated with CBD (1000nM)	CBD increased cell proliferation of astrocytes cultured in basal conditions; CBD protected astrocytes from H_2O_2 damage and reduced ROS production; CBD reduced astroglial apoptosis
Dos-Santos-Pereira, 2020[93]	Murine primary microglia	Cells were pretreated with CBD (0,1μM, 1μM and 10μM) or veh and 2h later, stimulated with LPS for 30min or 24h A cell-free assay, using DPPH as a stable free radical, was subjected to two reference antioxidants and CBD (1; 10μM)	CBD reduced LPS-induced microglial activation; CBD prevented LPS-induced increase in TNFα, IL-1β, glutamate and ROS levels; CBD did not affect the increase in the number of $DAPI^+$ nuclei caused by LPS; CBD reduced p-NF-κB expression; CBD decreased LPS-induced [^{3}H]-2-DG uptake; CBD did not affect lactate release levels of LPS-treated cells but reduced NADPH production

Table VI - Characteristics of studies addressing the combinatory effects of THC and CBD in animal models

Author/Year	Study Design	Subjects	Experimental Procedure	CNS Region	Results
Valdeolivas, 2012[100]	Non-randomized, placebo-controlled	Sprague Dawley rats	Rats were subjected to intrastriatal malonate injection or sham operation; 30min before and 2h after this injection, animals were administered with a 1:1 combination of THC + CBD (4.63mg/kg; i.p.) or veh	Striatum	THC+CBD reduced the volume of edema, the activation of microglia and astrocytes and the *iNOS* and *IGF1* gene expression, caused by malonate
Feliú, 2015[99]	Non-randomized, placebo-controlled	TMEV-IDD-susceptible SJL/J mice (MS model)	Mice were either sham-operated or inoculated with TMEV; mice were treated daily with veh or a 1:1 combination of THC + CBD (Sativex®, 10mg/kg; i.p.)	Spinal cord	THC+CBD reduced cell infiltration (particularly of lymphocytes) and the expression of VCAM-1 and ICAM-1, caused by TMEV; Sham operated mice treated with THC+CBD showed no infiltrates; THC+CBD reduced microglial activation, astrogliosis, *IL1b* mRNA levels and chondroitin sulfate proteoglycan release and showed a tendency to decrease *TNFα* and *IFNγ* expression, in TMEV-treated mice; THC+CBD increased *Arg1* expression and showed a tendency to increase *IL10* mRNA, in TMEV-treated mice
Al-Ghezi, 2019 [101]	Non-blind, randomized, placebo-controlled	C57BL/6 mice	On day 10, EAE mice were treated with a 1:1 combination of THC + CBD (10mg/kg each, i.p.) or veh	Whole brain	THC+CBD reduced the number of infiltrating cells and histopathological signs of tissue damage provoked by EAE
				Spinal cord	THC+CBD reduced cellular infiltration caused by EAE
Al-Ghezi, 2019[98]	Non-blind, randomized, placebo-controlled	C57BL/6 mice	EAE mice were treated with a 1:1 combination of THC + CBD (10mg/kg each; i.p.) or veh for 5 days	Whole brain	THC+CBD decreased the populations of total mononuclear cells, $CD3^+$ T cells and $CD3^+CD4^+$ Th cells caused by EAE THC+CBD down-regulated miRs targeting IL-10, FoxP3, SOCS1, Bcl2L11 and CCNG1; THC+CBD upregulated miRs targeting IL-17A, TNFα and IL-6 THC+CBD upregulated Treg related genes (*Foxp3*, *Stat5b* and *IL10*) and Th2 related genes (*Gata3* and *IL4*); THC+CBD downregulated Th17 related genes (*Stat3* and *IL17a*) and Th1 related genes (*Tbx21* and *Ifng*); THC+CBD resulted in reduced production of IL-17A,IFNγ, TNFα, IL-6 and IL-1β, and increased production of IL-10 and TGF-β

Table VII: Characteristics of studies addressing the effects of combinations of THC and CBD in *in vitro* models

Author/Year	Cell Culture	Experimental Procedure	Results
Feliú, 2015[99]	Rat primary astrocytes	Cells were treated with TGFβ-1 and basic FGF (bFGF) and with a combination of CBD + THC (0.1µM Sativex®: 0.05 CBD-BDS + 0.05 THC+BDS; 0.50µM: 0.25 CBD-BDS + 0.25 THC-BDS; 1µM: 0.5 CBD+BDS + 0.5 THC+BDS) or veh	THC+CBD reduced CSPGs synthesis and release

Table VIII - Characteristics of studies addressing the effects of non-THC/CBD cannabinoids in animal models

Author/Year	Study Design	Subjects	Experimental Procedure	CNS Region	Results
Chen, 2017[103]	Non-blind, randomized, placebo-controlled	Sprague Dawley rats	Rats were treated with EFC (200 or 400mg/kg, intragastrically) or veh, in combination or not to D-gal-induced aging, for 14 weeks	Hippocampus	EFC, alone or in combination with D-gal, resulted in lower number of GFAP-positive cells compared to D-gal treated mice, representing lower astrocytes' reactivity; EFC, alone or in combination with D-gal, resulted in higher levels of SOD activity and lower levels of malondialdehyde compared to D-gal treated mice, reducing by this means hippocampal oxidative stress
Nadal, 2017[104]	Single-blind, non-randomized, placebo-controlled	C57BL/6 mice	7 injections of 3-NP were administered to mice to induce striatal degeneration every 12h; control mice received PBS injections; Mice were treated daily with veh or Δ^9-THCA (20mg/kg; i.p.)	Striatum	Δ^9-THCA reduced the 3-NP-induced upregulation of *TNFα, iNOS, and IL-6* gene expression and the increased expression of *Cox2*; Δ^9-THCA prevented the 3-NP-induced astrogliosis and microgliosis
Alegre-Zurano, 2020[107]	Non-blind, non-randomized, placebo-controlled	CD1 mice	Mice were divided into 5 groups: a) veh, b) CBDA (0,001mg/kg; i.p.), c) CBDA (0,01mg/kg; i.p.), d) CBDA (0,1mg/kg; i.p.), and e) CBDA (1mg/kg; i.p.); drugs were acute or repeatedly (10 days) administered	PFC	Repeated treatment (10 days) with CBDA, but not acute treatment, increased IL-6 levels and showed a decreasing trend in PPARγ levels

Table IX - Characteristics of studies addressing the effects of non-THC/CBD cannabinoids in *in vitro* models

Author/Year	Cell Culture	Experimental Procedure	Results
Hassan, 2014[69]	BV-2 cell line	Cells were treated with CBDA (10μM), CBDV (10μM) or THCV (10μM) for 24h	None of these substances had any effect on microglial phagocytosis
Gugliandolo, 2018[105]	RAW 264.7 cell line NSC-34 cell line	NSC-34 cells were treated with the cell culture medium of LPS-stimulated RAW macrophages; NSC-34 cells were pretreated with CBG (7.5μM) for 24h Controls included NSC-34 cells treated with the cell culture medium of unstimulated RAW macrophages, and cells treated with veh or CBG alone	CBG reduced the LPS-stimulated macrophages induced increase in proinflammatory cytokines (IL-1β, TNFα, and IFNγ) and PPARγ protein levels; CBG reduced oxidative stress (by decreasing nitrotyrosine levels, *SOD1* and *iNOS* expression)
Wang, 2019[106]	BV-2 cell line	Cells were pretreated with Cannabisin F (0, 5, 10 and 15μM) for 1h and co-cultured in the absence or presence of LPS for 24h	Cannabisin F had no effect on cell viability; Cannabisin F decreased the LPS-induced increase in IL-6 and TNFα protein and mRNA levels; Cannabisin F reversed the LPS-induced production of ROS and prevented NF-κB activation; Cannabisin F increased the expression of *Nrf2* and *HO-1*
di Giacomo, 2020[88]	CTX-TNA2 rat astrocytes Prefrontal cortex *ex-vivo*	Cells were treated with H_2O_2 for 3h and with CBG (1-1000nM) Prefrontal cortexes were exposed to H_2O_2 followed by exposure to CBG (0,001-1μM) for 1h	CBG increased proliferation of astrocytes cultured in basal conditions; CBG protected astrocytes from H_2O_2 damage and reduced ROS production; CBG reduced astroglial apoptosis CBG inhibited the hydrogen-peroxide-induced increase in ROS production

Table X - Characteristics of studies addressing the effects of non-cannabinoid cannabis components in animal models

Author/Year	Study Design	Subjects	Experimental Procedure	CNS Region	Results
Zhou, 2018[108]	Non-blind, randomized, placebo-controlled	Kunming mice	Mice were divided into 5 groups: a) control (1-3 weeks; saline), b) model (3rd week; saline), c) TPA (1-3 weeks; 1 or 2g/kg/day), and d) piracetam (2-3 weeks; 0,4g/kg/day) After 2 weeks, groups b, c and d were injected with LPS for another week	Whole brain	TPA (1g/kg) reduced LPS-induced increased levels of IL-1β, IL-6 and TNFα, while TPA (2g/kg) had no effect

Table XI - Characteristics of studies addressing the effects of non-cannabinoid cannabis components in *in vitro* models

Author/Year	Cell Culture	Experimental Procedure	Results
Askari, 2019[110]	OLN-93 cell line	Cells were preincubated for 24h with veh (control group) or BCP (0.1-50μM); after, cells were treated with veh or BCP + LPS for another 24h	Pre-incubation of cells with BCP had no effect on cell viability; BCP decreased the LPS-induced increased levels of NO, ROS and TNFα but reversed the reduction in PPAR-γ levels; BCP increased HO-1, Nrf2 and GSH levels in comparison to cells treated with LPS alone
Wang, 2019[106]	BV-2 cell line	Cells were pretreated with veh or CLG (5, 10 or 15μM) for 1h and then LPS was added	CLG had no effect on cell viability; CLG decreased the LPS-induced increase in *IL1b* and *IL6* protein and mRNA levels and reduced LPS-induced ROS production; CLG decreased the LPS-induced increase of TLR4 and NF-κB and AMPK phosphorylation
Rodriguez-Martin, 2019[109]	BV-2 cell line	Cells were exposed to veh or LPS, alone or in combination with HPI or HPH; 50 or 100μg/ml)	HPI/HPH had no effect on cell viability; HPI and HPH reduced the LPS-induced increase in *IL6* and *TNFα* mRNA levels; HPH also reduced the LPS-induced *IL1b* mRNA levels and upregulated *IL10* mRNA expression

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