Study the Effect of Cannabidiol Topical on Antinociceptive and Anti-inflammatory Activities in Animal Model

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Article

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Abstract

Introduction: Cannabidiol (CBD) is a non-psychoactive compound of cannabis. Due to the therapeutic potential of CBD, there were given drugs through oral administration to treat pain and anti-inflammatory. The bioavailability of CBD has been reported to be poor when given through oral administration because of the high first-pass effect with cytochrome P450. Transdermal delivery systems of CBD may increase bioavailability and decrease first-pass metabolism with cytochrome P450. This study aimed to evaluate the antinociceptive and anti-inflammatory activities of CBD cream in an animal model. Formalin test and Antinociceptive activity.

Materials and Methods: We examined the antinociceptive and anti-inflammatory of CBD cream in an animal model. Formalin and writhing tests were used for the antinociceptive activity, and Acute inflammatory was used carrageenan-induced edema test.

Result: In this study, we tested the efficacy of CBD topical for antinociceptive and anti-inflammatory in an animal model. For the formalin test, in the early phase, AUC values in all treatments were significantly decreased when compared with placebo cream (P<0.0001, P<0.0001, P<0.0001, respectively), which were the same results in the late phase. Moreover, mice treated with CBD and CBD+levomenthol group showed less pain than with diclofenac usage. For the acetic induce writhing response test, The results have demonstrated that diclofenac, CBD, and CBD+levomenthol cream showed an ability to reduce writhes compared with a placebo group. Carrageenan-induced edema, The 1% CBD cream could significantly decrease paw volume from 1 to 4 h compared to the placebo group. Overall, 1% CBD cream treatment may have a high efficacy in decreasing paw volume from 1 to 4 h.

Conclusion: The study demonstrated that 1% CBD cream has potential effects for analgesia and anti-inflammation. Even though the mechanism of the therapeutic effect of a new formulation of CBD has not been completely understood, the topical of 1%CBD cream may also be a good candidate for treatment for analgesic and anti-inflammatory conditions.

Introduction

Cannabis sativa L. has been widely used in traditional medicine for centuries. There are over 100 different cannabinoid compounds in this herbaceous flowering plant. Two bioactive cannabinoid compounds, namely Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have been mainly found in Cannabis [1]. THC is a psychoactive compound, while CBD is non-psychoactive. Those two compounds have been reported as counter pain reliever [2, 3]. CBD and THC interact with specific cannabinoid receptors: cannabinoid receptor type 1 (CB1) and receptor type 2 (CB2). CB1 receptors can be found in the central nervous system such as glial cells and neurons in various brain parts, whereas the CB2 receptor is mainly found in the peripheral nervous system or the body's immune system [4, 5]. Although CBD showed lower affinity to receptors than THC (ca. 100-fold) [6], it could exert multiple pharmacological actions through CB1 and CB2, involving intracellular pathways that play an important role in neuronal physiology [7, 8]. In

particular, many actions of CBD seem to be mediated by binding with G protein-coupled receptor 55 (GPR55) [6, 9], transient receptor potential vanilloid type 1 (TRPV1), and 5-hydroxytryptamine receptor subtype 1A (5-HT1A) [10].

In clinical use, there are many commercially available CBD products; however, only two products: Sativex® [11], and Epidiolex® were approved by U.S. FDA [12]. In addition, these products can be used only in oral administration for reducing seizure activities in Lennox-Gastaut and Dravet Syndrome. The bioavailability of CBD has been reported to be low when given drugs through oral administration in both animals and humans because of the high first-pass effect with cytochrome P450 through the liver [13, 14]. Due to the therapeutic potential of CBD, there were many publications studying delivery systems to treat pain. Millar et al. summarized promising delivery systems such as oral administration, pulmonary administration (inhalation), transmucosal administration, dermal and transdermal route, and ophthalmic delivery [15]. In this paper, we are interested in dermal and transdermal-drug administration through the skin because of its easy accessibility. To conduct clinical research in this study, we therefore, formulated in-house CBD cream and evaluated the activities of CBD (antinociceptive and anti-inflammatory) in an animal model.

Materials And Methods

Animals

Ten male ICR mice (30–40 g body weight) and eight Sprague Dawley rats (300–320 g body weight) were purchased from Nomura Siam International, Thailand. Animals were housed under standard conditions of 12 h/12 h light/dark cycle with 130–325 Lux, temperature 22 ± 1°C, and 30–70% relative humidity at the Laboratory Animal Center of Thammasat University. Prior to experiments, all animals were kept under laboratory conditions for one week. Water and food were available ad libitum. All animal procedures followed the recommendations in the ARRIVE guidelines and were approved by the Institutional Animal Care and Use Committee of Thammasat University, Thailand (Protocol Approval No. 010/2021). All methods were carried out in accordance with relevant guidelines and regulations.

Chemicals and drugs

Ethylenediaminetetraacetic acid, lambda-carrageenan, acetic acid, Hematoxylin & Eosin staining solution, and absolute ethanol were purchased from Sigma-Aldrich, Milano, Italy. Diclofenac, formaldehyde, and levomenthol were purchased from Tokyo Chemical Industry, Tokyo, Japan. Cannabidiol (CBD) powder was purchased from Suranaree University of Technology, Thailand.

In-house CBD cream formulation

Pure CBD, Ethanol, Propylene glycol, and Paraben were dissolved in water and then mixed with Cosmedia® Ace during the cream-forming process.

Formalin test

A standard method for inflammatory pain tests in animals is the formalin test. The method was performed as previously described with minor modifications [16–18]. Prior to the experiment, placebo cream (without any drugs additive), 1% diclofenac, 1% CBD, and a mixture of CBD and 4% levomenthol cream (0.3 mL each) were prepared and applied on an individual right hind paw of mice and handled in a restrainer for 1 h. Then, mice were injected with 20 µL of 2.5% formalin solution into the dorsal surface of the right hind paw using a Hamilton microsyringe with a 30-gauge needle. After formalin injection, mice were placed in a glass cylinder (15 cm in diameter and 20 cm in height) and observed behavior. Nociceptive behavior in mice was observed from 0 to 5 min (phase I, early phase) and 15 to 30 min (phase II, late phase). Mirror glass was placed under the floor at a 45^0 angle to observe the nociceptive behavior such as biting, licking, or shaking paw. The number of times of bite, licking, and shaking paw were counted and recorded. The nociceptive responses were divided into two phases: early phase (neurogenic pain) and late phase (inflammatory pain). The number of times of response was plotted versus time (minute). The area under the curve (AUC) was calculated from the graph using the trapezoidal rule [19].

Writhing test

The acetic acid-induced writhing response was performed as described by Fontenele et al.[20] Prior to the experiment, placebo cream, 1% diclofenac, 1% CBD, or 1% CBD combination with 4% levomenthol cream (0.3 mL each) were applied on the abdominal of mice, and handled in a restrainer for 1 h. Mice have induced nociception by injecting 0.6% of acetic acid solution (10 mL/kg) using the intraperitoneal (i.p.) method. After injection, mice were placed in a glass cylinder (15 cm in diameter and 20 cm in height) and observed writhing responses such as pelvic rotation and abdominal stretching of a least one hind limb. The numbers of writhing responses were counted 5 mins intervals for 30 mins after the i.p. injection.

Carrageenan-induced edema

Carrageenan-induced edema is the standard method for anti-inflammatory activity screening. Prior to inducing inflammation, Placebo cream, 1% diclofenac, or 1% CBD cream were individually applied on the right hind paw of the rat and handled in a restrainer for 1 h. The inflammation was then induced by intra-plantar injection of 0.1 mL of λ-carrageenan (1% w/v in saline). Paw volume was immediately measured using a plethysmometer (Ugo Basile, Varese, Italy) at 1, 2, 3, and 4 h after injection. If the inflammation occurred, the paw would show edema. The volumes of paw after injection were compared before injection.

Statistical analysis

Data were analyzed with GraphPad Prism version 9 (GraphPad Software, San Diego, CA). One-way ANOVA was used followed by a post hoc Tukey for multiple comparisons. The confidential level was set at 95% (p-value < 0.05) and each data set was shown as mean ± SD.

Results

In this study, we tested the efficacy of CBD topical for pain relief in animals using three methods: formalin test, writhing test, and carrageenan-induced edema test was used.

For formalin test, it was used to investigate the antinociceptive activity of CBD. Four treatments: placebo, 1% diclofenac, 1% CBD, and CBD combination with 4% levomenthol cream were tested. Nociceptive pain was induced by injection with 1% formalin. Data were collected for two phases: early phase (0–15 mins) and late phase (15–30 mins). For the early phase, it showed that diclofenac and CBD cream helped to reduce pain in mice, resulting in a significant decrease of AUC values when compared with placebo cream ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, respectively) (Fig. 1A). Interestingly, CBD gave the same AUC level as diclofenac, which was used as a drug standard. Considering a combination of CBD and levomenthol, mice had less pain than in other treatments, resulting in AUC values lower than those three treatments. This result showed that CBD combined with levomenthol cloud significantly improves the efficiency of pain relief. For the late phase, AUC values in all treatments were significantly decreased when compared with placebo cream ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, respectively) which were the same results in the early phase (Figure. 1B). Moreover, mice in treated with CBD and CBD + levomenthol group showed less pain than diclofenac usage.

For the acetic-induced writhing response test, this method is used to examine the peripheral nociceptive activity. Mice were divided into four groups. Each group was tested with a placebo, 1% diclofenac, 1% CBD, and a combination of CBD and 4% levomenthol cream. The results have demonstrated that diclofenac, CBD, and CBD + levomenthol cream showed the ability to reduce writhing when compared with the placebo group (Fig. 2). Furthermore, these three treated groups did not show a difference. These results illustrated that CBD might be used as an alternative counter-pain reliever.

For the carrageenan-induced edema test, the results illustrated that 1% CBD cream could significantly decrease paw volume from 1 to 4 h, compared to the placebo group (Fig. 3). Unlike diclofenac cream, it affected paw volume after 3 h. After 3 h, paw volumes from diclofenac were equal to CBD treatment. This might be suggested that CBD was more effective than diclofenac. Considering the physiology of rat paw after 4 h treatment, Overall, treatment with 1% CBD cream may have a high efficacy in decreasing paw volume from 1 to 4 h (Fig. 4).

Discussion

The formalin test in mice is a reliable and valid model of nociception. The noxious stimulus was injected with 1% formalin in saline under the right hide paw. In this study, we first found that the treatment of CBD cream effectively decreases nociceptive behaviors in mice. Moreover, we found that the treatment of 1% CBD combination with 4% levomenthol cream effectively decreases nociceptive behaviors and higher treatment with 1% CBD cream alone in the early phase. In addition, we found that 4% levomenthol was a synergistic antinociception response of 1% CBD in the early phase (neurogenic pain) while the late phase (inflammatory pain) was antagonistic 1% CBD. Levomenthol is a cooling effect and vasoactive agent [21], acting as a sodium channel blocker. [22] Low concentration of levomenthol depresses cutaneous

nociception and may even desensitize nociceptive C-afferent fibers [23], but it did not have anti-inflammatory activity as shown in the late phase.

The nociceptive mechanism of the formalin test produces the inflammatory mediator's distinct biphasic phases. The early phase of the formalin test represents acute pain and occurs immediately after formalin injection. The nociceptor mediators, including bradykinin, glutamate, and substant P activate the nociceptor through C fiber [18, 24]. The late phase of the formalin test activates inflammatory mediators, including prostaglandins, histamine, bradykinin, serotonin, and substance P [25]. Moreover, inflammatory mediators of the late phase were activated transient receptor potential subfamily A (TRPA) and transient receptor potential vanilloid (TRPV) indirectly or directly via the activation of downstream signaling pathways [26, 27]. The meta-analysis and a systematic review of modulators of the endocannabinoid system found verity in CBD treatment outcomes based on the inflammatory and pain model. The studies CBD has significantly decreased nociceptive behaviors with mixed results in neuropathic and inflammatory pain models [28, 29]. Other pre-clinical and clinical studies have found CBD to be a promising antinociceptive agent for decreasing neuropathic and inflammatory pain [22, 30–34].

The acetic acid-induced writhing test, which model represents a nociceptive chemical test. This test is widely employed to measure peripheral analgesic activity or visceral inflammatory pain [35]. Nociception is intraperitoneal injection with acetic acid and leads to the stimulation of nociceptive neurons by increasing inflammatory mediators such as histamine, prostaglandin, and bradykinin in peritoneal fluid. The inflammatory mediators stimulate peripheral nociceptive receptors and nociceptive behavior such as abdominal constrictions, pelvic rotation, and subsequent stretching of at least one hind limb [36 37]. However, the nociceptive behavior was probably similar to the result of peritonitis [38]. The study found that CBD cream had an anti-inflammatory effect and decreased nociceptive behavior in the mice model. However, the effect of combination with 4% levomenthol cream is not synergistic CBD to reduce nociceptive behavior.

Carrageenan-induced inflammation is a well-established inflammation model for evaluating the acute anti-inflammatory [32, 39]. This method is susceptible to Non-steroidal anti-inflammatory drugs (NSAIDs) and has been accepted as helpful in investigating new anti-inflammatory drugs [40]. The effect of carrageenan has been a descript biphasic event that involves various inflammatory agents. The first phase (0–2 h after carrageenan injection) is rapid-release pro-inflammatory mediators such as serotonin, histamine, and bradykinin [41]. The second phase (3–6 h after carrageenan injection) is associated with the release of prostaglandins, inflammatory cytokines, nitric oxide, and other COX products respectively [42]. The effect of paw edema occurs immediately and reaches a maximum in the third hour [43, 44].

The study found that 1% CBD cream had rapid onset to decrease paw edema at 1 h and remained the anti-inflammatory effect until 4 h. In contrast, 1% diclofenac cream decreased paw edema at 3 h. Moreover, the literature review of CBD has been shown to be a potential anti-inflammatory and analgesic in animal models [45, 46]. CBD provides various anti-inflammatory mechanisms and regulates immune cell

functions and cell cycle [47]. CBD could suppress products of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), tumor necrosis factor (TNF)-α, growth factors, chemokines, as well as inhibition of immune cell, migration, activation, maturation, proliferation, and antigen presentation [48, 49]. CBD also showed potential action of oxidative inhibition stress, modulating the expression of inducible nitrotyrosine and nitric oxide synthase (iNOS) and decreasing the production of reactive oxygen species (ROS) [50]. However, the mechanism of topical CBD did not been well-identify yet, but it might be an alternative drug for relieving pain and anti-inflammatory conditions [51, 52].

Conclusion

In summary, the topical administration of CBD cream could be used to decrease nociceptive behavior and inflammation in an animal model. Even though the mechanism of the therapeutic effect of a new topical formulation of CBD has not been completely understood, CBD might also be a good candidate for topical treatment for inflammatory conditions. To ensure its safety in clinical use, further studies are required.

Declarations

Funding Information

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

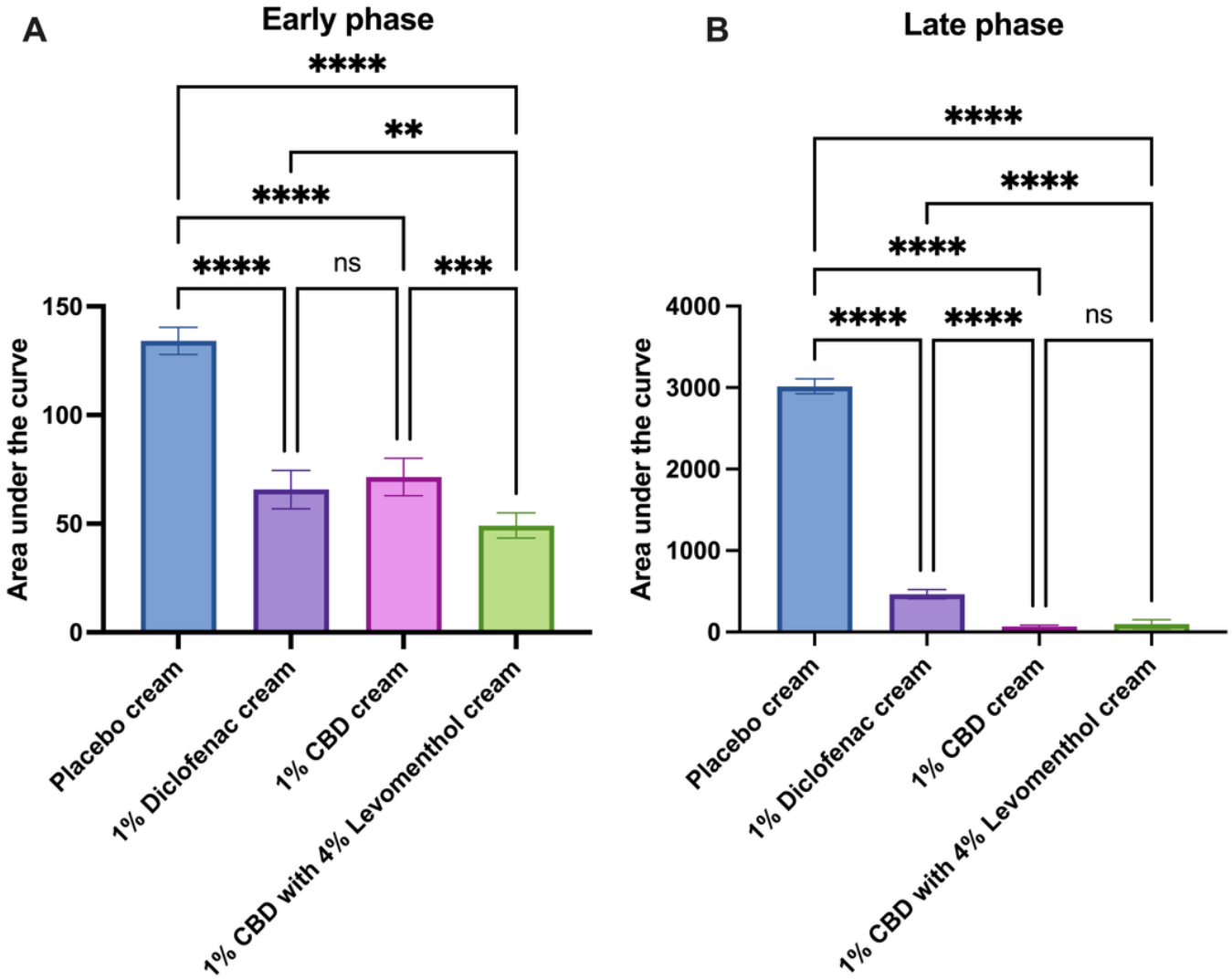

Figure 1

Formalin test. Mice (n=10 per group) were treated with placebo, 1% diclofenac, 1% CBD, and CBD combining with 4% levomenthol cream. Nociceptive behaviors were reported as area under curve (AUC) values in (A) early phase (phase I: 0-5 mins) and (B) late phase (phase II: 15-30 mins). Each data set was plotted as mean of AUC with standard deviation (SD). **, ***, and **** indicate *p*-values < 0.01, < 0.001 and, <0.0001respectively.

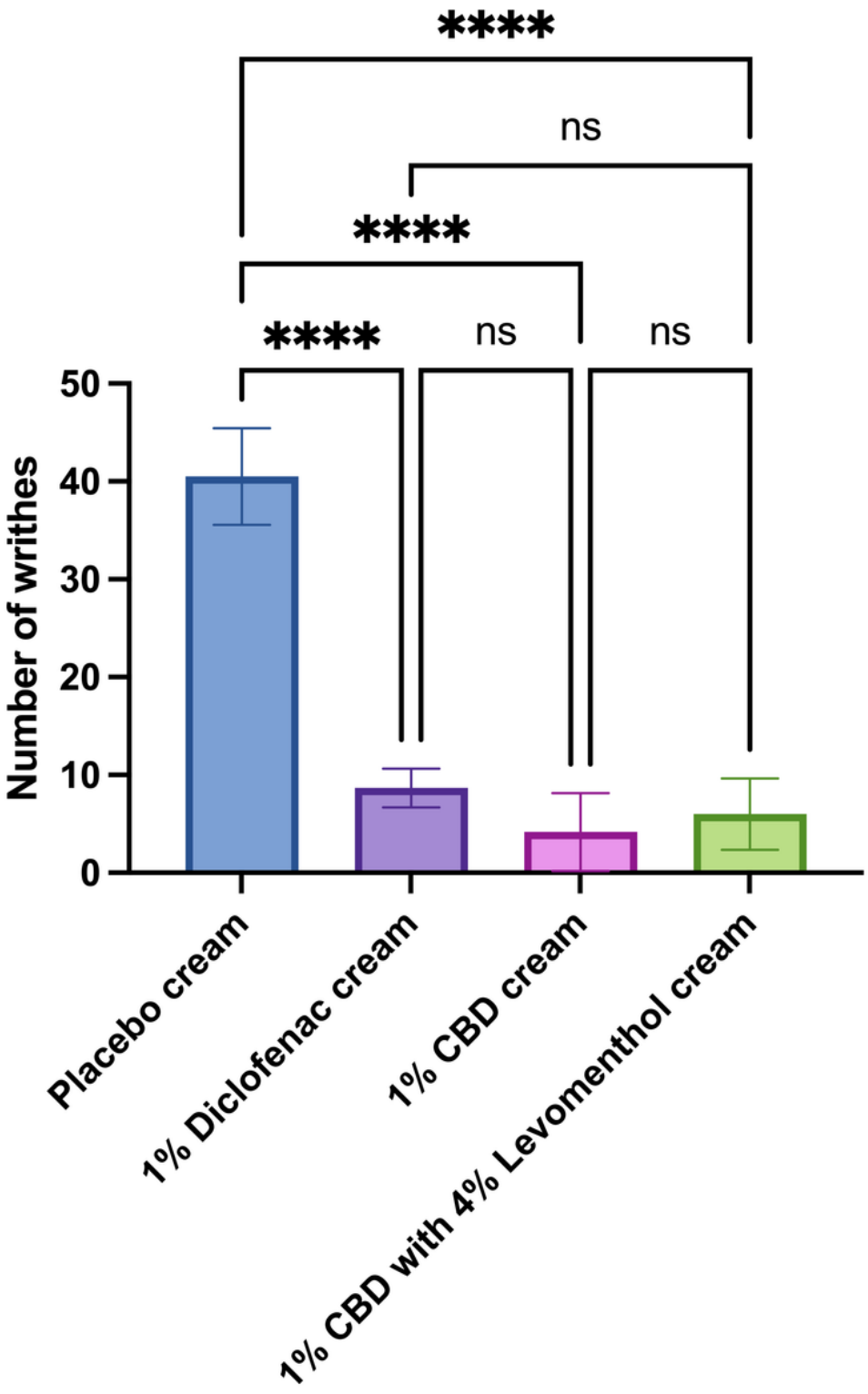

Figure 2

Writhing test. Mice (n=10 per group) were tested an antinociceptive activity by treating with placebo, 1% diclofenac, 1% CBD, 1%CBD combining with 4% levomenthol cream.

**** indicates *p*-values < 0.0001. Each data set was plotted as mean of number of writhes with standard deviation (SD).

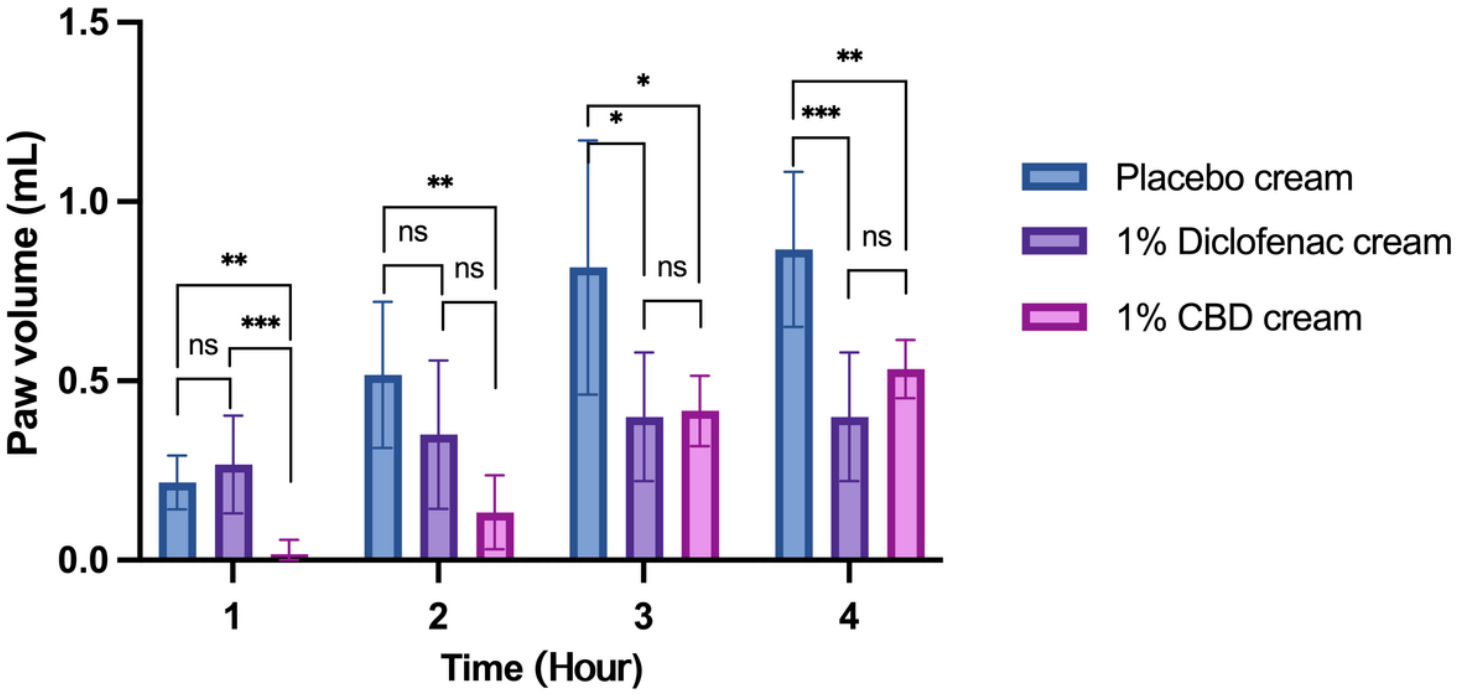

Figure 3

Paw volume after λ carrageenan-induced paw edema. Rats (n = 8 per group) were treated with placebo, 1% diclofenac, and 1% cream. Paws were measured volume at 1, 2, 3, and 4 h after induction. *, **, *** indicate p-values < 0.05, < 0.01, and < 0.01, respectively.

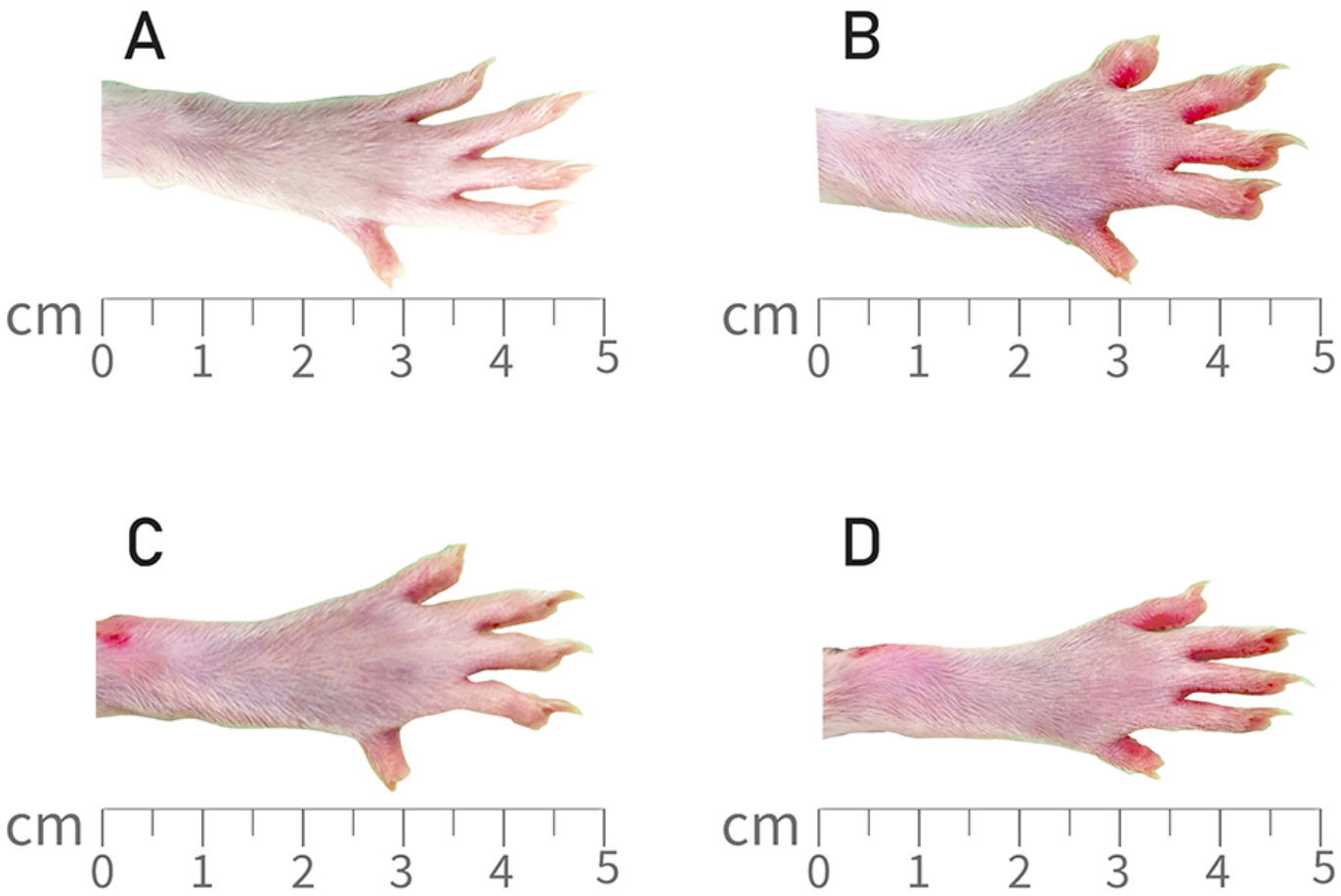

Figure 4

Carrageenan-induced paw edema. (A) Rat paw without induction was used to compare with carrageenan-induced group. Rats were treated with (B) placebo gel (control), (C) 1% diclofenac cream, and (D) 1% CBD cream after injected with λ carrageenan.