



Cannabidiol and Beta-Caryophyllene: Chronic inflammatory pain

Mohammed Alnoud^a, Mohammad A. Hussain^b, Jose Rios^a, Emmanuel Franco^a, Justin Mills^a, Joshua Nwose^a, Maria Sophia Malbas^a, Hiram Garcia^a, Sophia Leslie^a, Manish K. Tripathi^b, Khalid Benamar^{a,*}

^a Institute of Neuroscience and Department of Neuro-behavioral Health, University of Texas Rio Grande Valley, School of Medicine, Biomedical building, McAllen, TX 78504, United States

^b Medicine and Oncology ISU, University of Texas Rio Grande Valley, School of Medicine, Biomedical building, McAllen, TX 78504, United States

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ABSTRACT

While chronic pain is challenging to manage, it always co-exists with depression. Currently, chronic pain and depression are usually treated separately with distinct approaches, yet effectiveness remains elusive. Consequently, the development of integrated therapeutic strategies for pain while addressing depression is a high public health priority and unmet need that affects millions of people. This study aims to determine if the combination of the two phytocannabinoids Beta-Caryophyllene (BCP) and cannabidiol (CBD) is effective for chronic pain while simultaneously showing antidepressant effects. We used a chronic inflammatory pain model (Complete Freund's Adjuvant, CFA) and a battery of pain and depression-like behavior tests in mice. Proteomics and immunohistochemistry (IHC) were used to explore the potential mechanisms of the effect of the combination on pain and depression. We found that mice treated with the CBD and BCP combination produced a synergistic pain-relieving effect in the chronic inflammatory pain model and exhibited antidepressant properties. Our IHC data also show that the CBD and BCP combination significantly reduced the neuroinflammation produced by CFA, and the proteomics showed downregulation of selected proteins involved in inflammation by the combination, compared to the individual effects of CBD and BCP. In conclusion, our current findings show that, in the CFA pain model, the combination of CBD and BCP produces a synergistic pain-relief effect while also having antidepressant properties. Additionally, our data show that the anti-inflammatory action of this combination may explain its beneficial effects on pain and depression. Therefore, our data suggest this combination as a potentially effective treatment for chronic pain and related depression.

1. Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage [1]. Chronic pain contributes to an estimated \$560 billion each year in direct medical costs, lost productivity, and disability programs [2,3]. Pain, one of the most common reasons adults seek medical care [4], and the treatment options are limited and often ineffective, highlighting the urgent need for compelling novel analgesic agents.

Depression is a common mental health condition characterized by a persistently low mood, loss of interest or pleasure in activities, and other symptoms that interfere with daily functioning [5]. Chronic pain is often comorbid with depression. On average, between 30 % and 60 % of chronic pain patients report concurrent depression [6–9]. Comorbid

chronic pain in patients with depression has negative impacts on both depression and pain outcomes [10]. Patients with chronic pain and depression are more likely to have greater severity and longer duration of pain, and patients with higher pain scores experience longer time to response and remission of depression compared with patients with lower pain scores [10]. Depression not only is prevalent, but it imposes an annual economic burden on society of US \$326.2 billion [11]. Thus, the consequences of depression and chronic pain are exorbitantly costly to the individual sufferers, their families, and society.

The identification of novel pain management strategies for treating pain while addressing depression is a high public health priority and unmet need. Natural products have historically been sources of novel analgesic compounds developed into pharmaceuticals. There is increasing interest in the potential utility of cannabis. More than 560

* Corresponding author.

E-mail address: khalid.benamar@utrgv.edu (K. Benamar).

constituents have been identified in cannabis [12–14]. Besides the major psychoactive component delta-9-tetrahydrocannabinol (Δ^9 -THC), there are also non-psychoactive cannabinoids such as cannabidiol (CBD). CBD is a minor cannabinoid found in the cannabis plant that has gained attention as a therapeutic agent over the past several years due to its lack of cannabinoid receptor 1 central nervous system (CNS) side effects (e. g., hypoactivity, hypothermia, and catalepsy) [15]. While preclinical data showed an analgesic effect of CBD [16–23], clinical trials to date have failed to demonstrate significant analgesic effects with pure CBD [24,25].

It has been suggested, however, that compounds in the *cannabis* plant function more efficiently in concert with each other rather than alone [26], a concept introduced by Dr. Mechoulam [27]. This concept brought valuable information that helped to explain how botanical drugs were often more efficacious than their isolated components [28].

In addition to CBD, there are other non-psychoactive cannabinoids in cannabis plants, such as Beta-Caryophyllene (BCP) [29]. BCP is a natural bicyclic sesquiterpene that is a common constituent in many essential oils, including those derived from *Cannabis sativa*. BCP is a natural selective agonist for the cannabinoid type 2 receptor (CB₂) [30]. BCP is an FDA-approved food additive [31] that has several beneficial effects, such as analgesia, antioxidation, and anti-inflammation [26,30,32–39].

Using the inflammatory pain model, the primary goal here was to test the effect of the CBD and BCP combination on pain and associated depression.

2. Materials and methods

2.1. Animals

Male and female C57BL/6 J mice (20–25 g) were purchased from Jackson Laboratories (Bar Harbor, Maine, USA). Mice were group-housed (5 per cage) under a 12:12 h light-dark cycle (lights on 07:00, lights off 19:00) and provided with standard mouse chow *ad libitum*. All animal care and experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas Rio Grande Valley and conducted in accordance with the National Institutes of Health accepted guidelines found in the Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

CBD and BCP were purchased from Cayman Chemical (Ann Arbor, MI, USA).

2.3. Complete Freund's adjuvant (CFA) test

Unilateral injection of 20 μ L (CFA, Sigma Aldrich) into the hind paw produces lasting pain-like behaviors [40].

2.4. Pain-like behaviors

Mechanical allodynia was assessed using the digital Vonfrey method (IITC Life Sciences, Woodland Hills, CA), as we previously reported. Mice were placed in individual plastic cages on an elevated wire mesh platform and allowed to habituate to the testing apparatus until exploratory behavior was no longer observed. A semi-flexible filament was applied with increasing force (grams) until a paw withdrawal response was elicited. The force at which this response occurs is recorded automatically by the apparatus and is designated as the paw withdrawal threshold. Three thresholds were taken at each time point.

Cold Allodynia was assessed using the acetone drop method [41]. Animals were placed in plexiglass boxes and allowed to acclimatize. A drop of acetone applied to the plantar surface of the paw produces a cold sensation upon evaporation leading to a nocifensive response by the animals displayed as paw elevation, licking, biting, and shaking, which

is enhanced in the inflammatory pain model. Time (seconds) spent elevating, licking, biting, and shaking the stimulated paw was recorded.

2.5. Tail suspension test

The test was initiated by suspending the mouse by its tail from a rod positioned at a height of 50 cm above the surface. We recorded the mouse's behavior on video and precisely measured the latency to the first instance of immobility, as well as the total duration of immobility within a 5-minute window, with all scores recorded manually.

2.6. Forced swim test (FST) [42,43]

This test is performed by placing mice in a clear cylindrical container measuring 55 cm in height and 20 cm in diameter, filled with water. Mice were allowed to spend a total of 5 min in the water, and the total amount of time the animal spent immobile in the water was recorded and analyzed using a video tracking system (Noldus).

2.7. Isobologram

Isobolographic analysis was performed to determine whether the combined anti-nociceptive effects of were sub-additive, additive, or synergistic (super-additive). An isobologram was derived by plotting an XY graph with the ED₅₀ for CBD on the Y axis, the ED₅₀ for BCP on the X axis, and connecting the two points diagonally, through the so-called "line of additivity" [44]. If drug synergy is displayed, the ED₅₀ for the drug combination falls below the line of additivity, indicating drug synergy.

2.8. Immunohistochemistry (IHC)

Paraffin-embedded brain tissues were sectioned at a thickness of 10 μ m. The primary antibodies used were Anti-Iba1 antibody (ab178846) at a dilution of 1/1000, and Anti-GFAP antibody (ab68428) at a dilution of 1/250. The secondary antibody employed was MACH 4 (Polymer) Universal HRP-Polymer (M4U534H). DAB (3,3'-diaminobenzidine) served as a chromogen to visualize antibody binding, resulting in a brown precipitate at the site of antigen expression. Stained sections were scanned using Panoramic Scanner 2.2.0-MIDI for digital image acquisition, and all images were analyzed with 3DHISTECH CaseViewer software.

2.9. Proteomics

Mouse brain tissues were flash-frozen in liquid nitrogen during brain extraction. Mouse brains were homogenized to obtain a fine powder, as we previously reported. The powdered tissue was transferred to 1.5 mL tubes, mixed with 400 μ L of lysis buffer (containing 1 μ L of protease inhibitor), and incubated on ice for 30–60 min. The extract was sonicated with 2-second on/off pulses for 1–2 min and then centrifuged at 14,000 rpm for 1 h at 4°C. The supernatants were stored at –20 °C. Fifty micrograms of mouse brain extracts (Bradford assay) from four groups (triplicate) were used for Liquid Chromatography-Mass Spectrometry analysis. Extracts were resuspended in lysis solution (Thermo Scientific, Cat# A40006), reduced, and alkylated for 20–30 min each, then heated at 95 °C for 10 min. The samples were trypsinized with a Trypsin/Lys-C mix at 37 °C overnight. Digested peptides were purified with peptide clean-up columns (Thermo Scientific) and finally eluted in 300 μ L of elution buffer. Eluates were vacuum-dried and reconstituted in 100 μ L of 0.1 % formic acid. One microliter of each sample was analyzed on an Orbitrap Exploris 240 mass spectrometer (Thermo Scientific) using data-independent acquisition (DIA) over a 120-minute gradient run. Standard HeLa extract (Thermo Scientific) was used as a reference. Raw data were analyzed using Proteome Discoverer software (versions 2.5 and 3.1).

2.10. Statistics

Statistics were performed in Graph Pad Prism 9 (GraphPad Software; San Diego, USA). One-Way ANOVA with appropriate Post Hoc Tests. A *t*-test was used to compare the means of the responses of two groups in which a single variable is affected. Significance was set at $p < 0.05$.

3. Results

3.1. Dose-response (D-R) of CBD

Fig. 1A shows the experiment design. CBD administered intraperitoneally (i.p.) at doses of 0.1–100 mg/kg produced a dose-dependent reduction in mechanical allodynia and thermal hypersensitivity (Fig. 1B and C, One-Way ANOVA with Dunnett's Post Hoc Test). The vehicle was dimethyl sulfoxide (DMSO), cremaphor, and saline (1:1 18).

3.2. D-R of BCP

The BCP administered at 0.1–100 mg/kg doses induced a dose-dependent reduction in the mechanical allodynia and thermal hypersensitivity (Fig. 2A and B, One-Way ANOVA with Dunnett's Post Hoc Test).

3.3. D-R of CBD and BCP in combination

Next, the combination of the two compounds was tested at fixed-dose ratio of 1:1.44 (based on each compound's ED_{50}). We tested four dose combinations: 1/2, 1/4, 1/8, and 1/16 of ED_{50} of CBD and BCP. The combination reduced mechanical allodynia (Fig. 3A, One-Way ANOVA with Dunnett's Post Hoc Test). Of great interest, we found that the combination of CBD and BCP at a lower dose (8.4 mg/kg, Fig. 3B, One-Way ANOVA with Dunnett's Post Hoc Test) produced the same effect as a higher dose of CBD (50 mg/kg) and BCP (50 mg/kg) individually. These data suggest a synergistic effect. Using isobolographic analysis confirmed that this combination is synergistic (Fig. 3C).

Similarly, tested at this fixed-dose ratio of 1:2.62 (based on each compound's ED_{50}) in the acetone test, the combination reduced cold allodynia (Fig. 3D, One-Way ANOVA with Dunnett's Post Hoc Test), and isobolographic analysis demonstrated that this combination is

synergistic (Fig. 3E).

Furthermore, the ED_{50} of this combination at 0.93 ± 0.12 mg/kg (Vonnrey test) was lower than the ED_{50} of the individual effects of CBD (7.2 ± 3.9 mg/kg) and BCP (10.38 ± 2.01 mg/kg). These results indicate that this combination has greater potency than the individual effects in the vonfrey test. In the acetone, the ED_{50} of this combination (1.8 ± 1.4 mg/kg) was lower than the ED_{50} of CBD (3.5 ± 2.8 mg/kg) and BCP (9.2 ± 2.1 mg/kg). These findings demonstrate that this combination is more potent than the individual treatments in the acetone test.

Finally, we tested this combination in female mice. The combination of CBD and BCP attenuated inflammatory pain in Vonnrey (Fig. 4A, *t*-test) and the acetone test (Fig. 4B, *t*-test).

3.4. CBD and BCP in combination produced an antidepressant effect in the CFA pain model

We also tested the effect of this combination (8.7 mg/kg) on depression-like behaviors using the tail suspension and FST tests. The depression associated with chronic pain in CFA model was significantly reduced by the CBD and BCP combination compared to the vehicle group (Fig. 3F, *t*-test). Similarly, this combination showed an antidepressant effect in FST (Fig. 3G, *t*-test). Similarly, this combination showed an antidepressant effect in female mice in the tail suspension test (Fig. 4C, *t*-test) and FST (Fig. 4D, *t*-test).

3.5. Effects of BCP and CBD combination on glial cells

We conducted histological staining using two specific markers: ionized calcium-binding adapter molecule 1 (Iba1) for microglia and glial fibrillary acidic protein (GFAP) for astrocytes.

3.5.1. Effect of BCP and CBD combination on microglia

The CFA treatment resulted in a notable increase in Iba1 expression in the brain (Fig. 5A, One-Way ANOVA with Tukey's Post Hoc Test). The treatments with CBD, BCP, and their combination led to a significant decrease in Iba1 expression in mice with inflammatory pain induced by CFA compared to the vehicle control (Fig. 5A, One-Way ANOVA with Tukey's Post Hoc Test). When comparing the individual effects of CBD and BCP, the combination treatment showed an even greater reduction

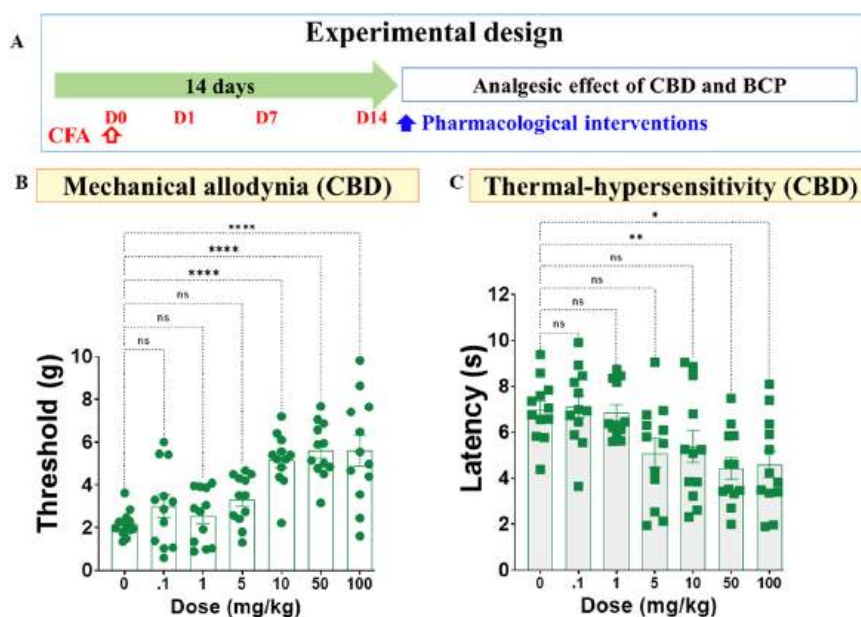


Fig. 1. Experimental design (A). Effect of CBD in CFA-induced mechanical allodynia (B) and thermal hypersensitivity (C). Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. N = 12 mice per group. ns, not significant.

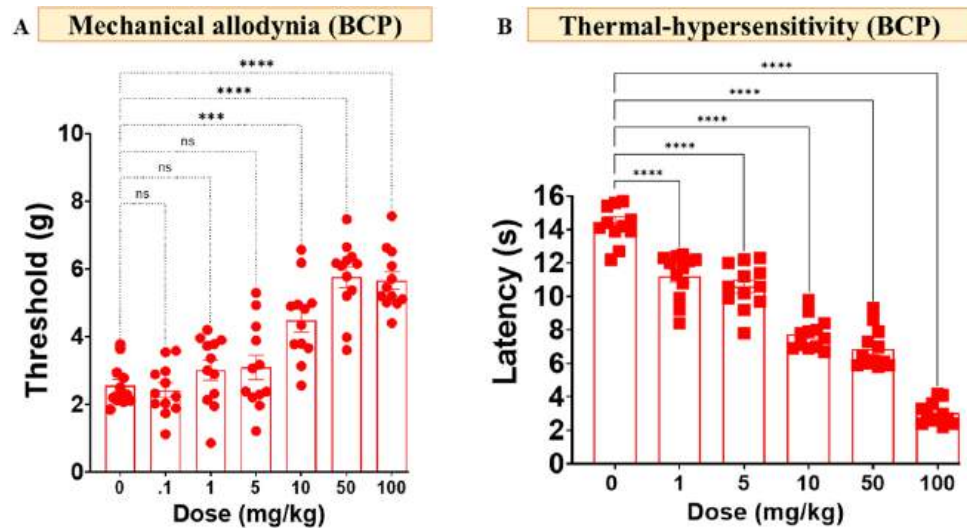


Fig. 2. Effect of BCP on CFA-induced mechanical allodynia (A) and thermal hypersensitivity (B). Data are presented as mean $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. $N = 12$ mice per group.

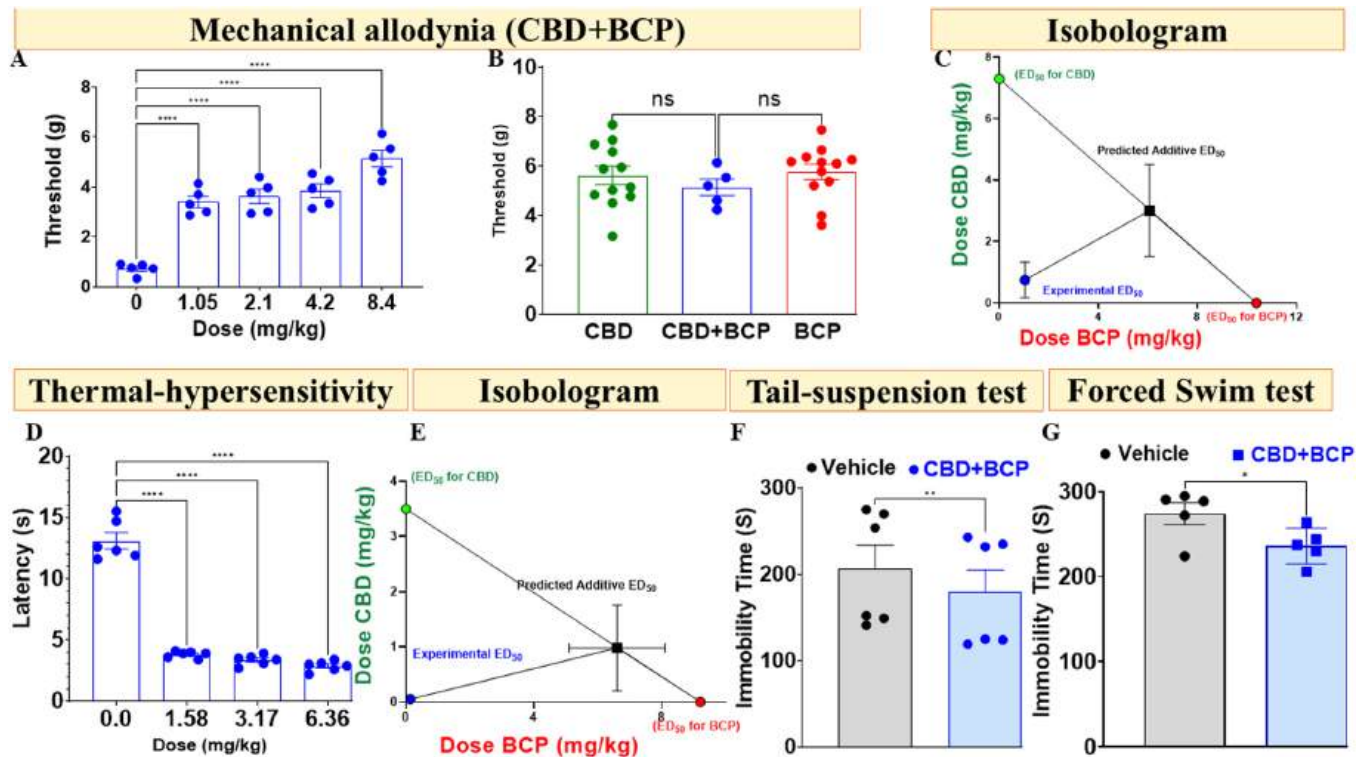


Fig. 3. Effect of CBD and BCP in combination on CFA-induced mechanical allodynia (A). The impact of CBD and BCP combined compared to their individual effects (B). The dose and effect of CBD (50 mg/kg, $N = 12$) and BCP (mg/kg, $N = 12$) were taken from Figs. 1B and 2A and compared to the combination of CBD and BCP (8.7 mg/kg, $N = 5$). Isobolographic analysis of CBD and BCP in combination (C). CBD and BCP in combination fixed ratio ($N = 6$) produce analgesic effect in CFA-induced cold allodynia (D). Isobolographic analysis of CBD and BCP in combination (E). Administration of the combination shows antidepressant effect compared to vehicle-treated animals in the tail suspension test (F) and FST (G). Data are presented as mean $\pm$ SEM. ED₅₀: median effective analgesic dose. ** $p < 0.01$, **** $p < 0.0001$. (compared to the vehicle). $N = 6$.

in Iba1 expression (Fig. 5A, One-Way ANOVA with Tukey's Post Hoc Test). Representative sections of the periaqueductal grey (PAG) were immuno-stained with anti-Iba1 in vehicle (B), CBD (C), BCP (D), and CBD+BCP (E). The PAG is a key descending pain modulatory system component.

3.5.2. Effect of BCP and CBD combination on astrocytes

The treatments with CBD, BCP, and their combination led to a

significant decrease in GFAP expression in mice with inflammatory pain induced by CFA compared to the vehicle (Fig. 5F, One-Way ANOVA with Tukey's Post Hoc Test). The effect of the combination on the expression of GFAP was similar to the effect of the individual effects of CBD and BCP (Fig. 5F). Representative PAG sections were immuno-stained with anti-Iba1 in control vehicle (G), CBD (H), BCP (I), and CBD+BCP (K).

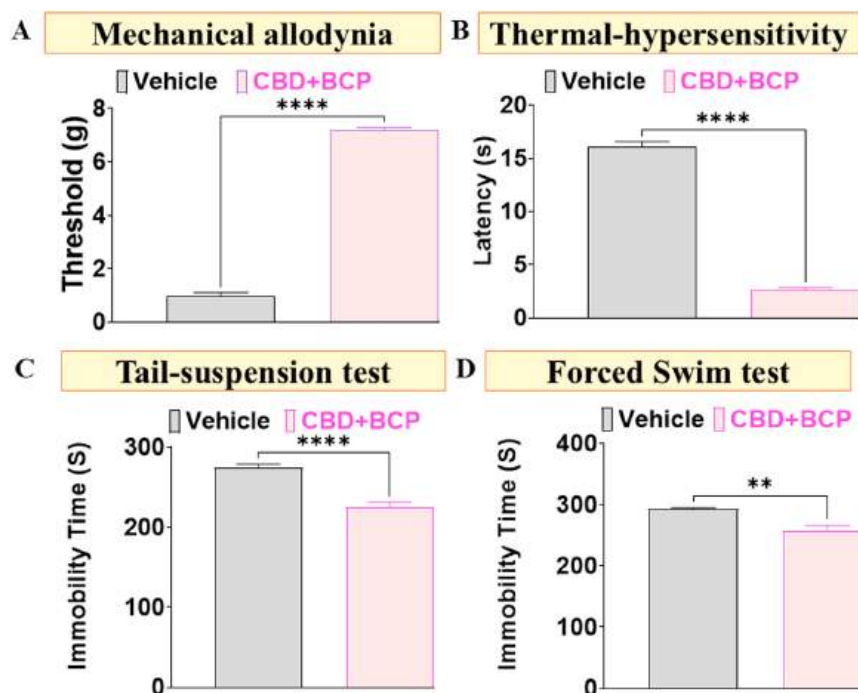


Fig. 4. Effect of CBD and BCP in combination on CFA-induced mechanical allodynia (A), thermal hypersensitivity (B), tail suspension (C), and FST (D) in female mice. Data are presented as mean $\pm$ SEM. ** $p < 0.01$, **** $p < 0.0001$ (compared to the vehicle). $N = 5$.

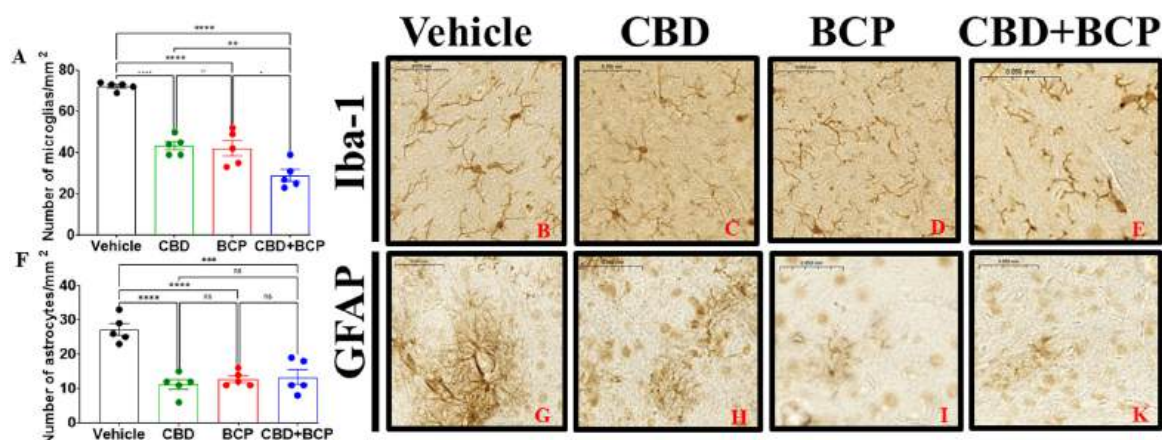


Fig. 5. Treatment with BCP, CBD, and their combination reduced the expression of Iba1 (A) and GFAP (F) in the brain. Representative PAG images of Iba1 (B, C, D and E) and GFAP (G, H, I, and K) immunostaining. These images are captured from the periaqueductal gray area, a key brain region involved in the descending pain modulatory system. ** $p < 0.01$; *** $p < 0.001$; * $p < 0.05$. ns indicates not significant. Data are presented as means $\pm$ SEM. $N = 5$ mice per group. Scale = 0.05 mm.

3.6. Proteomics

3.6.1. Heatmap of differential protein expression in Mice's brain tissues (Fig. 6A)

The clustering heatmap illustrates the expression profiles of differentially expressed proteins identified across brains from four experimental groups: Vehicle/CFA, BCP-treated/CFA, CBD-treated/CFA, and BCP+CBD combination-treated/CFA mice. Clear clustering patterns highlight treatment-specific proteomic signatures. Each row represents a unique protein, and each column indicates a treatment group (with three replicates per group). Proteins with high abundance (red) and low abundance (green), along with a gradient, are visible. This suggests that the regulation of multiple proteins (or a specific set) varies depending on the treatment conditions. Overall, the heatmap's clustering reveals distinct expression patterns and potential group-specific molecular signatures associated with each treatment. Clear differences in group

clustering of protein expression levels (red or green) indicate treatment-specific changes in the brain proteome.

3.6.2. The Venn diagram (Fig. 6B)

The Venn diagram shows the overlap and differences of proteins found in each experimental group. Many proteins are unique to specific treatments, with 163 for BCP, 133 for CBD, 138 for BCP+CBD, and 176 for the vehicle group. A smaller number of proteins were found in all four groups ($n = 15$), and there were also smaller shared subsets among pairs of triads of the groups (Fig. 6).

3.6.3. Volcano plots of differentially expressed proteins in treatment groups (Fig. 6C)

Volcano plots show the significant expression of overlapping proteins between two specific groups of analysis. They illustrate the distribution of proteins that are significantly upregulated and

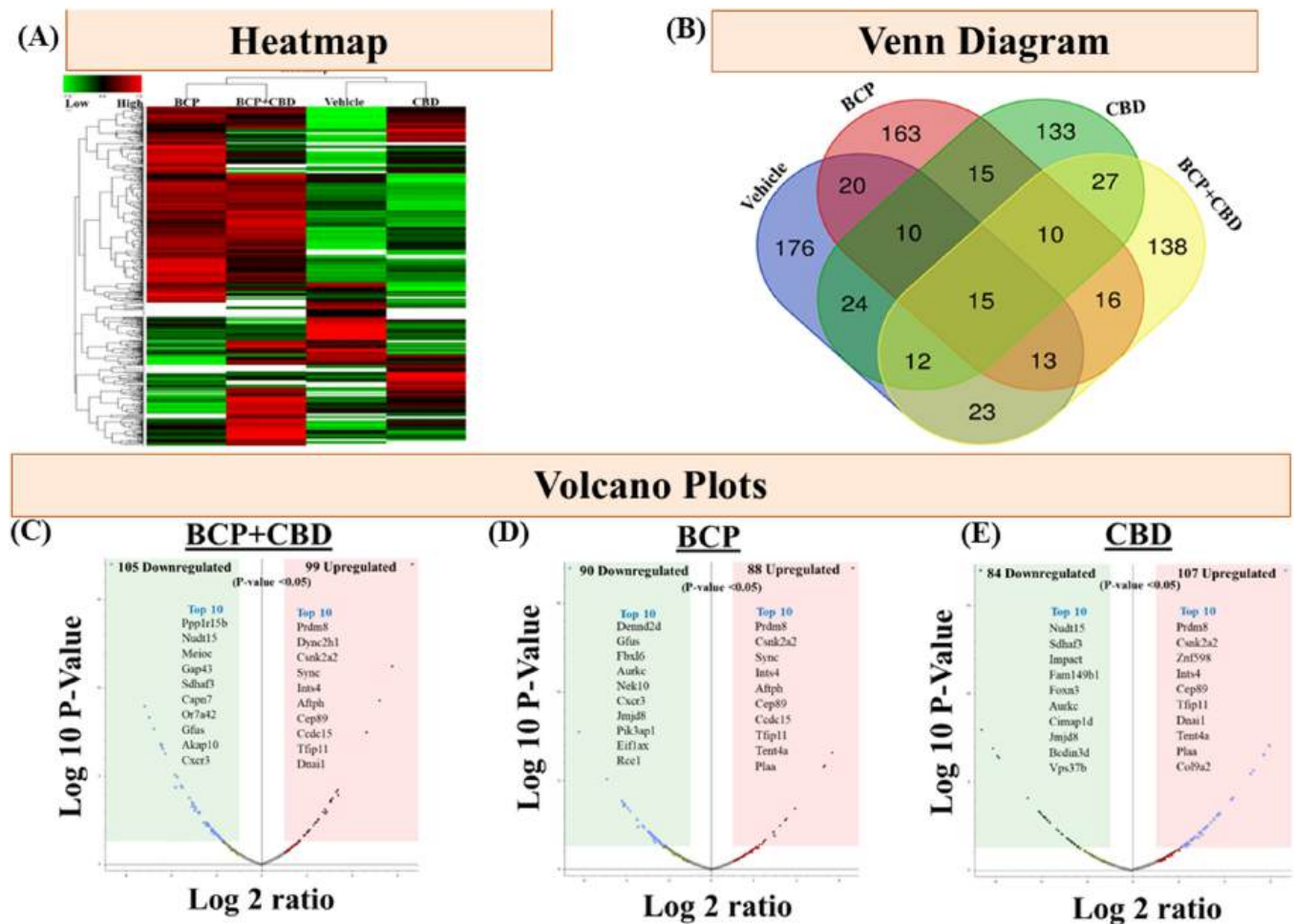


Fig. 6. Heatmap (5 A). The heat map shows the expression patterns of identified proteins in brain tissues from four experimental groups: Vehicle, BCP-treated, CBD-treated, and the combination of (BCP + CBD). The color intensity indicates the relative abundance of proteins, highlighting different protein expression patterns and clustering across treatment groups and conditions. Venn Diagram (5B): The Venn diagram displays the distribution and unique proteins among the four groups, with protein names listed on the diagram. This comparison emphasizes the unique proteins within each treatment group. Volcano Plot (C, D, and F): The volcano plot illustrates the distribution of differentially expressed proteins based on P value < 0.05 and fold change with statistical significance across the experimental groups for the combinations of C. (BCP + CBD), D. (BCP), and E. (CBD). We also highlight the top 10 up-regulated and top 10 down-regulated differentially expressed proteins.

downregulated ($p < 0.05$) across the treatment comparisons. BCP vs. vehicle: 105 proteins were downregulated, and 99 were upregulated. CBD vs. vehicle: 90 proteins were downregulated, and 88 were upregulated. BCP+CBD vs. vehicle: 84 proteins were downregulated, and 107 were upregulated. Each treatment condition (compared to the vehicle control) showed a unique set of differentially expressed proteins. These findings indicate that each treatment caused distinct proteomic changes in brain tissue, supporting the presence of unique molecular mechanisms underlying their pharmacological effects. Here, we presented the top 10 up-regulated and top 10 down-regulated differentially expressed proteins by each group. In particular, CBD+BCP caused the downregulation of GAP-43, Akap10, and Capn7, PP1R15B, MEIOC, DCXR, CCN1, RCE1, and TEPsin, which are distinctly influenced by this treatment.

3.6.4. Gene association network (Fig. 7)

The top 10 downregulated genes mentioned above in the combination treatment were further analyzed for their overlap with those downregulated by the individual CBP and BCP treatments. Five genes were uniquely downregulated by the combination, as shown in the table (arrows). The figure displays a network analysis (using the GeneMANIA database) of five of these genes: PP1R15B, MEIOC, GAP43, CAPN7, and AKAP10. (Note: OR7A42, encoding olfactory receptor 8, was not

included in the analysis due to its absence from the database).

4. Discussion

While chronic pain is challenging to manage, it always co-exists with depression. Currently, chronic pain and depression are usually treated separately with distinct approaches, yet effectiveness remains elusive. Consequently, the development of integrated therapeutic strategies for pain while addressing depression is a high public health priority and unmet need that affects millions of people.

This study presents two important findings that demonstrate the potential of CBD and BCP combination therapy: 1) it shows a synergistic pain-relieving effect in the chronic inflammatory pain model. 2) This combination also exhibits antidepressant properties.

First, we examined the effect of the CBD and BCP combination on CFA-induced chronic inflammatory pain and determined the nature of the interaction (e.g., additive, synergistic).

Our findings indicate that co-administering CBD and BCP in a fixed ratio leads to a significant dose-dependent reduction of mechanical allodynia using the Vonfrey test. Notably, when CBD and BCP are administered at doses that do not produce analgesic effects on their own, their combination yields a significant analgesic response. These results suggest a synergistic effect. Furthermore, isobolographic analysis

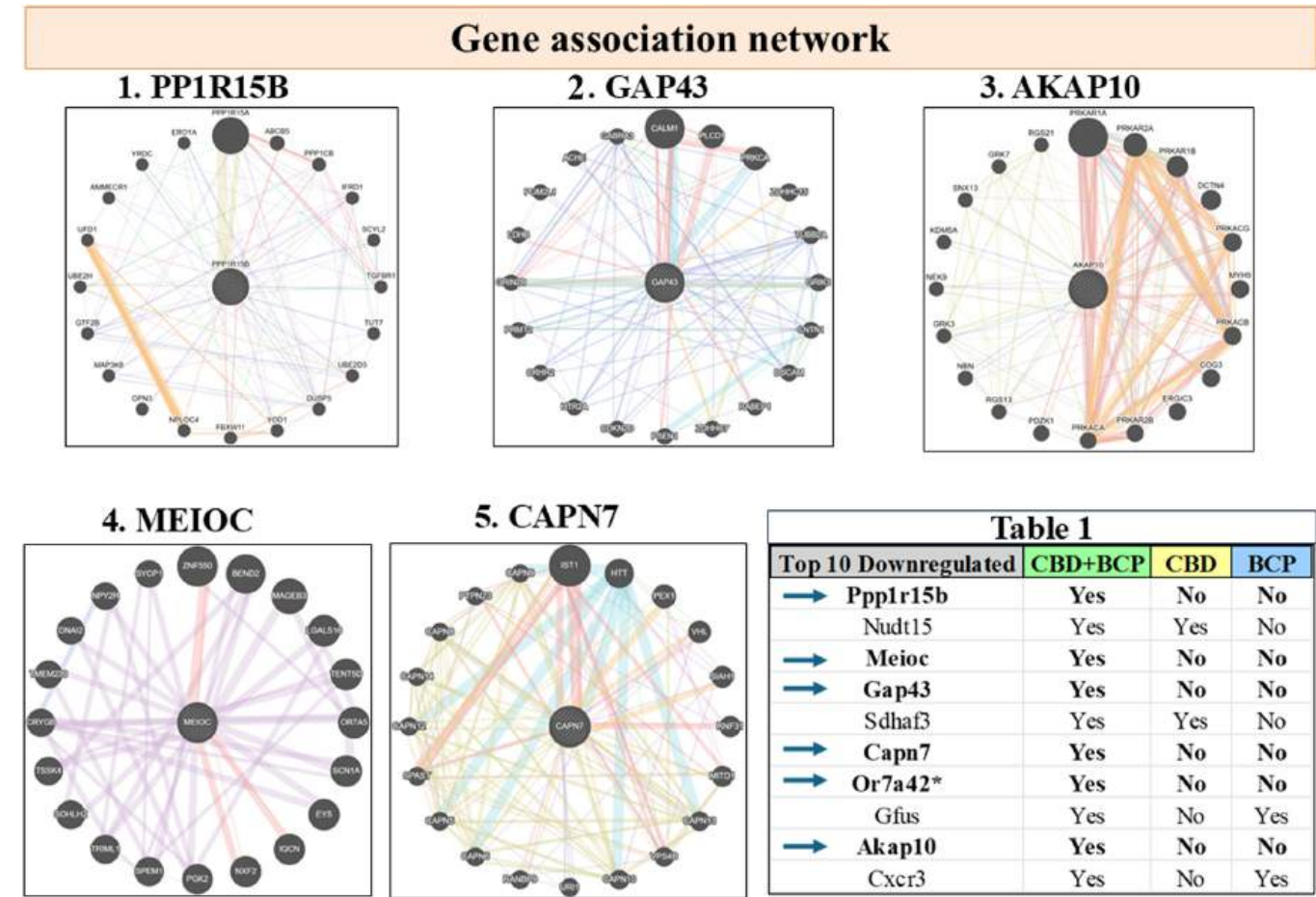


Fig. 7. Network analysis of genes uniquely downregulated by combination treatment. The figure shows a network analysis (using the GeneMANIA database) of five of these genes: PP1R15B, MEIOC, GAP43, CAPN7, and AKAP10. (Note: OR7A42, encoding olfactory receptor 8, was not included in the analysis due to its absence from the database). The top ten downregulated genes in the combination group were verified in the CBP and CBD groups to identify genes that are uniquely downregulated in the combination group only. Arrows indicate those uniquely downregulated by the combination group.

confirmed that the mechanical antiallodynic effect of this combination is indeed synergistic using Vonfrey test.

Similarly, using behavioral and pharmacological approaches, along with isobolographic analysis, we demonstrated that the combination of CBD and BCP also produced a synergistic cold antiallodynic effect in the acetone test.

Additionally, in both tests, the ED50 of this combination was lower than the ED50 of either CBD or BCP alone. These data indicate that this combination has greater potency than the individual effects of its components.

The findings support the idea that CBD and BCP work in synergy to alleviate inflammatory pain and underscore the efficacy of combining different constituents of the cannabis plant for effective pain management. This strongly supports the concept of the *entourage effect* [26] and validates the potential of cannabis-based therapies in addressing pain.

Next, we examined the effects of combining CBD and BCP on depression related to CFA chronic pain. CFA-induced inflammatory pain is associated with depression-like behavior (Fig. 3F). Our data indicate that the treatment with CBD and BCP combination produced a significant antidepressant effect, as seen in improved performance in the tail suspension test, a standard measure for depressive behaviors in animal studies.

Taken together, these results highlight the potential of this combination as an effective treatment option for chronic inflammatory pain and related depression.

Finally, we examined whether the beneficial effect of this combination in pain and depression involved anti-inflammatory pain. The reason for focusing on inflammation is that it plays a key role in the induction and maintenance of chronic pain but is also implicated in depression [45–48]. Inflammation not only serves as a driving force for chronic pain but is also implicated in depression [47,49,50]. Since depression and chronic pain mechanistically share neuroinflammation, a potential effect of the combination of CBD and BCP in inflammation may account for the mechanism by which this combination simultaneously attenuates pain and the related depression.

The activation of glial cells accompanies the development of chronic inflammatory pain [51,52]. In our current study, we found that CFA induced strong microglial activation in the brain as indicated by increased expression of Iba1. Furthermore, we tested the effect of the CBD and BCP in combination on the Iba1 expression induced by CFA-treated mice. We found that the combination treatment inhibits the expression of Iba1, and this effect was greater than the individual effect of CBD and BCP. These results are of particular interest because they could explain the synergic analgesic effect of the combination.

We also studied how the combination treatment affects astrocytes. Our results showed that GFAP expression was significantly higher in the brains of mice treated with CFA. The combination treatment reduced GFAP levels, indicating it also impacts astrocytes.

We also used a proteomics approach to gain insight into the mechanism by which this combination produces its beneficial effect in pain and depression. The proteomic analysis offers valuable insights, showing that while the combination shares a target with CBD and BCP, it

specifically affects certain unique proteins. Notably, this combination causes the downregulation of GAP-43, Akap10, Capn7, PP1R15B, and MEIOC, which are distinctly influenced by this treatment. These proteins play important roles in pain and inflammation, indicating they may play a key role in the beneficial effects of the CBD and BCP combination in pain and depression. Further investigation of these findings could help improve understanding of pain management strategies.

In conclusion, our current findings show that, in the CFA pain model, the combination of CBD and BCP produces a synergistic analgesic effect while also having antidepressant properties. Additionally, our data show that the anti-inflammatory action of this combination may explain its beneficial effects on pain and depression. Therefore, our data suggest this combination as a potentially effective treatment for the co-occurrence of chronic pain and depression.

Authors' contributions

Khalid Benamar wrote and designed the research. Jose Rios, Emmanuel Franco, Justin Mills, Maria Sophia Malbas, Joshua Nwose, Hiram Garcia conducted behavioral tests. Mohammed alnoud performed IHC, and Manish Tripathi and Mohammad Hussein performed proteomics studies.

CRediT authorship contribution statement

Mohammed Alnoud: Methodology, Investigation. **Mohammed Hussain:** Methodology, Investigation. **Manish Tripathi:** Methodology. **Khalid Benamar:** Writing – original draft, Funding acquisition, Conceptualization. **Justin Mills:** Investigation. **Jose Rios:** Methodology, Investigation. **Emmanuel Franco:** Investigation. **Hiram Garcia:** Investigation. **Sophia Leslie:** Investigation. **Joshua Nwose:** Investigation. **Maria Sophia Malbas:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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