https://doi.org/10.31925/farmacia.2022.6.24 **ORIGINAL ARTICLE**

EVALUATION OF THERAPEUTIC POTENTIAL OF CANNABIDIOL-BASED PRODUCTS IN ANIMAL MODELS OF EPILEPTIC SEIZURES, NEUROPATHIC PAIN AND CHRONIC INFLAMMATION

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Abstract

Cannabidiol (CBD) is one of the main cannabinoids (CBi) found in *Cannabis* species. The purpose of this study is to present the evaluation of the anticonvulsant effect in an animal model of epileptic seizures induced by the administration of electric shocks to mice for acute treatment with 3 vegetable oils with CBD. We also evaluated the antihyperalgic effect in a neuropathy model, and anti-inflammatory and analgesic potential of the CBD-containing emulsion after local administration. Significant anticonvulsant and antihyperlagic effects were observed for CBD-containing oils.

Rezumat

Canabidiolul (CBD) este unul dintre principalii canabinoizi (CBi) ce se regăsesc în speciile de *Cannabis*. Scopul acestui studiu constă în prezentarea evaluării efectului anticonvulsivant într-un model de convulsii induse prin administrarea de șocuri electrice la șoareci pentru tratamentul acut cu 3 uleiuri vegetale cu CBD. De asemenea am evaluat efectul antihiperalgic într-un model de neuropatie, dar și potențialul antiinflamator și analgezic al emulsiei cu conținut în CBD după administrare locală. Au fost observate efecte anticonvulsivante și antihiperalgice semnificative pentru uleiurile cu conținut de CBD.

Keywords: anticonvulsant, anthyperalgic, cannabidiol, epileptic seizures, neuropathic pain, chronic inflammation

Introduction

The therapeutic properties of the *Cannabis* plant are attributed to cannabinoids (CB), a class of compounds comprising more than 150 substances with terpeno-phenolic structure, which accumulate mainly in the resin secreted from the trichomes of the female plants of *Cannabis sativa* L. [1-3].

The main active compound in the plant is Δ-9-tetrahydrocannabinol (Δ-9-THC), largely responsible for psychoactive effects. Cannabidiol (CBD) is another important compound with high therapeutic potential that could bring new hope to chronic patients suffering from pain, inflammation, epilepsy, sleep disorders, multiple sclerosis, anorexia, schizophrenia or even cancer [4, 5]. In recent years, CBD has become a compound believed to have broad therapeutic spectrum, and the isolation of endocannabinoids (eCBs) in the early 1990s has renewed interest in studying CB. CBD is available in a growing number of pharmaceutical forms, but the best known is CBD oil, which in just a few years, has become extremely popular world-wide [6].

Non-clinical studies on laboratory animals have shown that cannabinoid derivatives are effective for antagonising chemical-induced seizures [7].

Previous studies have demonstrated the beneficial effects on animal models with neuropathy for cannabis extract constituents such as CBD and THC, with a synergistic effect between the two substances. Thus, CBD in different doses can prevent the development and improve neuropathy induced by treatment with cytostatics [8-10].

In several models of chronic pain induced by inflammation, CBi, including CBD, can exert an analgesic and anti-inflammatory effect [11]. Studies on rodents revealed an important role for CBD and its derivatives in alleviating chronic pain. In the 2018 review on this topic, Donvito *et al*. discussed the antinociceptive effects in neuropathic pain in rodents. It has also been shown that CBD can exert its therapeutic effects through a reduced activation mechanism of the amygdala during negative emotional processes by modulating the concentration of dopamine and serotonin [12]. Therefore, it is an option in the treatment of chronic pain. There is the possibility of

off-label use of CBD, which includes pain treatment [13]. Previous non-clinical studies have shown that oral administration of CBD in rats improved thermal and mechanical hypersensitivity and reduced inflammation in a model of chronic inflammation [14].

Gamble *et al*. in their study on the analgesic efficacy of CBD oil treatment demonstrated that it can help increase comfort and increase activity in dogs with osteoarthritis (OA) [15, 16].

The purpose of this study was to evaluate the anticonvulsant effect in a model of seizures induced by the administration of electric shocks to mice for acute treatment with 3 vegetable oils from *Cannabis sativa* having different concentrations in CB [17] and without pesticide content [18] . Moreover, we evaluated the antihyperalgic effect in an animal model of neuropathic pain [19], but also the anti-inflammatory and analgesic potential of an emulsion containing CBD after local administration, in a model of chronic inflammation in rats, induced by the intraplantar administration of complete Freund adjuvant (CFA).

Materials and Methods

All experimental procedures have been carried out in compliance with the bioethics norms for research on experimental animals for scientific purposes, in accordance with Law 43/2014 on the protection of animals used for scientific purposes and Directive 2010/63/EU of the European Parliament on the protection of animals used for experimental or other scientific purposes. The experimental protocol was approved by the Bioethics Commission of "Carol Davila" University of Medicine and Pharmacy in Bucharest, Romania.

Animals were housed in plexiglass cages with free access to food and water. The temperature and relative humidity were maintained at 21 - 24ºC and 45 - 50% and were recorded using a hygro-thermometer. Animals were brought to the research room an hour before the start of the experiment so that the animals could get used to the new environment.

Anticonvulsant activity of three CBD oils in mice

The experiment was conducted on 50. 8 - 12 weeks, old male NMRI mice with an average weight of 37.74 ± 2.21 g.

The threshold parameters recommended to induce seizures in a mouse of 25 - 30 g are: electric current intensity, 15 mA; shock duration 3 s; frequency 100 pulses/s; pulse with/duration 0.5 s.

Five experimental groups (10 animals/group) were put together and the convulsive stimulus was applied using the electroconvulsive-therapy (ECT) apparatus, with the before mentioned parameters.

One hour before the application of electric shock, the mice received the following oral treatments: distilled water 0.1 mL/10 g (control, CTL); phenobarbital sodium 100 mg/kg (reference, PHB); CBD oil 1.35% 15 mg/kg CBD (CBD1); CBD oil 2.5% 25.77 mg/kg CBD (CBD2); CBD oil 8% 47 mg/kg CBD (CBD3). The 3 types of oil were previously analysed in terms of content in cannabinoids [17] and pesticides [18].

After the application of the convulsive stimulus, the total duration of tonic-clonic seizures was measured for all experimental groups. The mean seizure durations for each treatment group were compared with the control group to determine the anticonvulsant effect. The incidence of seizures and lethality were also assessed.

Antihyperalgic effect of three CBD oils in a rat neuropathic pain model

Were used a total of 36 Wistar rats, 8 - 12 weeks old male, with average weight of 356.94 ± 56.18 g. Rats were divided into 6 experimental groups (6 animals/group) and the following substances were administered for 4 consecutive days [19]: saline 0.1 mL/100 g intraperitoneally (i.p.) and distilled water 0.1 mL/100 g orally (p.o.) (control-CTL); paclitaxel 2 mg/kg i.p. and distilled water 0.1 mL/100 g p.o. (PTX); paclitaxel 2 mg/kg i.p. and gabapentin 60 mg/kg p.o. (reference treatment, PTX+GPN); paclitaxel 2 mg/kg i.p. and CBD oil 1.35% (0.75 mg/kg CBD) p.o. (PTX+CBD1); paclitaxel 2 mg/kg i.p. and CBD oil 2.5% (1.29 mg/kg CBD) p.o. (PTX+CBD2); paclitaxel 2 mg/kg i.p. and CBD oil 8% (2.35 mg/kg CBD) p.o. (PTX+CBD3) [17].

Treatment with low doses of PTX was intended to induce neuropathy in rats. After the 4 treatment days, PTX treatment was discontinued and distilled water, GPN and CBD oils were further administered for 14 days to assess the antihyperalgic effect of the substances administered compared to the control. Mechanical hypersensitivity and cold allodynia were assessed before the start of treatment (initial testing to assess homogeneity between experimental groups, day 0), on the fourth day of treatment with PTX (one hour after administration, day 4), one week after discontinuation of PTX (day 11) and two weeks (day 18) after discontinuation.

Tactile hypersensitivity. Tactile hypersensitivity (mechanical allodynia) was determined by measuring the withdrawal thresholds of the hind paws using von Frey filaments (Ugo Basile, Gemonio, VA, Italy). The manual von Frey filaments are 20 individual nylon monofilaments constructed in such a way that a force between 0,008 (using the smallest diameter filament) and 300 g/mm^2 (using the largest diameter filament) can be applied at hind paw level. Tactile hypersensitivity was assessed using the Dixon "up and down" method [20] applied and validated by Chaplan *et al*. [21]. The animals were placed in individual plexiglass compartments above a sieve-type platform that allows full access to the plantar area of the hind paws. The test was initiated with

the fourth filament in the series (force 8 g). Von Frey filaments with increasing rigidity were applied to the level of the plantar surface of the hind paws, with enough pressure to bend the filaments, for 8 seconds [19]. The forces exerted by the von Frey filaments used in the experiment were: 2, 4, 6, 8, 10, 15, 26 and 60 g. The filaments were chosen in such a way that the last filament, with the greatest force exerted, did not exceed 10% of the average weight of rats. The absence of lifting of the paws (reaction) was considered a negative response (denoted by O), and in this case the next filament with greater rigidity ("up") was used. Paw retraction was considered a positive response (denoted by X) and led to the use of the next filament, with lower rigidity ("down"). In total, 6 responses were determined from the moment of obtaining an OX or XO sequence or 4 consecutive positive or negative responses. Subsequently, the 50% response threshold was determined, by using the following formula:

$$50\%\ withdrawal\ threshold\ (g) = {}^{10^{Xf+k\delta}}/_{10000}, \quad (1)$$

where Xf (in logarithmic units) = the last filament used; k = tabular value corresponding to the obtained sequence of X and O; δ = the mean value of the difference (in logarithmic units) between stimuli (in this case δ = 0.220271) [20].

Cold allodynia. Hypersensitivity to a cold stimulus was assessed using the acetone evaporation test. The experimental model involves applying a drop of acetone to the base of the hind paws with a syringe, its evaporation producing a cold, painful thermal stimulation [21].

Rats were placed in transparent plexiglass cages, provided with a floor consisting of a metal sieve used for nociceptive stimulation tests at plantar level (Hargreaves apparatus, Ugo Basile, Gemonio, VA, Italy). The experimental animals were allowed to accommodate in the environment (enclosure of the apparatus) for an hour. The acetone drop was applied to each of the hind paws, 3 times alternately, at an interval of 5 minutes between applications. After the application of the stimulus, the nociceptive behaviour was monitored for 50 seconds, and the assessments were carried out after a period of 10 seconds after the application of acetone, this interval corresponding to physiological reactions. The pain sensitivity of mice was assessed by calculating the total score of pain reactions for each rat, resulting from the sum of the measurements in the 6 determinations. Thus, the total score was determined by calculating the sum of all the individual scores, specific to each type of reaction, as follows: lack of reaction to pain (0); paw lifting, gently shaking or avoiding leaving body weight on the paw (1); prolonged withdrawal of the paws or vigorous shaking (2); licking of the hind paws (3). If the rat showed more than one sign of pain sensitivity in a test, then only the reaction sign with the highest score was taken into account [23].

Evaluation of analgesic and anti-inflammatory effects of an emulsion containing CBD in a rat model of chronic inflammation

This stage of the research was conducted on 32 male Wistar rats 12 - 15 weeks old, with an average body weight of 410 ± 33.14 g. Rats were divided in to 4 equal experimental groups (n = 8 *per* group) and were subjected to the following procedures [14, 24]: (i) Evaluation of basal sensitivity to thermal nociceptive stimulus, determination of initial hind paws volumes) at week 0, before treatment; (ii) Induction of chronic inflammation by intraplantar administration in the right hind paw of the inflammatory agent (complete Freund adjuvant-CFA 0.1 mL); (iii) Evaluation of thermal sensitivity and paw edema a week after induction of inflammation (week 1); (iv) Initiation of topical treatment, at the level of the right hind paws, once a day: massage without treatment for control group (CTL); massage without treatment for CFA group (CFA); CFA and hydrocortisone, (CFA+ HCZ) 2 mL HCZ ointment 10 mg/g, massaging; 2 mL CS-PBS and CBD emulsion in volumetric ratio (v/v) = 1: 4 [25], massaging (CFA + CBD); (v) Evaluation of thermal sensitivity and paw oedema a week after initiation of treatment (2 weeks after induction of inflammation).

Animals were anesthetized prior to hind paw volume measurements and intraplantar injections with 1300 mg/kg urethane (10%) solution administered intra-peritoneally.

Evaluation of analgesic effect. The analgesic effect of the CBD-containing emulsion was evaluated using the Hot Plate apparatus, the sensitivity of animals to thermal stimuli being investigated to test the supraspinal-mediated pain behaviour. The response of the animal to the application of thermal stimulus may consist in: shaking the hind limbs; licking of the hind limbs; jumping from the plate (attempted escape). The quantification of the pain response to the application of the painful stimulus was achieved by measuring the time elapsed (latency) to the appearance of one of the above-mentioned responses. The first observed response with the least variability was chosen [26]. Animals were positioned on the heated plate at a temperature of 56ºC. Latency was measured until the first reaction to pain occurred. The test was performed before the induction of inflammation, one week and 2 weeks after the administration of the inflammatory agent.

Evaluation of anti-inflammatory effect. The investigation of the anti-inflammatory action of the CBD-containing emulsion was carried out using the plethysmometric method. The anti-inflammatory effect was evaluated using the animal model of chronic inflammation induced with CFA in rats. After intraplantar administration of the reagent, local oedema was quantified by measuring

the volumes of both hind paws using the plethysmometer [27]. Before carrying out the determinations, the rats were anesthetized by intraperitoneal administration of 1 g/kg urethane. The volume for both hind paws was determined, before inducing inflammation and one week and 2 weeks after the administration of the inflammatory agent, respectively. The volumes determined after the occurrence of oedema were related to the initial volumes and the percentage change in volumes was calculated, using the following formula:

$$Var.\% = \frac{Vx-V0}{V0} \times 100,$$

where Vx – the volume determined after 1 week, respectively 2 weeks after the induction of inflammation; V0 – the initial volume.

Substances and apparatus

The following substances were used: hydrocortisone ointment 10 mg/g (Antibiotice Iași, Iași, Romania); acetone (Chemical Company, Iași, Romania); gabapentin (Aurobindo, Hyderabad, Telangana, India); ringer solution; phenobarbital sodium, urethane (Sigma-Aldrich, St. Louis, MO, USA); paclitaxel (Accord Healthcare, Durham, NC, USA) 3 variants of oils sold on the Internet - 1350 mg/100 mL CBD (content declared by the manufacturer - Lady Mary Farm, Călărași, Romania), 2.5% CBD (content declared by the manufacturer HempMed Pharma, Buzău, Romania), 8% CBD (4 mg/drop, content declared by the manufacturer, Dutch Natural Healing, Pater Zen Producties B.V., Castricum, The Netherlands). The CBD emulsion was prepared as follows: initially, a mixture of chitosan (CS) 1% was prepared in 1% acetic acid solution with phosphate-buffered saline solution (PBS) in volumetric ratio (v/v) = 1:9 and pH 7 (CS-PBS); then CS-PBS emulsion was mixed with 1.35% CBD oil in volumetric ratio (v/v) = 1:4 [25].

The following apparatus were used: plethysmometer, cages corresponding to the Hargreaves device for plantar stimuli, von Frey filaments, apparatus for induction of convulsions by electroshock (ECT unit), Hot Plate (Ugo Basile, Gemonio, VA, Italy).

Statistical analysis

The statistical interpretation of the results was performed using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA). The distribution of experimental data was determined using the D'Agostino & Pearson test.

The obtained results were analysed using the ANOVA unifactorial test, followed by the Bonferroni post hoc test for parametric data, respectively the Kruskal-Wallis test followed by the Dunn post hoc test for nonparametric data. Post hoc Tukey test was used for the data obtained in the initial assays, comparing all the groups with each other to test the homogeneity. For the rest of determinations, comparison was made between the control groups (CTL) and those given inflammation (CFA) or neuropathic pain (PTX) inducing agents and placebo, respectively between the groups treated with the combinations of PTX or CFA with the test substances. In case of evaluation of the anticonvulsant effect, the comparation was performed against the control group (CTL). Also, Fisher exact test was performed to compare the incidence of seizures and lethality to the control group [27, 28].

The results were expressed as mean ± standard error of the mean (SEM). The statistical significance threshold was $p < 0.05$.

Results and Discussion

Evaluation of the anticonvulsant effect

The ANOVA test showed a significant difference between groups ($p < 0.0001$, Figure 1A). Treatment with 100 mg/kg phenobarbital reduced the duration of seizures by 91% ($p = 0.0005$). Although acute treatment with 15 mg/kg of CBD produced a decrease in the duration of tonic-clonic seizures by 40%, the effect was not statistically significant ($p > 0.05$). In contrast, administering oils containing doses of 25.77 mg/kg and 47 mg/kg decreased seizure duration by 85% and 87%, respectively, the observed effects being statistically significant ($p = 0.0049$; $p = 0.0014$). Mice in the control group had a frequency of occurrence of seizures of 100% (Figure 1B). Mice treated with the reference substance, phenobarbital, had a frequency of seizures of only 20%. Also, mice treated with 15 mg/kg of CBD had a seizure frequency of 100%, similar to the control group, and the groups treated with 25.77 mg/kg and 47 kg/mg CBD showed a seizure frequency of 40% and 30%, respectively. The Fisher exact test denoted a statistically significant difference between phenobarbital and the control group on the incidence of seizures ($p = 0.0003$). The reduction in the frequency of seizures for mice treated with 25.77 mg/kg and 47 mg/kg CBD was also statistically significant ($p = 0.0034$; $p = 0.001$). The lethal effect of seizures administered by electro-shock was found in 30% of the animals in the control group (Figure 1C). Lethality was not observed among mice treated with phenobarbital, 25.77 mg/kg CBD and 47 mg/kg CBD, but the Fisher exact test showed no statistical significance ($p = 0.0603$). On the other hand, the group treated with 15 mg/kg CBD showed a lethality of 10%.

The results of other studies showed that CBD was more effective in partial and generalized seizures than in seizures localized in the temporal lobe. After i.p. administration, CBD at a dose of 100 mg/kg b.w. decreased the likelihood of tonic-clonic seizures [28, 29].

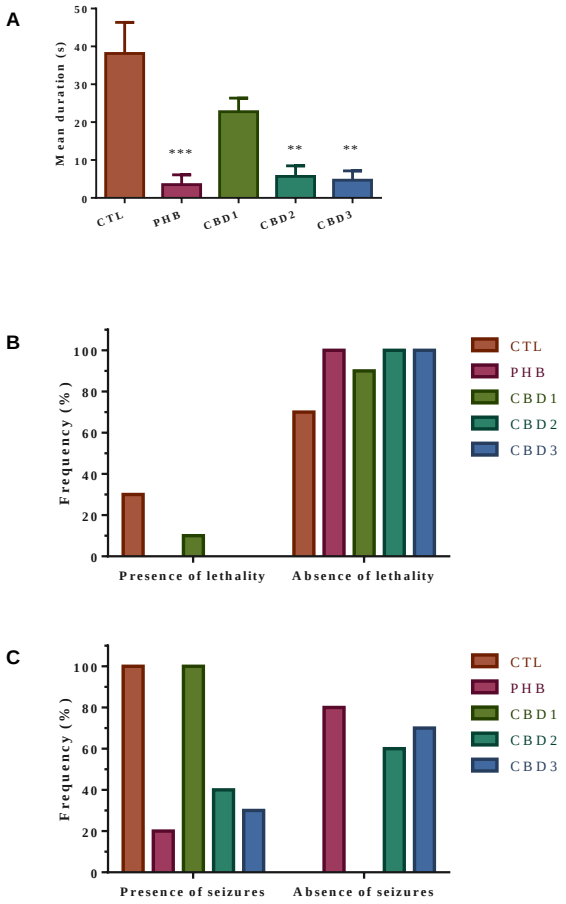

Figure 1.
Anticonvulsant effects of CBD oils
A – duration of seizures after application of electrical stimulus (mean ± SEM); B – Effects of administered substances on lethality; C – Effects of administered substances on seizure frequency;
**p < 0.01, ***p < 0.01 *vs* CTL

Evaluation of the antihyperalgic effect of three CBD oils with different CBD concentrations in a rat model of neuropathic pain

Mechanical allodynia was determined in rats under physiological conditions and at 4, 11 and 18 days of treatment, respectively. No statistically significant differences between the groups were observed in the initial test before administration of the substances (Figure 2A), the experimental groups being homogeneous in terms of tactile sensitivity.

After administration of the 4 consecutive doses of PTX, the rats showed a 26% lower average response threshold compared to the control group, but the difference was not statistically significant (Figure 2B). Also, simultaneous treatment with GPN produced an increase in the response threshold by 40% compared to the group treated with PTX and distilled water. Only the dose of 2.35 mg/kg CBD produced an increase in the response threshold compared to the group treated with PTX and water (23%), but the result was statistically insignificant ($p < 0.05$).

A week after the administration of the cytostatic agent, the neuropathy became slightly more pronounced, with rats treated with PTX and water showing a tactile sensitivity 39% higher than the control group, but the results were not statistically significant (Figure 2C). GPN treatment produced a decrease in tactile sensitivity by 56%, but the effect was not statistically significant. An antiallodynic effect could be observed for doses of 0.75 and 1.29 mg/kg CBD, which produced a statistically insignificant decrease in sensitivity compared to the PTX group by 19% and 21%, respectively. The dose of 2.35 mg/kg CBD improved tactile hypersensitivity by 35% compared to PTX ($p < 0.05$).

ANOVA unifactorial test revealed a statistically significant difference between the groups on day 18 (Figure 2D, $p = 0.0185$). The neuropathy induced by the administration of PTX was significant 2 weeks after the last dose, with tactile sensitivity increasing by 51% ($p = 0.0138$ *vs.* control, Bonferroni post hoc test). The combination of GPN with PTX treatment reduced the mechanical allodynia by 143%, the difference being statistically significant ($p = 0.0041$). Although administration of 0.75 and 1.29 mg/kg of CBD led to a reduction in tactile hypersensitivity by 57 and 61%, the differences were not statistically significant ($p > 0.05$). On the other hand, treatment with 2.35 mg/kg CBD reduced mechanical allodynia by 107%, the effect being significant ($p = 0.0409$).

Sensitivity to cold stimulus was also assessed in rats under basal conditions and at 4, 11 and 18 days of treatment, respectively. The statistical analysis did not reveal any significant differences between the groups in the initial testing, before the induction of neuropathy (Figure 3A), the experimental groups being homogeneous in terms of sensitivity to the cold stimulus.

After the 4 days of PTX treatment, the rats had a total pain reaction score 138% higher than the control group, but the increase in sensitivity to the cold stimulus was not statistically significant (Figure 3B). The combination of GPN reduced hypersensitivity by 63% when compared to the group treated with PTX and distilled water, but the result was not statistically significant. The 3 doses of CBD produced a decrease in the total pain response score by 21, 26 and 32%, respectively, compared to PTX group, the result being statistically insignificant ($p < 0.05$).

One week after PTX treatment, thermal hypersensitivity to the cold stimulus was relatively higher, with rats showing a total pain response score 150% higher than the control group, but the variation was not statistically significant (Figure 3C). Co-administration of GPN led to a decrease in thermal sensitivity by 80%, and treatment with CBD 0.75, 1.29 and 2.35 mg/kg reduced cold stimulus sensitivity by 20, 30 and 40%, respectively, compared to the PTX group.

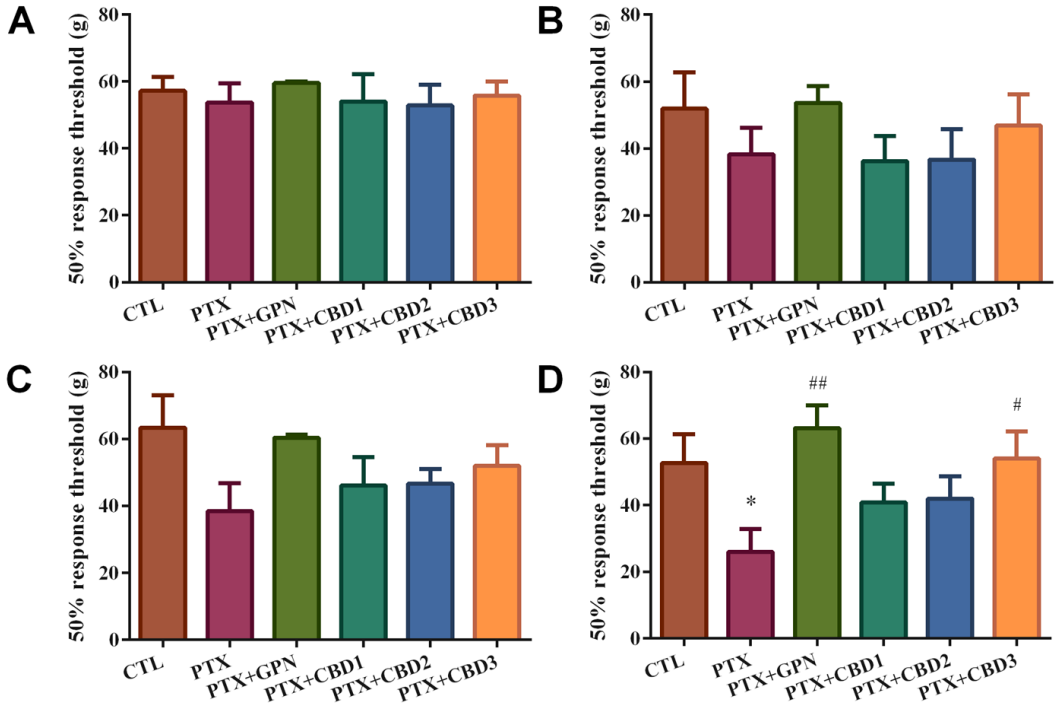

Figure 2.

Effects on tactile allodynia in paclitaxel-induced neuropathy

A – Baseline 50% response thresholds (day 0); B – 50% response thresholds recorded after 4 days; C – 50% response thresholds recorded after 11 days; D – 50% response thresholds recorded after 18 days;

Results are expressed as (mean ± SEM); *p < 0.05 vs. CTL, $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 *vs.* PTX.

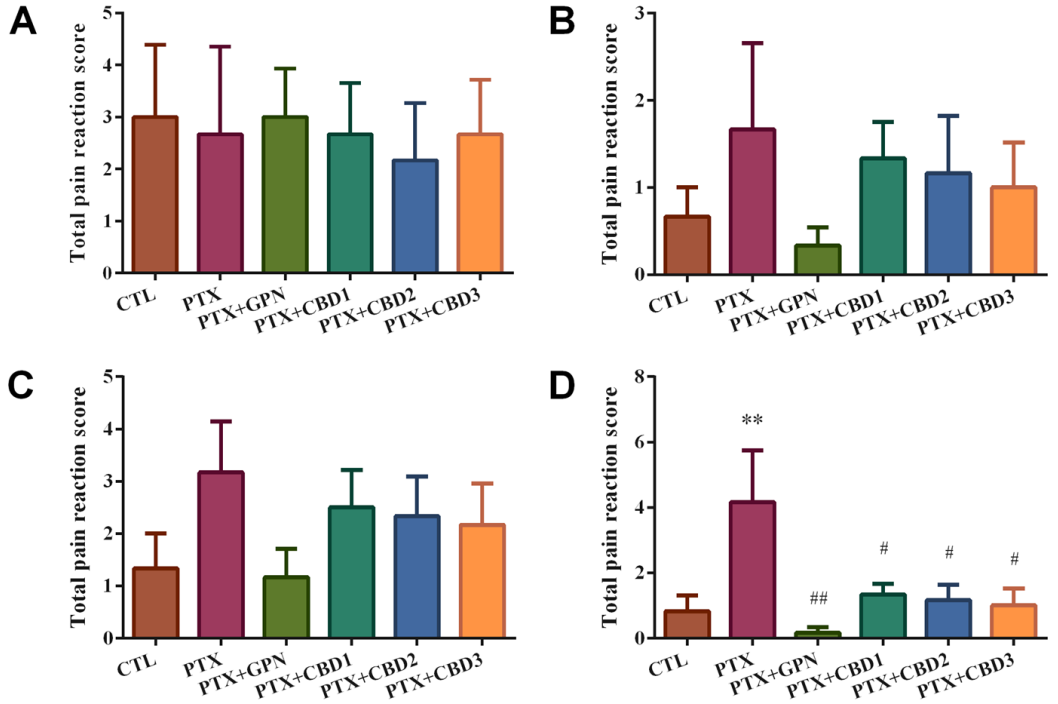

Figure 3.

Effects on cold allodynia in paclitaxel-induced neuropathy

A – Baseline total pain reaction score (day 0); B – Pain reaction score recorded after 4 days; C – 5Pain reaction score recorded after 11 days; D – Pain reaction score recorded after 18 days;

Results are expressed as (mean ± SEM); *p < 0.05 vs. CTL, $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 *vs.* PTX

The unifactorial ANOVA test revealed a statistically significant difference between treatment groups on day 18 of the experiment (Figure 3D., $p = 0.0136$). Two weeks after the last dose of PTX, significant neuropathy was observed, with sensitivity to cold stimulus increasing by 400% compared to the control ($p = 0.0037$, Bonferroni post hoc test). Simultaneous administration of GPN reduced the total pain response score by 96%, the variation being statistically significant ($p = 0.0028$ *vs*. PTX). CBD treatment at doses of 0.75, 1.29 and 2.35 mg/kg significantly alleviated thermal hypersensitivity, with the total pain response score being reduced by 68 ($p = 0.0475$), 72 ($p = 0.0324$) and 76% ($p = 0.0291$), respectively.

The antihyperalgic effect of CBD oil was dose-dependent in the case of cold allodynia, which increased depending on the dose at all moments of determination. However, the variations in effects depending on the dose administered were relatively small.

Treatment for 4 consecutive days with PTX produced a neuropathy highlighted by the mechanical allodynia and thermal hypersensitivity to cold stimulus, which can be observed as early as day 4 of the experiment. On the other hand, the hypersensitivity to painful stimuli induced by PTX was statistically significant only after 2 weeks after treatment, when rats also showed the most intense hypersensitivity.

Concomitant treatment with 60 mg/kg GPN prevented the development of neuropathic pain, the antihyperalgic and antiallodynic effects being statistically significant after 18 days of treatment.

Doses of 0.75 and 1.29 mg/kg CBD had similar effects on tactile hypersensitivity, and the antiallodynic effect was not statistically significant in this case. On the other hand, the dose of 2.35 mg/kg significantly reduced the mechanical allodynia after 18 days of treatment.

Evaluation of the effect of an emulsion containing CBD in an animal model of chronic inflammation

Painful sensitivity to thermal stimulus was determined in rats before induction of inflammation, and 1 and 2 weeks after administration of the inflammatory agent. No statistically significant differences between the groups were observed in the initial test prior to intraplantar administration (Figure 4A), the groups being homogeneous in terms of painful sensitivity to the thermal stimulus.

Although a reduction in latency was observed until the appearance of signs of reaction to pain a week after induction of inflammation, the variations were not statistically significant (Figure 4B). Compared to the blank group, the latency was 35% lower for the CFA group, 7% lower for the CFA+HCZ group, and 17% lower for the CFA+CBD group.

It was observed that the group to which CFA was administered exclusively, a thermal hypersensitivity occurred 2 weeks after induction of inflammation, the latency being 45% lower compared to the control group (Kruskal-Wallis, $p < 0.05$, Figure 4C). The group treated with HCZ had a latency 32% higher than the CFA group, but the difference was not statistically significant. Also, the difference between the average latencies of the CFA+HCZ and CTL groups was not statistically significant (Kruskal-Wallis, $p > 0.05$). The group treated with CFA and CBD emulsion had a 38% lower latency when compared to the control group (Kruskal-Wallis, $p < 0.05$) and 13% higher when compared to CFA group ($p > 0.05$). Thus, the emulsion administered topically, once a day, did not have an antihyperalgic effect.

The anti-inflammatory effect of the CBD emulsion was investigated using the plethysmometer, the local inflammation being induced by intraplantar administration of CFA in rats.

No significant variations in the volumes of the left hind paws were observed during the 2 weeks for any experimental group. On the other hand, significant variations in volumes can be observed for the paws at the level of which the pro-inflammatory agent was administered (Figure 4D). For the control group, relatively constant paw volumes were recorded during the experiment. In contrast, for all groups to which the adjuvant was given, the appearance of significant oedema is observed after a week. Also, after the application of local treatments, decreases in oedema are observed, except for rats that have not received drug treatment.

One week after the induction of inflammation, significant variations in the volumes of the right hind paws are observed for all 3 groups to which the inflammatory agent was administered (Fig. 4E). Thus, the volumes of the hind paws increased compared to the control group by 1112% for the CFA group (ANOVA, $p < 0.0001$), 1016% for the CFA+HCZ group ($p < 0.001$), and 1417% for the CFA+CBD group ($p < 0.0001$).

Two weeks after induction of inflammation, the change in rat paw volumes was 360% higher compared to the control group, the difference being statistically significant (Kruskal-Wallis, $p < 0.001$). The local treatment with HCZ led to a 37% lower volume variation compared to the CFA group, this difference being statistically significant (Kruskal-Wallis, $p < 0.05$), thus highlighting the anti-inflammatory effect. Although local treatment with the CBD emulsion produced a decrease in volume variation by 21% compared to the CFA group, the difference was not statistically significant ($p > 0.05$). Also, the CBD-treated group had significantly higher variations in the volumes of hind paws when compared to the control group ($p < 0.05$).

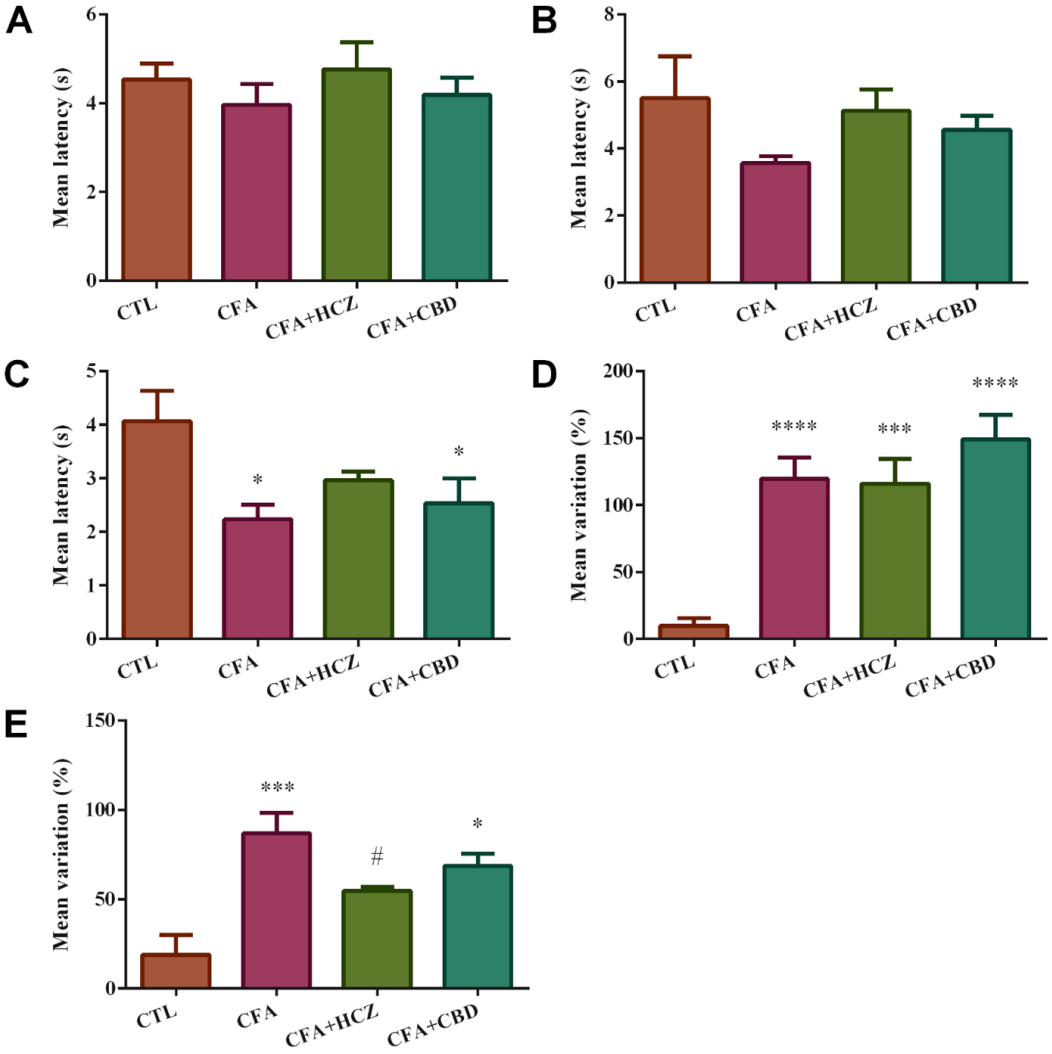

Figure 4.

Analgesic and anti-inflammatory effects in CFA-induced chronic inflammation

A – Baseline thermal sensitivity; B – Thermal sensitivity after 1 week, C – Thermal sensitivity after 2 weeks; D – Variation in right hind paw volume after 1 week; E – Variation in right hind paw volume after 2 weeks; Results are expressed as mean ± SEM; *p < 0.05, ***p < 0.001, ****p < 0.0001 *vs.* CTL, # p < 0.05 *vs.* CFA

Intraplantar administration of CFA produced significant thermal hypersensitization after 2 weeks. This effect has not been ameliorated by local treatments with HCZ and CBD-CS emulsion, respectively.

Inflammation induced by the CFA appeared a week after injection of the pro-inflammatory agent. Local treatment with HCZ significantly relieved CFA-induced oedema.

Conclusions

A significant anticonvulsant effect has been observed for 2.5% CBD and 8% CBD oils, the effect being almost similar to that produced by phenobarbital. Also, for 2.5% and 8% CBD oils, no lethal effect was observed, and the frequency of occurrence of seizures was lower than that observed in the control group.

All 3 doses of CBD have significantly reduced thermal hypersensitivity at the end of the treatment in the rat model of neuropathic pain. In the case of both tactile and thermal sensitivity, GPN exhibited therapeutic effects superior to the highest dose of CBD used in the experiment.

Local treatment with the CBD-CS emulsion produced a modest, statistically insignificant improvement in oedema. Therefore, it is probably necessary to increase the concentration of the active substance or repeated topical application, several times a day, or systemic administration of the CBD-CS emulsion for the development of a significant effect. Further studies are needed to investigate these issues.

Conflict of interest

The authors declare no conflict of interest.

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