



## REVIEW PAPER

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# Progress in study of *Cannabis sativa* leaves extracts without psychotropic cannabinoids in animal model of neuropathic pain

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### ABSTRACT

Neuropathic pain is a type of chronic pain caused by a lesion or disease of the somatosensory nervous system. Current therapy for this pain includes the use of pharmacological and nonpharmacological methods but due to the fact that a lot of therapy does not produce the analgesic results, it is necessary to search for new and more effective pharmacological strategy in relief of this type of pain. One of the interesting natural sources of compounds against this type of pain is extract of *Cannabis sativa* without psychotropic cannabinoids. Medicinal properties of *C. sativa* have been explored for centuries. It is well established that active compounds of this herb act through two cannabinoid receptors (CB1, CB2) as endocannabinoid system in the central nervous system. The present review addresses the recent advances in the study of pharmacological mechanisms on cellular and receptor level underlying non-hallucinogenic cannabinoid analgesic effect. In recent years, results of studies allow to state that special plant extract of *C. sativa* (without psychotropic cannabinoids) may be a promising source of drug used to relieve neuropathic pain.

**Keywords:** cannabinoids, analgesic effect, animal models.

## Introduction

The International Association for the Study of Pain [1] defines neuropathic pain (NP) as pain initiated or caused by a primary lesion or dysfunction in the nervous system, thus NP may be caused by any disease or injury to the nervous system. A nerve lesion leads to serious changes in the nervous system and makes it distinct NP

from other chronic pain types that have an intact nociceptive system [2]. Pain occurring in acute or chronic diseases, including NP, are a very common challenge in medical care. Thus, pharmacotherapy of this pain is one of the top priorities in developed countries. Currently, it is estimated that 7–8% of the general population in Europe suffer from NP [3], but in 30 to 40% of patients

with diabetes have symptoms suggesting neuropathy [1]. Moreover, different type of pain occur in as many as 90% of patients during cancer [4] and it is predicted that at least 15–20% of patients may suffer from NP during the course of the cancer [5]. The research issue becomes even more complex and socially urgent due to the fact that the anatomical classification of NP includes over 50 types of diseases associated with the pathogenesis of pain [2], for example phantom pain, trigeminal neuralgia, carcinoma-associated paraneoplastic peripheral neuropathy, acute or inflammatory polyradiculoneuropathy (Guillain-Barre syndrome), diabetic mononeuropathy, alcoholism, amyloidosis, multiple sclerosis, traumatic brain injury, Parkinson's disease, mastectomy. According Gondim et al [6], peripheral neuropathy in inflammatory bowel disease also belongs to NP. In particular, the inflammatory bowel diseases are an increasing challenge for pharmacotherapy, and this is because of contraindications to conventional analgesics. Due to the fact that a lot of therapy does not produce the expected results in the treatment of NP, it is necessary to search for new and more effective pharmacological solutions in relief of this type of pain [2, 7]. This is even more important, due to the fact that opioids and nonsteroidal anti-inflammatory drugs are among the most commonly used drugs in clinical practice, their use subsequently can induce unexpected drug interactions and/or several types of these medicines often produce several adverse reactions [8]. Nowadays, beside typical analgesics, also antidepressants, anti-convulsants [9], capsaicin, and memantine are used in pain relief [1]. In the recent years, the problem becomes even more complex because chemotherapy (e.g. use of such drugs as cisplatin, paclitaxel, vincristine) can also induce sensory neuropathies as an adverse drug effect (neurotoxicity) [10].

## Impact of cannabinoid receptors in pain

A very interesting option in this field may be plant extract from *Cannabis sativa* L. This is important for NP therapy, because results of many researches showed occurrence of high density cannabinoid receptors (CB1 and CB2) in many areas related to pain (endocannabinoid system, ED) [11, 12]. It was shown that deregulation of the ED underlies several neurological disorders including chronic pain [13]. In human brain two endocannabinoids were found out, N-arachidonoyl ethanolamine (anandamide), the first discovered and best studied endogenous lipid-signaling ligand and 2-arachidonoyl glycerol (2-AG) which acts through cannabinoid

receptors [14–16]. It is important to note, that other chemical compounds may belong to endocannabinoids such as dihomog- $\gamma$ -linolenylethanolamide, docosahexaenoylethanolamide, and possibly the CB1-selective agonist – 2-arachidonoylglycerol ether (noladin ether).  $^9$ N-arachidonoyl dopamine, N-oleoyl dopamine, and oleamide [17].

Studies have reported a similar distribution of CB1 cannabinoid and mu-opioid receptors in sites which are strongly involved in antinociception as the dorsal horn of the spinal cord [18], the caudate putamen, dorsal hippocampus, substantia nigra [19, 20], raphe nuclei, central medial thalamic nuclei and hypothalamus [21, 22]. A growing body of literature attests to the interaction between opioids and cannabinoids at the receptor and signal transduction levels [23]. Another study in animal model showed that cannabinoid receptor agonists increased proenkephalin gene expression in the caudate-putamen, nucleus accumbens, paraventricular and ventromedial hypothalamic nuclei and also medial mamillary nucleus [24]. Moreover, Vigano et al. [23] concluded that CB1 receptors may be necessary for the expression of several effects of opiates, so CB1 antagonists may offer a novel approach for treating opiate addiction and the cannabinoids use may help relieve the effects of opiate withdrawal. Several studies showed, that agonists for CB1 receptor exert an anti-inflammatory, anti-nociceptive and anti-hyperalgesic properties in the central and peripheral nervous system levels [25]. It was demonstrated furthermore that activation of peripheral CB2 receptors generates an antinociceptive response in situations of inflammatory hyperalgesia and NP by inhibiting the release of proinflammatory factors in non-neuronal cells located near nociceptive neuron terminals [26]. CB2 receptors are mainly located in the immune system, therefore representing a target in inflammatory pain processing [27]. Recent results of the research provided evidence of the involvement of cannabinoid receptors in the supraspinal modulation of pain in different models with use intra-cerebral microinjections of cannabinoid ligands or positive modulators for CB1 receptors [27]. The study has demonstrated that synaptically released glutamate, as a result of pain, stimulates mGlu5 receptor inducing endocannabinoid release, which in turn activates presynaptic cannabinoid CB1 receptor. Moreover, it was explained that CB1 receptor activation expressed on presynaptic GABAergic terminals reduces the probability of neurotransmitter release thus dis-inhibiting the periaqueductal grey-rostral ventromedial medulla-dorsal horn (PAG-RVM-DH) antinociceptive pathway.

According to Palazzo et al. [27], cannabinoids may increase a glutamate release (maybe as consequence of GABA decrease) and require a glutamate receptor activation to induce the antinociception phenomena.

## Neuroinflammation and NP

Very often the NP is disproportionately enhanced in intensity (hyperalgesia) or altered in modality (hyperpathia or allodynia) in relation to the stimuli leading to changes in some factors coupled with inflammation [28]. The inflammatory response initiates a cascade of events inducing the concentration and activation of innate immune cells at the site of tissue injury and as a consequence an infiltration of damaged peripheral nerves by mast cells, granulocytes, macrophages and T lymphocytes which leads to release of immunoactive substances such as cytokines, neurotrophic factors, and chemokines initiating a local actions and can result in a more generalized immune response [29]. Proinflammatory cytokines, eg. interleukin-1beta (IL-1 $\beta$ ) and tumor necrosis factor-alpha (TNF $\alpha$ ) secreted by activated glial cells in the spinal cord and the brain play probably prominent role in inflammation-induced nociception [30]. Recent study showed that a chronic constriction injury led to increase of TNF $\alpha$  level in the sciatic nerve [31]. These proinflammatory cytokines exert their actions, at least partially, through the activation of the transcription factor, nuclear factor kappaB (NF-kappaB), which, in turn, regulates the transcription of many inflammatory mediators by binding to the promoter region of various genes, [eg. tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin 1 $\alpha$  (IL1 $\alpha$ ), cyclooxygenase-2 (COX-2), inducible NO-synthase (iNOS) and proteases (eg. matrix metalloproteases (MMPs)) [32–34]. It is well documented that the dysregulation of NF-kB activity is associated with risk of neurodegeneration [35] and with alteration of synaptic plasticity [36, 37]. In dorsal root ganglia and the spinal cord of rats an increased number of activated NF-kB immunoreactive neurons and astrocytes was observed [36, 38]. Several studies indicate that the NF-kB activation pathway plays a crucial role not only in the immune responses, inflammation, and apoptosis but also in the development and processing of pathological pain [39]. The results indicate that NF-kappaB has an impact on nociceptive transmission and processing and that a number of substances that inhibit the NF-kappaB-activating cascade are capable of reducing the nociceptive response in different animal models. Therefore, a modulation of specific participants in the NF-kappaB signal transduction

might exert a useful approach for the development of pain release drugs [40]. Both experiments performed in in vivo model [41] and studies done in patients during acute migraine attacks [42] have revealed also the possibility of the NF-kB activation by transcriptional regulation of iNOS. Moreover, results from Wu et al. [43] suggest that reciprocal changes in the expression of ZAS3 (a member of zinc finger protein family) and NF-kappaB proteins might generate NP after peripheral nerve injury [43]. Recent experiments showed that glial NF-kB inhibition reduces galanin and CGRP expression, which are neuropeptides that correlate with pain behavior and inflammation after peripheral nerve injury [44, 45].

## Other receptor mechanism of NP pathogenesis

A relationship was demonstrated between the N-Methyl-D-Aspartate receptor (NMDAR)-mediated neuronal excitation and nociceptive behavior. There is considerable evidence that activation of NMDAR contributes to the mechanism of pathological pain, hence a NR2B-containing NMDA receptor is one of the best potential targets for NP [46, 47]. It is in line with information that hypoactivity of the spinal cannabinoid system results in an NMDA-dependent hyperalgesia and thus may participate in the etiology of certain chronic pain conditions [48]. Moreover, previously it was shown that NMDA-antagonists are effective in decreasing autotomy (self-injurious behavior) and relieving neuropathic symptoms in other types of experimental peripheral neuropathies [49], but based on the systematic review of clinical trials, no conclusions can yet be made about the efficacy of NMDA receptor antagonists on NP [50].

## Phytochemistry and biological activities of *Cannabis sativa* extracts

The *Cannabis sativa* L. has been known for centuries as a psychoactive plant and they has been used for thousands of years in ethnomedicine to relieve human suffering. The plant produces over 421 chemical compounds, including about 80 terpeno-phenol compounds named phytocannabinoids [51] and these cannabinoids and hemp extracts may exert different and promising pharmacological activities. Several studies have shown that the most important psychoactive compound of cannabis extract is considered to be  $\Delta^9$ -tetrahydrocannabinol (THC) [52–55]. However, there are also well known phytocannabinoids with very weak or no psychotro-

pic effects. These include cannabidiol (CBD) [56], cannabigerol (CBG), cannabichromene (CBC), D9-tetrahydrocannabivarin (D9-THCV), cannabidivarin (CBDV), D9-tetrahydrocannabinolic acid (D9-THCA) and cannabidiolic acid (CBDA) [51], and also the main compound of essential oil – beta-caryophyllene [57]. Phytochemical analysis of 11 cannabis varieties proved chemical differences between Cannabis spp. [58]. Many recent studies with use gas chromatography (GC) analyzing cannabinoids and terpenoids have been performed for chemotaxonomic purposes [58]. Chemotaxonomic evaluation of cannabis has led to the recognition of three chemotypes [59]:

1. Chemotype I – narcotic hemp, where delta 9 THC and carboxy THC to CBD and carboxy CBD ratio is much higher than 1 ( $> 1$ ),
2. Chemotype II – intermediary hemp, where delta 9 THC and carboxy THC to CBD and carboxy CBD ratio is about 1,
3. Chemotype III – fibrous hemp, where delta 9 THC and carboxy THC to CBD and carboxy CBD ratio is much lower than 1 ( $< 1$ ).

In accordance with review of Izzo et al. [51], non-psychoactive phytocannabinoids exert multiple pharmacological effects and the most recently investigated mechanisms of their biological actions involve the modulation of the endocannabinoid system, transient receptor potential channels, the peroxisome proliferator-activated receptor GPR55, the putative abnormal-CBD receptor, 5-hydroxytryptamine receptor subtype 1A, glycine  $\alpha 1$  and  $\alpha 1b$  receptors, the adenosine membrane transporter phospholipase A2, lipoxygenase (LOX) and cyclooxygenase-2 (COX-2) enzymes, and  $Ca^{2+}$  homeostasis. At present, it was shown that D9-THCV is a CB1 receptor antagonist [60]. It was found out that at low doses ( $< 3$  mg/kg) it antagonises D9-THC effects, but it acts as a CB1 agonist at higher doses (10 mg/kg) in vivo in mice [51]. In the study performed by Bolognini et al. [61] it was shown that D9-THCV can activate CB2 receptors in vitro and decrease signs of inflammation and inflammatory pain in mice partly via CB1 and/or CB2 receptor activation. In mouse models of carrageenan-induced inflammation and inflammatory pain and formalin-induced hyperalgesia, D9-THCV suppressed signs of these pathological processes [61]. In detail, the THCV reduced the formalin-induced pain behaviour when administered at a dose of  $5$  mg·kg $^{-1}$  and suppressed carrageenan-induced signs of inflammation and inflammatory pain in mice when it was injected 30 min before carrageenan. This investigation has demonstrated that the anti-edema activity exhibit-

ed by THCV appeared to be CB2, but not CB1 receptor mediated, its anti-hyperalgesic activity seemed to be mediated by both CB1 and CB2 receptors in the formalin model, but by neither of these receptors in the carrageenan model. Therefore it means that peripheral antinociception without CNS effects is consistent with the peripheral distribution of CB2 receptors [62]. Further research directed at identifying the mechanisms underlying these in vivo effects of THCV, is needed.

Results of Gertsch et al. [63] research proved that another non-psychoactive compound, (E)-beta-caryophyllene [(E)-BCP], which is one of the most abundant in the plant essential oil, has been shown to selectively target the CB2 receptor at nM concentrations and to act as a full agonist. Moreover, Gertsch et al. [63] demonstrated that (E)-BCP (500 nM) inhibited the lipopolysaccharide (LPS)-induced proinflammatory cytokine expression in peripheral blood and (E)-BCP at  $5$  mg kg $^{-1}$  strongly reduced the carrageenan-induced inflammation in mice. These data confirmed that different CB2 receptor-selective ligands, including CB2 receptor agonists, are able to inhibit carrageenan-stimulated edema formation in mice. According to Bento's results [64] it was found also that the (E)-BCP reduced cytokine levels (tumor necrosis factor- $\alpha$ , keratinocyte-derived chemokine, and macrophage-inflammatory protein-2) in a culture of macrophages stimulated with lipopolysaccharide. Klauke et al. [65] confirmed that (E)-BCP exerts its analgesic effects in mouse models of inflammation and NP (formalin-induced inflammation model) and a model of NP, which involves the partial ligation of the sciatic nerve in male wild type (CB2 $^{+/+}$ ) and CB2 $^{-/-}$  mice). It was observed that analgesic effects of (E)-BCP were absent in CB2 $^{-/-}$  mice and blocked by the CB2 antagonist SR144528. Moreover, using von Frey test and Hargreaves test they showed, that in mechanical allodynia (E)-BCP exerted a strong effect at a dose 10 mg/kg, but in second test only 1 mg/kg gradually reduced thermal hyperalgesia.

Another major psycho-inactive component of cannabis, CBD has been more thoroughly investigated. CBD has exerted analgesic and anti-inflammatory, antioxidant, neuroprotective and pro-apoptotic activities and might predict a possible future use for the treatment not only of pain, but also of neurodegenerative disorders, ischemia and cancer [51]. Additionally, Zuardi et al. [66] showed that the antipsychotic action of CBD was similar to atypical antipsychotics such as clozapine. Moreover, CBD has exerted anxiolytic-like effects by activating post-synaptic 5-HT(1A) receptors in the periaqueductal gray matter [67].



However, among published studies the most strong pharmacological effect showed that CBD has substantial anti-nociceptive and anti-inflammatory activities. Previously, Evans et al. [68] and Formukong et al. [69] found that CBD was more effective than other natural cannabinoids in the phenylbenzoquinone (PBQ)-induced mouse writhing test. They concluded that the compound was about 360 times more potent than aspirin and 590 times more potent than THC [68, 69]. There are also inconsistent results of studies, for example Sanders et al. [70] showed that orally administered CBD was inactive in the acetic acid stretching model and CBN was only effective at high concentrations. In the study performed by Costa et al. [71] in both neuropathic (sciatic nerve chronic constriction) and inflammatory pain (complete Freund's adjuvant intraplantar injection) in rats' model, it was found that treatment with CBD at dose of 2.5–20 mg/kg (neuropathic model) and at 20 mg/kg (adjuvant-injected rats) from day 7 to day 14 after the injury, reduced hyperalgesia to thermal and mechanical stimulation. A reduction was also demonstrated in the content of several mediators, e.g. prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), lipid peroxide and nitric oxide (NO) after CBD treatment. On the basis of these results one may conclude that the CBD exerts the therapeutic potential in NP. Moreover, when CBD was administered orally (5–40 mg/kg) once a day for 3 days after the carrageenan-induced inflammation in the rat, it exerted not only anti-inflammatory effect but also six hours after carrageenan injection, the lower doses of CBD abolished a hyperalgesia [72]. It was calculated that CBD at a dose of 5 mg/kg reduced edema to 50%, at 7.5, 5 and 10 mg/kg to about 65% and to 100% after 40 mg/kg. These authors demonstrated also that CBD in the dose of 10 mg/kg non-significantly reduced COX activity (21%) and the doses of 20 and 40 mg/kg of CBD brought COX activity down to the level of non-inflamed tissues. Although there is an increasing number of experimental works and clinical studies explaining the mechanism of extracts obtained from *Cannabis sativa*, the data defining whether its biologically active compounds influence the expression at mRNA and/or protein level is still insufficient. It was shown for example that oral administration of ajulemic acid (1'-1'-dimethylheptyl-THC-11-oic acid; AjA), a cannabinoid acid devoid of psychoactivity, reduces joint tissue damage in rats with adjuvant arthritis. Zurier et al. [73] in an *in vitro* study (on peripheral blood and synovial fluid monocytes – PBM and SFM – isolated from healthy subjects and from patients with inflam-

matory arthritis, respectively) have revealed that AjA in doses 0–30 microM did not influence TNF $\alpha$  production by activated cells but AjA reduced levels of IL-1 $\beta$  mRNA in a concentration-dependent manner which might be helpful to explain the therapeutic effects of AjA in the animal model of arthritis [73]. In CBD treated mice a significant reduction of plasma levels of the pro-inflammatory cytokines, IFN-gamma and TNF $\alpha$  was observed [74]. Furthermore, Costa et al. have examined whether CBD inhibited the production of nociceptive and inflammatory mediators involved in development and maintenance of NP and inflammation. In rats orally treated with CBD (2.5–20 mg/kg to neuropathic and 20 mg/kg to adjuvant-injected rats) from day 7 to day 14 after the injury no reduction in NF-kappaB activation and TNF $\alpha$  content was observed [71]. There has been some evidences on the efficacy of analgesic effect of cannabinoids originating not only from different animal models [75], but from clinical trials which included e.g. patients with peripheral neuropathy in inflammatory bowel disease [6], sclerosis multiplex [76] and also in cancer pain [77].

### Preparations containing the *C. sativa* extract

In 2005, Ministry of Health of Canada has approved SATIVEX<sup>®</sup> for the adjunctive treatment of symptomatic relief of NP in multiple sclerosis in adults. SATIVEX<sup>®</sup> contains Tetranabinex<sup>®</sup> and Nabidiolex<sup>®</sup> – *Cannabis sativa* extracts. In this product the principal active components are 2.7 mg of THC and 2.5 mg of CBD.

It is worth emphasizing, that several studies concerning assessment of the Sativex analgesic activity have been carried out [78, 79]. Recently, a double-blind, randomized, placebo-controlled clinical study showed significant difference between Sativex treatment group and placebo. This product demonstrated the potent analgesic effect and it was generally well tolerated [78]. Other clinical studies largely affirm that NP patients derive benefits from cannabinoid treatment, but evidence to date suggests that abuse on Sativex is likely to occur [79]. This indicates the need to develop new products derived from cannabis extract devoid of THC, therefore further studies should be performed in this field.

### Summary

According to several experimental and review articles [80] currently it can be considered that progress in

understanding the physiological effects and pharmacological activity of phytocannabinoids may provide new therapeutic opportunities in the treatment of NP. There are some evidences provided both by animal and clinical studies on the efficacy of analgesic effect of different cannabinoids, both hallucinogenic (delta9-tetrahydrocannabinol – THC) and non-hallucinogenic (cannabidiol, delta9-tetrahydrocannabivarin, beta-caryophyllene) compounds. Clinical studies largely affirm that NP patients derive benefits from cannabinoids treatment e.g in peripheral neuropathy, in inflammatory bowel disease, sclerosis multiplex and with cancer pain. It indicates the need to develop a new product derived from cannabis extract that will be devoid of THC, and further studies should be performed in this field. Some aspects of the analgesic and anti-inflammatory effects of *Cannabis sativa* extracts containing non-psychotropic plant-derived cannabinoids seem to be interesting in context of discovery and development of drugs for the treatment of NP.

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#### Conflict of interest statement

The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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