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Review

The molecular mechanisms that underpin the biological benefits of full-spectrum cannabis extract in the treatment of neuropathic pain and inflammation

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ABSTRACT

Cannabis has been shown to be beneficial in the treatment of pain and inflammatory diseases. The biological effect of cannabis is mainly attributed to two major cannabinoids, tetrahydrocannabinol and cannabidiol. In the majority of studies to-date, a purified tetrahydrocannabinol and cannabidiol alone or in combination have been extensively examined in many studies for the treatment of numerous disorders including pain and inflammation. However, few studies have investigated the biological benefits of full-spectrum cannabis plant extract. Given that cannabis is known to generate a large number of cannabinoids along with numerous other biologically relevant products including terpenes, studies involving purified tetrahydrocannabinol and/or cannabidiol do not consider the potential biological benefits of the full-spectrum cannabis extracts. This may be especially true in the case of cannabis as a potential treatment of pain and inflammation. Herein, we review the pre-clinical physiological and molecular mechanisms in biological systems that are affected by cannabis.

1. Introduction

Neuropathic pain is initiated by a damage to the nervous system which might be attributed to infectious agents such as human immunodeficiency virus (HIV), metabolic disease, neurodegenerative disease, multiple sclerosis (MS) and physical trauma [1,2]. Regardless of the cause, damage to the nervous system and subsequent neuropathic pain can be accompanied by dysesthesia (abnormal sensations) or allodynia (pain from non-painful stimuli) [2,3]. As the pathophysiology of neuropathic pain is complex [3–5], the current therapeutic modalities are still limited. Hence, it is imperative to find a new therapeutic agent that helps treat or minimize the symptoms associated with neuropathic pain disorder.

Cannabis is a promising plant-based medicine that has garnered much attention of late [6,7] for the treatment of various conditions associated with pain and inflammation [8,9]. The potential health implications of cannabis are accredited to Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) [10–13]. In the majority of studies to-date, THC and CBD alone or in combination have been examined for the treatment of various disorders, such as pain and inflammation [10–13]. However, few studies have investigated the biological benefits of full-spectrum cannabis plant extract [10–13]. Given that cannabis is known to produce a large number of cannabinoids along with numerous other biologically relevant products including terpenes and others [14], it stands to reason that studies involving purified THC and/or CBD may not accurately reflect the potential biological benefits of the full-spectrum cannabis extract especially with regard to their crucial role in the treatment of neuropathic pain and inflammation [7,15].

Therefore, the goal of this review is to discuss the current knowledge about the potential beneficial effects of full-spectrum cannabis extract in pre-clinical studies involving rodents with neuropathic pain and inflammation.

2. Overview of the endocannabinoid system and cannabinoids

In 1964, Dr. Raphael Mechoulam discovered THC, which was the first identified cannabinoid [16]. This groundbreaking work paved the way for the discovery of the endogenous cannabinoid system [17] of which anandamide and 2-arachidonoylglycerol are considered the main endogenous cannabinoids in higher order mammals, including humans [17]. Both anadamide and 2-arachidonoylglycerol regulate the sensitivity of serotonin, dopamine, gamma-aminobutyric acid (GABA) and glutamate in the central nervous system (CNS) [18], thus demonstrating how these endogenous cannabinoids regulate many physiological and pathological processes such as pain, immune response, appetite, thermoregulation, energy metabolism, depression, memory and fertility

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[18].

3. Endocannabinoids

Anandamide was the first endocannabinoid isolated and it is chemically characterized as N-arachidonoylethanolamine. The name of anandamide originates from Sanskrit term ananda, which refers to "bliss". Bliss is defined as euphoria that involves physiologic and psychologic harmony [17]. Anandamide is synthesized from the precursor N-arachidonoyl phosphatidylethanolamine by phosphodiesterase phospholipase D enzyme [19]. Once anadamide is synthesized, it is released from the neuronal terminal in a calcium ion-dependent manner and binds to presynaptic cannabinoid receptors (CB) [20]. Anandamide is then rapidly up taken by neurons and astrocytes where it is degraded by fatty acid amide hydrolase (FAAH) into ethanolamine and arachidonic acid [21]. The other endogenous cannabinoid is 2-arachidonoylglycerol, which is synthesized by the hydrolysis of an inositol-1,2-diacylglycerol by phospholipase C [19,22]. Similar to anadamide, 2-arachidonoylglycerol binds to CB receptors and undergoes rapid biological degradation and catalytic hydrolysis, which is mediated by monaoacylglycerol lipase (MGL) [20,23]. Of importance, MGL along with FAAH are considered potential therapeutic targets that can regulate endocannabinoid levels [20] (Fig. 1).

4. Phytocannabinoids

The most well characterized phytocannabinoids are THC, CBD, cannabinol (CBN), cannabigerol (CBG) and cannabichromene (CBC). These botanical cannabinoids exist as inactive monocarboxylic acids containing precursors referred to as tetrahydrocannabinoic acid (THCA), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), and cannabichromenic acid (CBCA), respectively. The presence of a carboxylic acid moiety on these chemicals precludes cannabinoids, particularly THC, from being bioavailable and binding to either CB receptors or other biological targets [24]. Thus, the conversion of THCA, CBDA, CBGA, and CBCA to THC, CBD, CBN, CBG, and CBG, respectively, through decarboxylation is necessarily before any biological effect can be observed [24]. Decarboxylation of these carboxylic acids can be promoted by heating the plant above 105 °C, which can be achieved during the smoking or baking process [24] (Fig. 2).

4.1. THC

THC is the primary psychoactive component of *Cannabis sativa* and chemically analogous to N-arachidonoylethanolamine [25,26]. THC is a euphoric agent that has anti-nociceptive, anti-inflammatory, sedative and muscle relaxant effects [27]. Additionally, THC increases appetite, dilates bronchial muscle and it has anti-emetic, anti-spasmodic, neuroprotective and anti-oxidant properties [28,29]. Mechanistically, the physiological effect of THC is mediated primarily through the activation of CB1 and CB2 receptors with preferential binding to CB1 receptors [27]. The major undesirable effect of THC is cognitive dysfunction particularly the loss of short-term memory consolidation [30]. This effect might be attributed to the ability of THC to inhibit *N*-methyl-D-aspartate (NMDA) receptor activity in addition to the decrease in the hippocampal acetylcholine release [31,32]. The decrease in acetylcholine release may be due to the activation of the CB1 receptor on parasympathetic neurons [27]. Intriguingly, it has recently been shown that a low dose of THC reversed the age-related decline in cognitive performance in aged but not young mice [33]. This effect was associated with increased expression of synaptic marker proteins and enhanced hippocampal spine density through glutamatergic CB1 receptors-dependent mechanism [33]. Thus, this study raises the possibility that THC or full-spectrum cannabis extracts may have the potential to reverse cognitive decline in the elderly and suggests an age-dependent effect of THC.

4.2. CBD

CBD is the primary non-psychoactive component of *Cannabis sativa* [25] and possesses sedative, anti-inflammatory, anti-convulsive and anti-psychotic actions, but does not have the typical THC side effects [34]. Of importance, the powerful anti-convulsant effect of CBD appears to be mediated through a CB receptor-independent mechanism [35]. Indeed, CBD mediates neuronal inhibition and anti-epileptic effects through gamma-aminobutyric acid A (GABA-A) and adenosine A1 receptors dependent mechanisms [35]. In addition, CBD has anti-psychotic and neuroprotective effects that are mediated via increasing the effect of dopamine and norepinephrine, activating the 5-hydroxytryptamine 1A (5-HT1A) receptor, inhibiting adenosine transporter, blocking T-type voltage-gated calcium channels and reducing glutamate induced-neurotoxicity [36–38]. Numerous additional effects of CBD have also been reported. For instance, in the heart, CBD inhibits THC-induced tachycardia through the activation of adenosine A1 receptor [35]. Moreover, it has been reported that CBD protects against cardiac dysfunction, fibrosis, oxidative stress, and cell death signaling pathways in diabetic cardiomyopathy and doxorubicin-induced cardiotoxicity [39–42]. In addition to the cardiac effects, CBD has recently been shown to be cytotoxic in estrogen receptor-positive and triple negative breast cancer cells through the induction of apoptosis [43] as

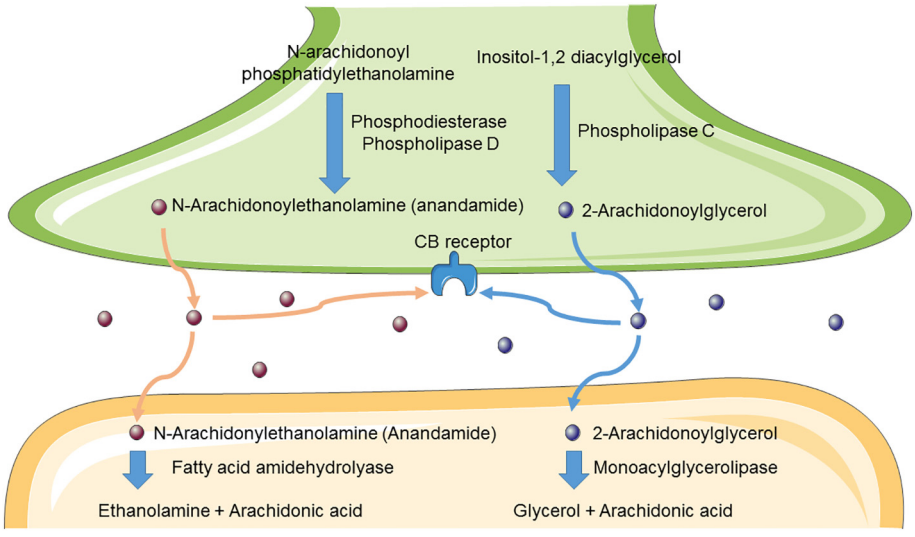

Fig. 1. The synthesis and the metabolism of endocannabinoids.
Anandamide is synthesized from the precursor N-arachidonoyl phosphatidylethanolamine by phosphodiesterase phospholipase D enzyme. Once anadamide is synthesized, it is released from the neuronal terminal in a calcium ion-dependent manner and binds to presynaptic cannabinoid receptors (CB). Anandamide is then rapidly up taken by neurons and astrocytes where it is degraded by fatty acid amide hydrolase (FAAH) into ethanolamine and arachidonic acid. The other endogenous cannabinoid is 2-arachidonoylglycerol, which is synthesized by the hydrolysis of an inositol-1,2-diacylglycerol by phospholipase C. Similar to anadamide, 2-arachidonoylglycerol binds to CB receptors and undergoes rapid biological degradation and catalytic hydrolysis, which is mediated by monaoacylglycerol lipase (MGL).

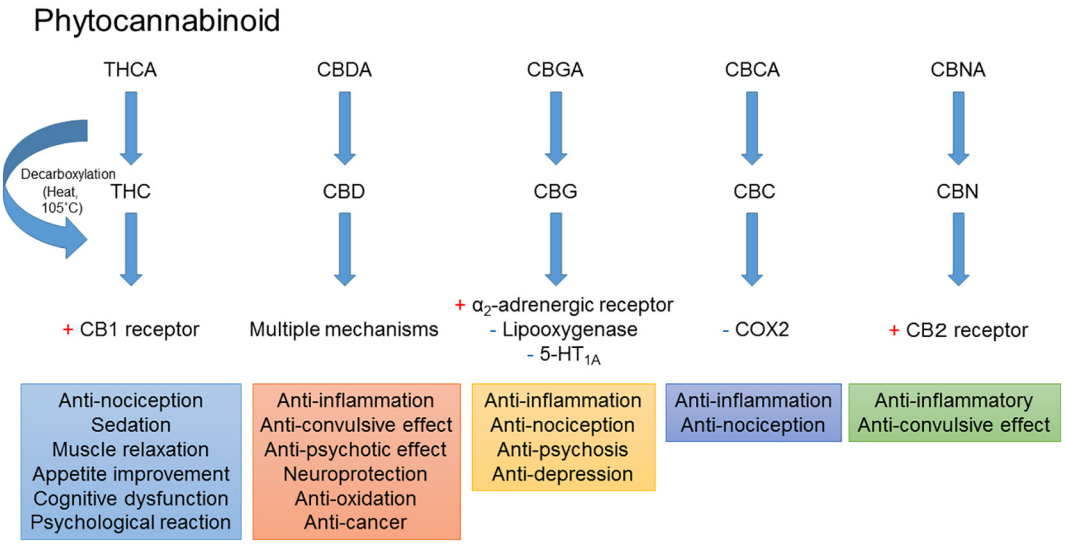

Fig. 2. The pharmacological effects of phytocannabinoids.
THC: tetrahydrocannabinol, CBD (cannabidiol), cannabinol (CBN), cannabigerol (CBG) and cannabichromene (CBC) are the most well characterized phytocannabinoids. These botanical cannabinoids exist as inactive monocarboxylic acids containing precursors referred to as tetrahydrocannabinoic acid (THCA), Cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), and cannabichromenic acid (CBCA), respectively. The presence of a carboxylic acid moiety on these chemicals precludes cannabinoids, particularly THC, from being bioavailable and binding to either CB receptors or other biological targets. Thus, the conversion of THCA, CBDA, CBGA, and CBCA to THC, CBD, CBN, CBG, and CBG, respectively, through decarboxylation is necessarily before any biological effect can be observed. Decarboxylation of these carboxylic acids can be promoted by heating the plant above 105 °C, which can be achieved during the smoking or baking process. + indicates activates. - indicates inhibits.

well as it increases the uptake of the chemotherapeutic agent, doxorubicin, to induce apoptosis in these cells through transient receptor potential vanilloid type-2 (TRPV2)-dependent mechanism [44]. Thus, the potential benefits of CBD are extensive, even independent from the classical endocannabinoid system involving CB receptors.

4.3. Cannabinol (CBN)

CBN is an oxidized by-product of THC produced in trace amounts by aged cannabis upon long exposure to air [45]. Studies have shown that while CBN is inactive when administered alone to healthy volunteers, it still can potentiate the sedative effect of THC [46]. Given that CBN is closely related to CBD in terms of the chemical structure, it shares the anti-convulsant and anti-inflammatory effects with CBD [45,47]. The physiological effect of CBN is attributed to the modulation of the CB2 receptor with lower affinity for the CB1 receptor in comparison to THC [48].

4.4. Cannabichromene (CBC)

CBC is one of the main phytocannabinoids and appears to have no affinity to CB1 and CB2 receptors [49]. Similar to CBD and THC, CBC possesses anti-inflammatory [50] and anti-nociceptive effects [51] through the inhibition of the cyclooxygenase enzyme and its associated prostaglandins [52]. In contrast to CBD, CBC neither has an anti-convulsant effect nor inhibits the activity of cytochrome P450 (CYP) [51,53].

4.5. Cannabigerol (CBG)

CBG is the precursor phytocannabinoid compound of THC, CBD and CBC and is only produced in trace amounts in cannabis. Although CBG has low affinity to CB receptors, it is still capable of reducing pain, erythema and inflammation through the inhibition of peripheral lipooxygenase enzyme and the activation of central α_2-adrenergic receptor [47,54]. Furthermore, CBG has an anti-depressant effect because it is a potent anadamide uptake inhibitor [55] as well as a moderate 5-HT1a antagonist [56].

5. Cannabinoid receptors and their role in regulating pain and inflammation

5.1. CB1 and CB2 receptors

A plethora of studies have demonstrated that phytocannabinoids mediate their pharmacological actions by binding to CB1 and CB2 receptors and through the regulation of the production and the degradation of endogenous endocannabinoids [57]. Both CB1 and CB2 receptors are 7-domain $G_{i/o}$-protein coupled receptors decreasing the level of cyclic-AMP by suppressing adenylate cyclase [57]. CB1 receptors are abundant and widely expressed throughout the CNS (i.e. the cerebellum, basal ganglia, hippocampus, cerebral cortex, and spinal cord) and they are responsible for the psychopharmacological and analgesic effects of THC [58,59]. Of particular interest, CB1 receptors have high expression level in areas of the brain that are implicated in nociceptive perception, such as the thalamus and amygdala [58,59], the midbrain periaqueductal grey matter cells [60], and the substantia gelatinosa of the spinal cord [61]. The presynaptic localization of CB1 receptors enables cannabinoids to modulate neurotransmitter release such as dopamine, noradrenaline, glutamate, GABA, serotonin and acetylcholine [62]. The activation of the CB1 receptors in the aforementioned brain areas modulates nociceptive thresholds and produces multiple biological effects by regulating the balance between excitatory and inhibitory neurotransmitters [63] (Fig. 3).

While the CB2 receptor has limited expression in sensory and CNS cells, it is mainly expressed in peripheral tissues, including keratinocytes and tissues of the immune system such as the lymphatic system [64]. The CB2 receptor was shown to contribute to analgesia through suppressing the release of inflammatory mediators by cells located adjacent to nociceptive nerve terminals [65]. In addition, activation of peripheral CB2 receptors blocks the transduction of pain signals into the CNS [66]. Given that CB2 receptors are expressed in several types of inflammatory cells and immunocompetent cells [65,66], it is reasonable to assume that the activation of peripheral CB2 receptors may

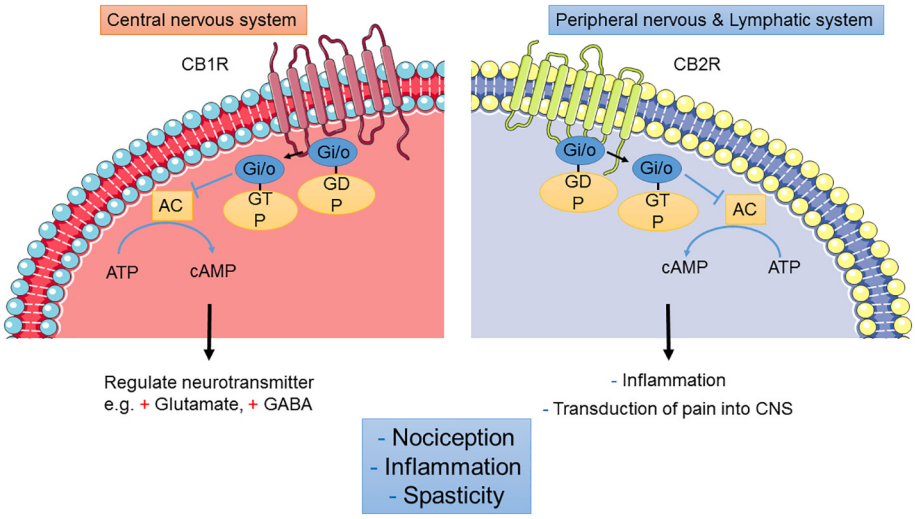

Fig. 3. Proposed anti-nociceptive and anti-inflammatory mechanism of cannabinoid receptors. Both CB1 and CB2 receptors are 7-domain Gi/o-protein coupled receptors decreasing the level of cyclic-AMP by suppressing adenylate cyclase. The activation of the CB1 receptors centrally modulates nociceptive thresholds and produces multiple biological effects by regulating the balance between excitatory and inhibitory neurotransmitters, glutamate and GABA, respectively. The activation of CB2 receptor peripherally suppresses the release of inflammatory mediators by cells located adjacent to nociceptive nerve terminals and blocks the transduction of pain signals into the CNS. CB1R: cannabinoid receptor type 1, CB2R: cannabinoid receptor type 1, Gi/o: G_i alpha subunit of G protein-coupled receptors, GDP: guanosine diphosphate, GTP: guanosine triphosphate, AC: adenylyl cyclase, ATP: Adenosine triphosphate, cAMP: cyclic-adenosine monophosphate. + indicates activates. - indicates inhibits.

contribute to analgesic effect in conditions of inflammatory hyperalgesia and neuropathic pain such as MS. Consistent with this notion, increased numbers of microglia/macrophage cells expressing CB2 receptor have been reported in spinal cords derived from MS patients relative to controls [67], suggesting the involvement of CB2 receptor in the regulation of pain and inflammation in MS patients. Based on these findings, it was proposed that cannabinoid-based pharmacotherapies might be effective therapies for the reduction of pain due to MS [68] (Fig. 3).

5.2. CB1/CB2-independent signaling pathways

The anti-nociceptive effect of cannabinoids might not necessarily be due entirely to the activation of CB1 and CB2 receptors. Indeed, the analgesic effects may be due to the modulation of the transient receptor potential vanilloid 1 (TRPV1). The evidence supporting this is based on the observation that the anti-nociceptive effect of CBD in neuropathic rats was completely reversed by capsazepine, a known TRPV1 activator [15]. Other receptor sites implicated in the action of CBD include the suppression of putative novel cannabinoid G protein coupled receptor GPR55, NMDAR [69,70] and α1-adrenoreceptors and the activation of 5HT1A, adenosine A2, and the peroxisome proliferator-activated gamma (PPAR-γ) receptors [71]. In addition, THC and CBD are positive allosteric modulators of the μ- and δ-opioid receptors [72], suggesting the involvement of these receptors in the anti-nociceptive effect of THC and CBD. Moreover, CBD has been shown to block low-voltage-activated (T-type) Ca^{+2} channels [73], stimulate the glycine-receptor, and modulate the activity of FAAH [74,75]. The action of CBD via these pathways may be responsible for the suppression of neuronal excitability and pain perception [76–78]. In addition, there is evidence that CBD inhibits synaptosomal uptake of dopamine, noradrenaline, GABA, serotonin in addition to cellular uptake of anandamide [55,79,80]. The modulation of these neurotransmitters might explain the neuroprotective and the anti-nociceptive effects of CBD. Moreover, CBD and THC have been shown to inhibit the cycloxygenase-2 enzyme and the production of arachidonic acid metabolites, prostaglandins, suggesting anti-inflammatory effects [40,81,82]. Of note, the inhibition of cycloxygenase-2 was associated with an increase in the level of endocannabinoids, anandamide and 2-AG [83]. This observation suggests that the suppression of cycloxygenase-2 enzyme by CBD and THC may not only decrease nociceptive and inflammatory prostaglandins but it may produce an indirect increase in the level of endocannabinoids, anandamide and 2-AG (Fig. 4).

6. Cannabinoids and the gut microbiome

Another important biological system that is affected by cannabinoids, at least when consumed orally, is the gastrointestinal microbiota (gut microbiome) [84]. The gut microbiota is known to produce various metabolites resulting from the fermentation of molecules of either exogenous source (i.e. from dietary intake) or from endogenous origin (i.e. bile acids) [85,86]. These metabolites can act as signals that can contribute to the maintenance of host immunity and physiology [87]. For example, the gut bacteria *Lactobacillus acidophilus*, metabolizes tryptophan from dietary sources such as eggs, milk, red meat, and vegetables into diverse metabolites, including indole propionic acid, which can signal through the aryl hydrocarbon receptor (AhR) [88,89]. Activation of AhR halts inflammation notably through induction of interleukin-22 (IL-22) [88]. Of interest, activation of AhR, dependent or independent of the gut microbiota, has been reported to limit the production of microglial pro-inflammatory mediators such as transforming growth factor alpha (TGFα) and vascular endothelial growth factor B (VEGF-B) in a mouse model of MS [90,91]. Interestingly, oral administration of *Lactobacillus acidophilus* was shown to combat inflammation and nociception through increasing the expression of the CB2 receptor in intestinal epithelial cells [92], suggesting that probiotics and cannabinoids might work together to halt inflammation and nociception. In support of this, it has recently been shown that THC reduces inflammation and adiposity in mice by increasing the accumulation of mucin-degrading bacteria, *Akkermansia municiphila* [84]. Of relevance, *Akkermansia municiphila* supplementation was shown to reduce systemic inflammation in mice [93], further supporting the notion that microbiota contributes to the anti-inflammatory and analgesic effects of oral cannabinoids.

7. Cannabis terpenoids

Terpenes are volatile unsaturated hydrocarbons that represent the largest group of plant organic chemicals with around 20,000 fully characterized compounds [94]. Terpenes account for a unique aroma of cannabis [94], but there is evidence that they may play more of a role in biology than simply affecting taste/aroma of cannabis. Indeed, while > 200 terpenes have been identified in cannabis, 3 monoterpenes, (myrcene, D-limonene and pinene), and sesquiterpenoid, (beta-caryophyllene), have been shown to have biological importance [7,15]. Indeed, sedation and a decrease in motility have been observed in mice upon inhalation exposure to terpenoids in a concentrations equivalent to 0.05% v/w for 1 h [95]. In addition to having their own independent effects, these cannabis-derived terpenoids have been

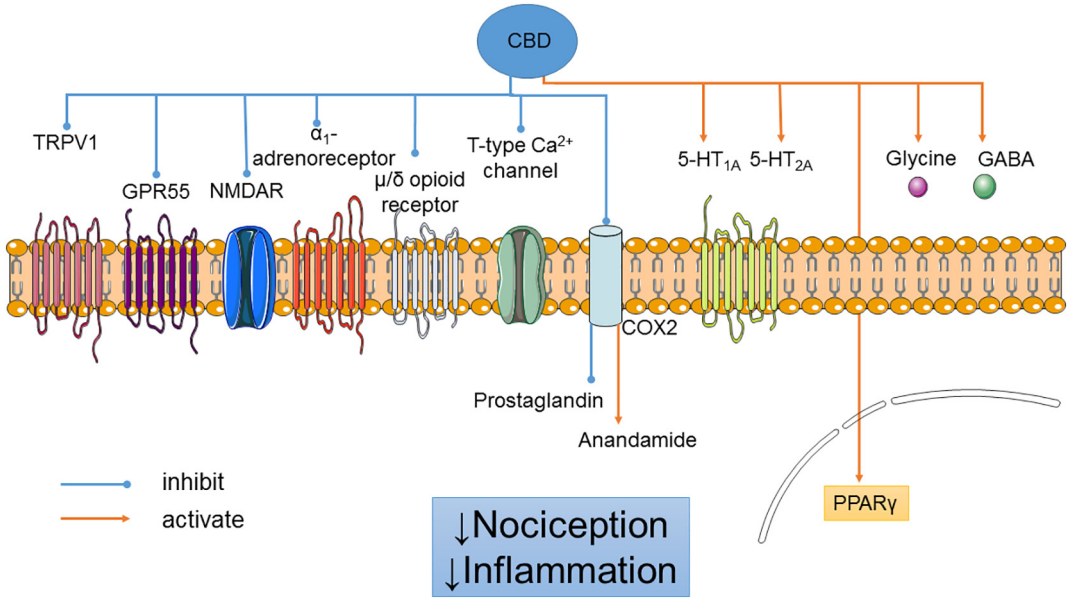

Fig. 4. Proposed analgesic and anti-inflammatory effect of CBD.
The analgesic effects of CBD may be due to the suppression of TRPV1, GPR55, NMDAR and α1-adrenoreceptors, T-type Ca^{+2} channels and μ-/δ-opioid receptors in addition to the activation of 5HT1A, adenosine A2, glycine, GABA, and PPAR-γ receptors. CBD may also decrease nociceptive and inflammatory prostaglandins and produce an indirect increase in the level of endocannabinoids, anandamide and 2-AG, via suppressing COX2. TRPV1: transient receptor potential cation channel subfamily V member 1, GPR55: G protein-coupled receptor 55, NMDR: The *N*-methyl-D-aspartate receptor, COX2: cyclooxygenase, 5-HT: 5-hydroxytryptamine receptors, PPARγ: peroxisome proliferator-activated receptor gamma.

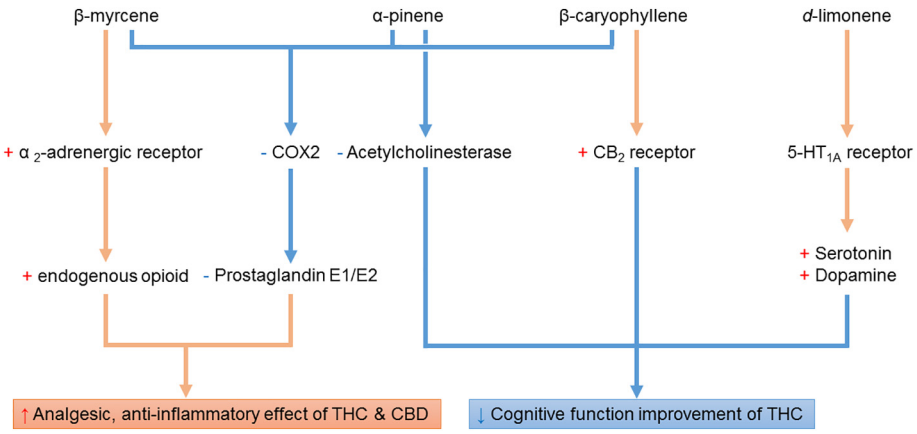

Fig. 5. The pharmacological effect of cannabis terpenoids.
β-myrcene may enhance the analgesic effect of THC and CBD centrally by stimulating the release of endogenous opioids through α2-adrenergic receptor dependent mechanism. β-myrcene, α-pinene and β-caryophyllene synergize the anti-nociceptive and anti-inflammatory effects of CBD peripherally by inhibiting cycloxygenase-2 (COX-2) dependent prostaglandin E2. α-pinene, β-caryophyllene and D-limonene minimize THC-mediated cognitive dysfunction via inhibiting the activity of acetylcholinesterase in the brain, activating CB2 receptor, and increasing serotonin and dopamine in the prefrontal cortex and hippocampus through 5-HT1A receptor, respectively.

postulated to modulate the effects of cannabinoids via what has been termed the "entourage effect" [7,15]. However, it is important to note that many terpenoids appear to modulate molecular/biological functions only when the concentration of the terpene in full-spectrum cannabis extract is above 0.05% v/w [96,97]. Given that terpenoids would have a pharmacological interest only if their concentration is above 0.05% v/w [96,97], we will review the literature on the terpenes that meet this threshold including, β-myrcene (0.47% v/w), β-caryophyllene (0.05% v/w), D-limonene (0.14% v/w) and α-pinene (0.049% v/w) [96–98] (Fig. 5).

7.1. β-myrcene

β-myrcene, a noncyclic monoterpene, has been extensively used for centuries as a pain-killer [99]. β-myrcene may enhance the analgesic effect of THC and CBD centrally by stimulating the release of endogenous opioids through α_2-adrenergic receptor dependent mechanism [99]. Of interest, β-myrcene appears to also synergize the anti-nociceptive and anti-inflammatory effects of CBD peripherally by inhibiting cycloxygenase dependent prostaglandin E2 [100]. In addition to this analgesic effect, β-myrcene has been shown to enhance the chemopreventive effects of CBD by inhibiting the metabolism of Aflatoxin B1, a poisonous carcinogen produced by *Aspergillus flavus*, through CYP2B1 enzyme [101], indicating multiple potential benefits of β-myrcene. Overall, due to the potent sedative and muscle relaxant effects of β-myrcene, it is thought that this terpenoid is involved in the "couch-lock" phenomenon of THC [102].

7.2. α-pinene

α-pinene is a bicyclic monoterpene that acts as an insect repellent in the cannabis plant [103]. In higher organisms, α-pinene may potentiate the peripheral anti-inflammatory and anti-nociceptive effects of CBD through the inhibition of cycloxygenase-2 and prostaglandine E2 [104]. Of particular interest, α-pinene was shown to inhibit the activity of acetylcholinesterase in the brain [105,106]. Thus, it has been postulated that such an effect would aid memory and minimize cognitive dysfunction induced by THC intoxication [106].

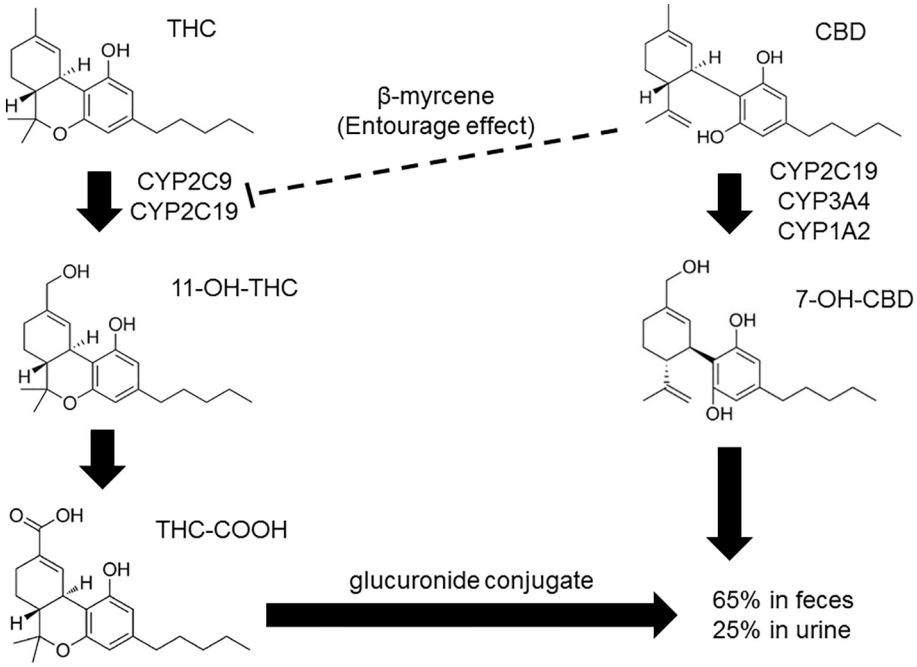

Fig. 6. Metabolism of THC and CBD.
CYP2C9 and CYP2C19 are the main enzymes responsible for the metabolism of THC into 11-OH-THC. While 11-OH-THC is psychoactive like THC, it is oxidized in the liver into an inactive metabolite THC-COOH which is eventually conjugated to glucuronic acid by UDP-glucuronosyltransferase. The presence of the glucuronide group increases the polarity and thus water solubility of this metabolite, which facilitates its excretion in the urine. While 25% of THC metabolites are excreted in the urine, > 65% of these metabolites are eliminated in the feces. Orally administered CBD is extensively metabolized into an inactive metabolite, 7-OH-CBD, by the CYP2C19, CYP3A4, CYP1A1, CYP1A2 and CYP2D6 enzymes in the liver. CBD and 7-OH-CBD are then eliminated in the feces, with a minor amount being excreted in the urine. CBD inhibits CYP2C9 and CYP2C19 mediated hydroxylation of THC to 11-OH-THC. The effect of CBD on hepatic CYP enzymes is enhanced by β-myrecene.

7.3. β-caryophyllene

β-caryophyllene is the most common sesquiterpenoid, a class of terpenes that contain three isoprene units, and one of the most predominant terpenoids in cannabis extracts [107]. There is evidence that β-caryophyllene may synergize the anti-inflammatory and the analgesic effect of THC through the inhibition of prostaglandin E1 and the activation of the CB2 receptor, respectively [108,109]. Therefore, the synergistic effect of β-caryophyllene with THC strongly suggests that the potential health benefits of complete cannabis extracts may be more evident in the treatment of inflammation compared to THC alone. Of interest, the anti-inflammatory activity of β-caryophyllene is comparable to the potency of the nonsteroidal anti-inflammatory drug, phenylbutazone [108], suggesting that this terpene is a potent anti-inflammatory molecule. β-caryophyllene also inhibits lipopolysaccharide (LPS)-induced proinflammatory cytokine expression through the activation of the CB2 receptor [109]. On the other hand, β-caryophyllene lacks any anti-inflammatory or anti-nociceptive activities in mice lacking CB2 receptors suggesting that β-caryophyllene exhibits cannabimimetic-dependent effects [109]. In support of this, β-caryophyllene has been identified as a natural selective agonist of the peripherally expressed CB2 receptor [109–111]. Of importance, oral administration of β-caryophyllene attenuates thermal hyperalgesia, mechanical allodynia and spinal neuroinflammation in a neuropathic pain model [112] and suppresses neuroinflammation in an animal model of MS suggesting that β-caryophyllene is an effective anti-inflammatory molecule for the treatment of MS [111].

7.4. D-limonene

D-limonene is the second most widely distributed terpenes found in nature. While D-limonene has a low affinity for cannabinoid receptors [113], it synergizes the anxiolytic, anti-stress and sedative effects of CBD by increasing serotonin and dopamine in the prefrontal cortex and hippocampus through 5-HT_{1A} receptor [114,115]. Similar to myrcene, D-limonene suppresses the metabolism of Aflatoxin B1 to its carcinogenic active metabolite [116] and thus it may act as a chemopreventive agent. Furthermore, D-limonene was shown to induce apoptosis in human breast cancer cells [117], and this effect has been postulated to potentiate the antitumor activity of CBD in advanced stages of breast cancer [44,118] (Fig. 5).

8. Metabolism of cannabinoids

Cannabis is commonly administered via inhalation (smoked cannabis or vaporization) or orally in the form of syrup (e.g. Epidiolex), oromucosal aerosol (e.g. Sativex) or capsule (e.g. Cannador) [57,119–122]. Since inhaled therapies involving cannabis may pose certain health risks, alternative routes of delivery such as oral administration have been studied. Perhaps one of the largest challenges associated with the use of cannabinoids as an orally available medical therapy is their low bioavailability. Although cannabinoids are highly absorbed when administered orally, they have a very low bioavailability due to the first pass metabolism [123]. Of interest, this issue might be less pronounced in the full extract [15,124], as it has been suggested that other components present in the full-spectrum cannabis extract modulate the bioavailability of THC and CBD [15,124].

The route of administration of cannabinoids determines their rate of absorption and bioavailability. The high absorption rate of THC upon inhalation either by smoking or vaporization results in a time to peak plasma levels of 6–10 min and a bioavailability of 10–35% [122,123]. In contrast, when THC is administered orally, the time to peak plasma levels is between 2 and 6 h and the bioavailability is very low (~6%) [123]. Similar to THC, CBD has poor oral bioavailability but CBD has a higher absorption rate than THC with a time to peak plasma level of 2 h [125]. Notably, the absorption of both THC and CBD can be improved using oil vehicle such as sesame oil or a glycocholate solution [123,126], suggesting that the administration of THC and CBD using oil based formulation might be the best existing oral dosage form for THC and CBD (Fig. 6).

The poor oral bioavailability of cannabinoids is largely attributed to the first pass metabolism in hepatic tissue [127,128]. CYP2C9 and CYP2C19 are the main enzymes responsible for the metabolism of THC into 11-OH-THC [127,128]. While 11-OH-THC is psychoactive like THC, it is oxidized in the liver into an inactive metabolite THC-COOH which is eventually conjugated to glucuronic acid by UDP-glucuronosyltransferase [57,129,130]. The presence of the glucuronide group increases the polarity and thus water solubility of this metabolite, which facilitates its excretion in the urine. Thus, the THC-COOH glucuronide conjugate in the urine is considered to be a good biomarker

for THC-containing cannabis use [131]. Interestingly, while 25% of THC metabolites are excreted in the urine, > 65% of these metabolites are eliminated in the feces [132,133] (Fig. 6).

Similar to THC, orally administered CBD is extensively metabolized into an inactive metabolite, 7-OH-CBD, by the CYP2C19, CYP3A4, CYP1A1, CYP1A2 and CYP2D6 enzymes in the liver [134,135]. CBD and 7-OH-CBD are then eliminated in the feces, with a minor amount being excreted in the urine [136]. Interestingly, CBD has been shown to bind to the catalytic site of CYP2C9 and CYP2C19 and competitively suppress the activity of these enzymes [137]. Thus, CBD has been reported to inhibit CYP2C9 mediated hydroxylation of THC to 11-OH-THC [138] providing a molecular mechanism that may explain why CBD can improve the oral bioavailability of THC [124] (Fig. 6). Intriguingly, β-myrecene and other terpenoids in cannabis have been shown to enhance the effect of CBD on hepatic CYP enzymes [101]. Importantly, the effect of CBD on CYP enzymes could have implications for the metabolism of other drugs and thus may be either additive or contraindicated for specific existing pharmacotherapies. Indeed, CBD was shown to decrease the metabolism and the excretion of anti-convulsant drugs, hexobarbital and clobazam in human subjects [138,139], which results in an increase in the plasma level of the aforementioned drugs and subsequently their side effects. Thus, care should be taken when CBD is co-administered with hexobarbital and clobazam.

9. Full-spectrum cannabis extract- pharmaceutical preparations

Epidiolex is an oil formulation of purified, plant-derived CBD that has been recently approved by FDA for the treatment of certain rare and catastrophic forms of childhood-onset epilepsy such as Lennox–Gastaut Syndrome and Dravet Syndrome [120]. Another clinically approved product derived from cannabis extract is Sativex. Sativex is an oromucosal spray containing a full-spectrum cannabis extract with a standardized ratio of THC and CBD in addition to other cannabinoids and terpenes in an aromatized water-ethanol solution [140]. Sativex has been approved in many countries such as Canada, UK, Spain and Germany for the treatment of symptoms associated with MS. In addition to Epidiolex and Sativex, Cannador is an oral capsule containing a full plant extract, with a standardized ratio of THC and CBD that has been used in several clinical trials for the treatment of symptoms associated with MS [11].

10. Potential health beneficial effects of full-spectrum cannabis extracts

While numerous studies have investigated the effects of cannabis extracts in multiple disease conditions, some of the most impactful effects appear to occur in the treatment of inflammation and neuropathic pain. For instance, the role of full-spectrum cannabis extract in the treatment of spasticity and neuropathic pain has been investigated in a mouse model of amyotrophic lateral sclerosis (ALS) [141]. In a mouse model of ALS, mice treated daily with 20 mg/kg Sativex for 20 weeks displayed significantly reduced progression of neurological deficits and had improved survival, particularly in females [141]. The protective effect of Sativex has also been confirmed in a mouse model of MS [142]. This study utilized the Biozzi ABH mice with chronic relapsing experimental allergic encephalomyelitis as a model of MS. These mice were treated with a full-spectrum cannabis extract, Sativex, baclofen (the anti-spastic drug used as a positive control) or vehicle and the stiffness of limbs of the mice were evaluated by measuring the force required to flex the hind limb [142]. In a manner similar to baclofen, Sativex significantly reduced neuropathy and spasticity by approximately 40% compared to control [142]. Overall, the study proposes that Sativex is as effective as baclofen in the treatment of symptoms associated with MS [142] (Table 1).

The neuroprotective effect of Sativex has also been studied in the Theiler's murine encephalomyelitis virus-induced demyelinating disease model of MS [143]. Treatment of these mice with 10 mg/kg i.p. of Sativex significantly improved the neurological deficits associated with MS [143]. Specifically, Sativex significantly improved motor activity, reduced axonal damage and restored myelin morphology in MS mice [143]. Mechanistically, Sativex was shown to act as an anti-inflammatory agent as it suppressed microglial reactivity, the expression of proinflammatory cytokine IL-1β and adhesion molecules, and it upregulated the anti-inflammatory cytokines, arginase-1 and IL-10 [143]. Furthermore, Sativex was able to decrease the accumulation of chondroitin sulfate proteoglycans and astrogliosis in the spinal cord of MS mice and in astrocyte culture *in vitro* [143]. In order to explore whether or not the protective effects observed with Sativex were due to extracted CBD, THC or both, MS mice were treated with extracted CBD or THC alone. In a manner similar to Sativex, extracted CBD was sufficient to significantly lessen the motor deterioration and axonal damage whereas extracted THC induced much weaker effects [143]. Together, these findings suggest that the neuroprotective and anti-inflammatory effects of Sativex are mainly due to CBD.

Contrary to the previous study [143], another study found evidence that the neuroprotective effect of Sativex was due to THC [144]. In that study, female C57BL/6 mice with a bacteria-induced experimental autoimmune encephalitis and MS were treated with Sativex, extracted THC, or extracted CBD [144]. Notably, while administration of Sativex, extracted THC, and extracted CBD all produced beneficial effects in neurological function, only Sativex and extracted THC maintained improvement of the neurological function along with reduced cell infiltrates in the spinal cord and thus slowed disease progression [144]. Importantly, the beneficial effect of extracted THC was abolished by the treatment of mice with the CB1 receptor antagonist, rimonabant, suggesting a CB1-dependent mechanism [144]. Although the discrepancy between the two studies discussed above is unknown, it is possible that the differences may be attributed to different environmental conditions for each study and/or sex differences [143,144]. For instance, the first study was performed on male mice using virus-induced demyelinating disease model of MS [143], while the later study was conducted on female mice with bacteria-induced experimental autoimmune encephalitis disease-related MS [144]. Thus, it is possible that the effects of Sativex, THC and/or CBD are dependent on sex, species, or pathogenesis of MS. Nevertheless, given that the beneficial effect of full-spectrum cannabis extract was consistent in both studies, this would highlight a crucial role of full-spectrum cannabis extract, in this case Sativex, in the treatment of neuropathic pain and inflammation associated with MS.

As mentioned, in addition to MS, cannabis extracts have also shown promise in the treatment of neuropathic pain associated with other conditions. Indeed, an important role of full-spectrum cannabis extract in the treatment of neuropathic pain compared to purified THC or purified CBD has been studied in a rat model of chronic constriction injury of the sciatic nerve (CCI) [15]. In this study, rats were treated with full-spectrum cannabis extract, purified CBD or purified THC. Of note, the full-spectrum cannabis extract used in this study contained 64.5% CBD, 4% THC, < 4% of other cannbinoids (e.g. CBG, CBC, cannabidivarin, CBDA) and additional minor components (e.g. terpenes, sterols, triglycerides, alkanes, squalene, tocopherol, carotenoids) [15]. Importantly, the full-spectrum cannabis extract totally relieved thermal hyperalgesia, mechanical allodynia and withdrawal latency in CCI rats [15]. In contrast, treatment with purified CBD or purified THC, given at the same dose existing in the extract, showed only a partial anti-nociceptive effect [15]. Thus, this study suggests that full-spectrum cannabis extract has a better analgesic effectiveness than CBD or THC alone in rats with neuropathic pain [15]. Intriguingly, the authors also found that the anti-nociceptive effect of the full-spectrum cannabis extract was independent of CB1 and CB2 receptors [15]. Indeed, the anti-nociceptive effect of the full-spectrum cannabis extract was mainly due to the activation of the vanilloid receptor, TRPV1 [15]. This conclusion was supported by the finding that treatment of animals with

Table 1
Effect of cannabis extract in rodents with neuropathic pain and inflammation.

Model	Dose	Duration	Effect	References
SOD1-(G93A) B6SJL transgenic mice (Amyotrophic lateral sclerosis)	Sativex 20 mg/kg	20 weeks	Reduced the progression of neurological deficits, improved animal survival, particularly in female mice	[141]
Chronic relapsing experimental allergic encephalomyelitis in Biozzi ABH mice	Sativex 10 mg/kg	7–8 months	Improved neuropathy and spasticity	[142]
Theiler's murine encephalomyelitis virus-induced demyelinating	Sativex 10 mg/kg	10 days	Improved the neurological deficits, improved motor activity, reduced axonal damage, restored myelin morphology	[143]
Bacteria-induced experimental autoimmune encephalitis	Sativex 10 or 20 mg/kg	7 days	Improved neurological dysfunction, reduced the cell infiltrates in the spinal cord	[144]
Chronic constriction injury of the sciatic nerve in rats	CBD extract 10 mg/kg	7 days	Relieved thermal hyperalgesia, mechanical allodynia and withdrawal latency	[15]
Streptozotocin-induced diabetic neuropathy in rats	CBD extract 15 or 30 mg/kg	8 days	Ameliorated mechanical allodynia and the physiological thermal pain perception	[64]

CB1 and CB2 receptor blockers could not abolish the protective effect of the full-spectrum cannabis extract [15]. Conversely, TRPV1 receptor antagonist, capsazepine, completely blocked the effect of full-spectrum cannabis extract suggesting a TRPV1-dependent mechanism [15]. Overall, this study clearly demonstrated that the beneficial effects of the full-spectrum cannabis extract was superior to purified THC or CBD in the treatment of neuropathic pain and that this effect was not mediated via the classical CB receptor-mediated signaling.

The anti-nociceptive effect of full-spectrum cannabis extract [15] has been confirmed in streptozotocin-induced diabetic neuropathy in rats [64]. Treatment of these animals with full-spectrum cannabis extract significantly ameliorated mechanical allodynia and the physiological thermal pain perception [64]. Of importance, the observed effect was independent of hyperglycemia [64], suggesting a direct neuronal effect. Indeed, evidence suggests that the anti-nociceptive effect may be due to the activation of the neurotrophic factor, nerve growth factor (NGF), by one or more components within the cannabis extract [64]. In addition to the anti-nociceptive effect, full-spectrum cannabis extract protected against oxidative stress-induced neuronal damage in these diabetic rats [64]. Collectively, this study supports the concept that the combination of cannabinoid and non-cannabinoid compounds, as present in the aforementioned extract, produces a profound benefit in the treatment of neuropathic pain (Table 1).

In addition to the pre-clinical studies, full spectrum cannabis extract such as Sativex has been investigated in numerous clinical trial on patients with MS-related symptoms [12,145–156]. These trials are either double-blind randomized placebo-controlled parallel-group trials, an uncontrolled open-label or non-interventional trials that have studied the effect Sativex as a monotherapy or as an add-on therapy on patient with MS-related symptoms [12,145–156]. Notably, Sativex reduces neuropathic pain [12,145–156], muscle stiffness and spasticity [147,149–152,154–158], bladder dysfunction [150–152,155], and improves sleep quality [12,146,151] in MS patients. Importantly, the effect of Sativex on MS-related neuropathic pain was more pronounced when administered as an adjuvant therapy [154–156]. Overall, these trials confirm the notion that full spectrum cannabis extract such as Sativex is effective for the treatment of MS-related neuropathic pain.

While small scale clinical studies suggest that full-spectrum cannabis extract like Sativex is safe and effective in the treatment of MS-associated symptoms such as neuropathic pain [12,145–156], this might not necessarily hold true for all other cannabis products. Indeed, purified oral THC lacks beneficial effects for the treatment of neuropathic pain associated with MS [159]. In addition, oral products with purified or high THC content produces cognitive dysfunction, undesirable psychological effects and tachycardia [160–162]. Thus, care should be taken in the interpretation of the effectiveness and safety of the types of cannabis products used in treating neuropathic pain.

11. Potential adverse effects of full-spectrum cannabis extracts

While the major undesirable effects of THC containing products are cognitive dysfunction, particularly the loss of short-term memory consolidation, anxiety, tachycardia and hunger [30], these are obviously not common adverse effects of full spectrum cannabis extract like Sativex [12,151,156]. In fact, given that full spectrum cannabis extract consists of a variety of cannabinoids and terpenes, we postulate that these cannabinoids and terpenes can help minimize the undesirable effects of THC. In support of this notion, CBD was shown to reduce unpleasant THC-induced effects such as psychological reactions, anxiety, tachycardia and hunger [34,163–166] through the more traditional CB receptor-mediated pathway. Indeed, the reduction of the unpleasant THC effects are mediated by the following mechanisms: (a) CBD appears to compete with THC for CB1 receptor binding site and acts as a CB1 receptor antagonist or reverse agonist [48,167] and (b) CBD suppresses the activity of CYP2C and CYP3A enzymes involved in the metabolism of THC in the liver, which subsequently inhibits the hydroxylation of THC to its 11-hydroxy metabolite [168]. Of note, 11-OH-THC is 4 times more psychoactive compared to THC [169,170], and thus reducing the formation of 11-hydroxy metabolite by CBD should minimize the unpleasant psychological reactions of THC. In addition to CBD, α-pinene, a bicyclic monoterpene, was shown to aid the memory and minimize cognitive dysfunction via blunting the activity of acetylcholinesterase in the brain [105,106]. Together, the absence of the major undesirable effects of THC is an important advantage of full spectrum cannabis extract like Sativex [12,151,156]. Nevertheless, side effects such as somnolence, dizziness, confusion, fatigue, dry mouth, white and red buccal mucosal patches and nausea have been reported in patients on Sativex [12,145,149].

In contrast to Sativex, the presence of CBD in some cannabis extracts, particularly the oral extracts, can sometimes exacerbate some of the psychological effects of THC (e.g., being “stoned”) which might be due to the profound effect of CBD on the hepatic first pass metabolism of THC whereby CBD elevates the blood level of THC [171]. Thus, oral broad spectrum CBD extract (i.e. CBD extract along with all other compounds and cannabinoids except THC) might be safer than oral THC or THC/CBD cannabis extract products. Nevertheless, while highly purified CBD extract lack any psychoactive adverse effects, a risk of hepatotoxicity, in addition to suicidal ideation have been reported with a chronic high dose of extracted CBD [172]. Other adverse effects such as fatigue, somnolence and gastrointestinal disturbances have been also reported [172].

12. General conclusion

Pre-clinical studies using full-spectrum cannabis extract have demonstrated several convincing beneficial anti-inflammatory and

analgesic effects [141–144]. In some instances, these findings have provided the foundation needed for understanding the biological effects of full-spectrum cannabis extract compared to purified THC and CBD. Indeed, the presence of various terpenes and cannabinoids in full-spectrum cannabis extract can modulate the binding of both endogenous endocannabinoids and exogenous THC and CBD [173], demonstrating the importance of understanding how all of these molecules interact to produce specific biological effects. Thus, full-spectrum cannabis extract may represent a promising therapeutic agent that seems to benefit a variety of conditions associated with pain and inflammation.

Declaration of competing interest

JRBD is on the board of directors of Aurora Cannabis Inc., which is a for-profit, company licensed for the cultivation and sale of cannabis. Aurora Cannabis Inc. was not involved in any aspect of the review. Thus, the authors have declared that no conflict of interest exists.

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