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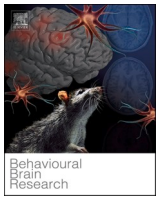

Research report

Medicinal cannabis oil improves anxiety-like and depressive-like behaviors in CCS mice via the BDNF/TRPC6 signaling pathway

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ABSTRACT

Background: Post-traumatic stress disorder (PTSD) refers to a chronic impairing psychiatric disorder occurring after exposure to the severe traumatic event. Studies have demonstrated that medicinal cannabis oil plays an important role in neuroprotection, but the mechanism by which it exerts anti-PTSD effects remains unclear.
Methods: The chronic complex stress (CCS) simulating the conditions of long voyage stress for 4 weeks was used to establish the PTSD mice model. After that, behavioral tests were used to evaluate PTSD-like behaviors in mice. Mouse brain tissue index was detected and hematoxylin-eosin staining was used to assess pathological changes in the hippocampus. The indicators of cell apoptosis and the BDNF/TRPC6 signaling activation in the mice hippocampus were detected by western blotting or real-time quantitative reverse transcription PCR experiments.
Results: We established the PTSD mice model induced by CCS, which exhibited significant PTSD-like phenotypes, including increased anxiety-like and depression-like behaviors. Medicinal cannabis oil treatment significantly ameliorated PTSD-like behaviors and improved brain histomorphological abnormalities in CCS mice. Mechanistically, medicinal cannabis oil reduced CCS-induced cell apoptosis and enhanced the activation of BDNF/TRPC6 signaling pathway.
Conclusions: We constructed a PTSD model with CCS and medicinal cannabis oil that significantly improved anxiety-like and depressive-like behaviors in CCS mice, which may play an anti-PTSD role by stimulating the BDNF/TRPC6 signaling pathway.

1. Introduction

In 1980, the American Psychiatric Association (APA) developed the Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (DSM-3), which established the concept and diagnostic criteria for PTSD. PTSD refers to a psychiatric disorder that follows a major traumatic event, such as a sudden, threatening, or catastrophic event, that results in the delayed onset (typically within 3–6 months of the event) and persistence of the individual. Typical symptoms of PTSD include repeated recollections and reenactments of traumatic events, avoidance of trauma-related situations and events, and increased alertness [1]. The prevalence of PTSD is around 6 % in the general population, but can be as high as 25–35 % in individuals who have experienced severe trauma, such as veterans, refugees, and victims of assault, and women have more risk factors for PTSD and a higher likelihood of developing PTSD compared to men [2,3]. Stress and mental disorders, especially PTSD is higher in military personnel. Research has shown that submarine personnel who are unable to perceive circadian shifts of sunlight due to living in enclosed environments are more likely to suffer from anxiety and depression [4]. Long voyages cause sailors to suffer from undue fatigue, low working capacity, disturbed sleep, abnormal irritability, and depressed mood [5]. However, there are no effective medicines for

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PTSD caused by special circumstances. Therefore, it is urgent to clarify the pathogenesis of PTSD and find the most direct and effective therapeutic drugs.

Currently, the main therapeutic drugs for PTSD are sertraline and paroxetine, selective 5-hydroxytryptamine reuptake inhibitors approved by the American Food and Drug Administration (FDA), which have significant efficacy but suffer from inefficiency (about 40 %-60 %), high price, slow onset of action, and serious adverse effects [6]. With the wide application of Chinese medicine, its advantages of multi-components, multi-targets, multi-pathways, and few side effects will be the best choice for the treatment of PTSD with complex pathogenesis [7]. There have been more studies related to the treatment of PTSD with Chinese medicine: (1) Chemical composition of Chinese medicine: Salidroside could attenuate PTSD-like behavior in rats by inhibiting hippocampal neuronal apoptosis, enhancing hippocampal synaptic plasticity, and reducing neuroinflammatory responses [8]. Studies have shown that tanshinone IIA improves PTSD-like symptoms through multi-targeted modulation [9]. Crocin ameliorated depressive-like behavior in CUMS mice through the Wnt/β-catenin signaling pathway without affecting sexual function. (2) Chinese Medicine Extracts: The ethanolic extract of Aralia continentalis significantly improved the abnormal expression of BDNF and pro-inflammatory factors TNF-α and IL-6, which attenuated cognitive deficits and behavioral abnormalities in PTSD rats [10]. Study showed that *Radix Polygalae* Extract increased the expression of BAG1 in the hippocampus of mice, thereby improving PTSD-like behavior in a mouse model of single prolonged stress and conditioned fear [11]. (3) Compound prescription of Chinese medicine: A randomized, double-blind controlled trial comparing the Chinese herbal formula Xiao-Tan-Jie-Yu-Fang (XTJYF) with placebo for the treatment of PTSD in survivors of the 2008 Sichuan earthquake showed that XTJYF significantly improved obsessive-compulsive behaviors, depression, anxiety, hostility, and sleep disturbances in patients with PTSD without serious adverse events [12]. Anshen Dingzhi's prescription could improve PTSD-like behavior in mice by improving hippocampal synaptic function [13]. Due to the late recognition of PTSD and the relative lack of research on its pathogenesis and therapeutic drugs, there is still a lot of space for future research on Chinese medicine for the treatment of PTSD.

Hemp (*Cannabis sativa L.*), commonly called hanja, or fire hemp, is an annual herbaceous flowering plant belonging to the genus Cannabis in the cannabis family (Cannabaceae). Cannabis contains more than 100 active compounds known as cannabinoids [14]. Cannabis is classified according to its cannabinoid content, with species containing less than 0.3 % tetrahydrocannabinol (THC) and higher levels of cannabidiol (CBD) classified as non-psychoactive medicinal cannabis, and cannabis varieties with a THC content of less than 0.3 % known as industrial hemp. Research has shown that THC is the main psychoactive ingredient in cannabis, with appetite-stimulating, anti-inflammatory, analgesic, and antiemetic properties, while CBD is a non-psychocannabinoid with neuroprotective, analgesic, sedative-antiemetic, antispasmodic, anti-inflammatory and anxiolytic properties [15–17]. There has been a gradual increase in research on cannabinoids (including THC and CBD) for the treatment of disorders related to mental disorders, and clinical data have confirmed that cannabinoids have particular medicinal value. However, there are still relatively few studies on medicinal cannabis oil for the treatment of PTSD.

Brain-derived neurotrophic factor (BDNF), is an important regulator for many different physiological and pathological functions in the nervous system, and plays a crucial role in the development and correct maintenance of brain circuits and synaptic plasticity as well as in neurodegenerative diseases [18]. It was found that veterans with PTSD had significantly decreased plasma BDNF concentration and cognitive decline [19]. In addition, several studies have shown that PTSD can lead to altered calcium homeostasis [20,21]. Typical transient receptor potential channel 6 (TRPC6) is a non-selective Ca^{2+} permeable cation channel in the central nervous system (CNS) that plays a key role in growth cone guidance, neuronal survival, and synaptic plasticity. It was shown that TRPC6 channels have a protective role in a rat retinal ischemia/reperfusion-induced cell death model, while demonstrating that the protection of TRPC6 takes place through BDNF-dependent pathways [22]. Yan Li [23] reports that in cultured cerebellar granule cells, TRPC channels contribute to the BDNF-induced elevation of Ca^{2+} at the growth cone and are required for BDNF-induced chemo-attractive turning. Meanwhile, it was indicated that the BDNF/TRPC6 signaling pathway plays a neuroprotective role in perimenopausal depression (PD) [24]. However, the role of the BDNF/TRPC6 signaling pathway in the development of PTSD remains unclear.

In this study, a mouse PTSD model was constructed using chronic restraint stress and shaking, accompanied by sound and light stimulation to simulate long voyage stress, and then medicinal cannabis oil was administered for treatment. By exploring the efficacy and mechanism of action of medicinal cannabis oil in exerting anti-PTSD, to provide a certain experimental basis for the anti-PTSD of traditional Chinese medicine.

2. Materials and methods

2.1. Animals

A total of 48 SPF-grade C57BL/6 J male mice with a body mass of 18 g to 20 g. They were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., Company Licence No.: SCXK (Beijing) 2021–0006, and Animal Experimentation Ethics Licence No.: IACUC-DWZX-2022–688. Mice were housed in standard environments with 12 h/12 h alternating light and dark illumination, temperature controlled at 22 ± 2℃, relative humidity 50 ± 10 %, and given a free diet.

48 mice were adapted to a standard environment for 7 days, and then randomly divided into Control group (Control), Model group (CCS), Sertraline group (Ser), medicinal cannabis oil low dose group (MC-L), medicinal cannabis oil medium dose group (MC-M), and medicinal cannabis oil high dose group (MC-H), with 8 mice in each group.

2.2. CCS model preparation and drug delivery

In this study, the chronic complex stress method was used to construct a mouse PTSD model (Fig. 1A). CCS modeling method is optimized based on previous modeling [25]: The mice were individually placed into a well-ventilated 50 mL centrifuge tube without food and water and then placed in an oscillator with low-speed vibration at a speed of 40 R/min for 6 h a day lasting 4 weeks. Meanwhile, the bound mice were exposed to noise and unnatural light during this process. After a series of stimuli, the animals were returned to their cages and were accessible to food and water free.

Mice in each group were treated with drug administration starting on day 15 of modeling. Mice in the Control and CCS groups were given corn oil (Changshouhua Foods Co., Shandong, China), and mice in the Ser group were given 1.5 mg/mL of sertraline hydrochloride solution (Macklin, Shanghai, China); MC groups were given 0.5 mg/mL (low dose), 1 mg/mL (medium dose), and 2 mg/mL (high dose) medicinal cannabis oil solutions (presented by Jilin Sihuan Aokang Pharmaceutical Co., Ltd., Jilin, China), and the above solutions were given with corn oil as solvent. Mice were administered intragastric once daily in a volume of 10 mL/kg for 14 days.

2.3. Behavior test

2.3.1. Open field test (OFT)

On day 29, the anxiety-like behavior and spatial exploration ability of mice were measured using an open field test box (50 cm × 50 cm × 35 cm, Shanghai Xinruan Information Technology CO., Ltd, Shanghai, China). The bottom of the box is equally divided into 25 squares, and the

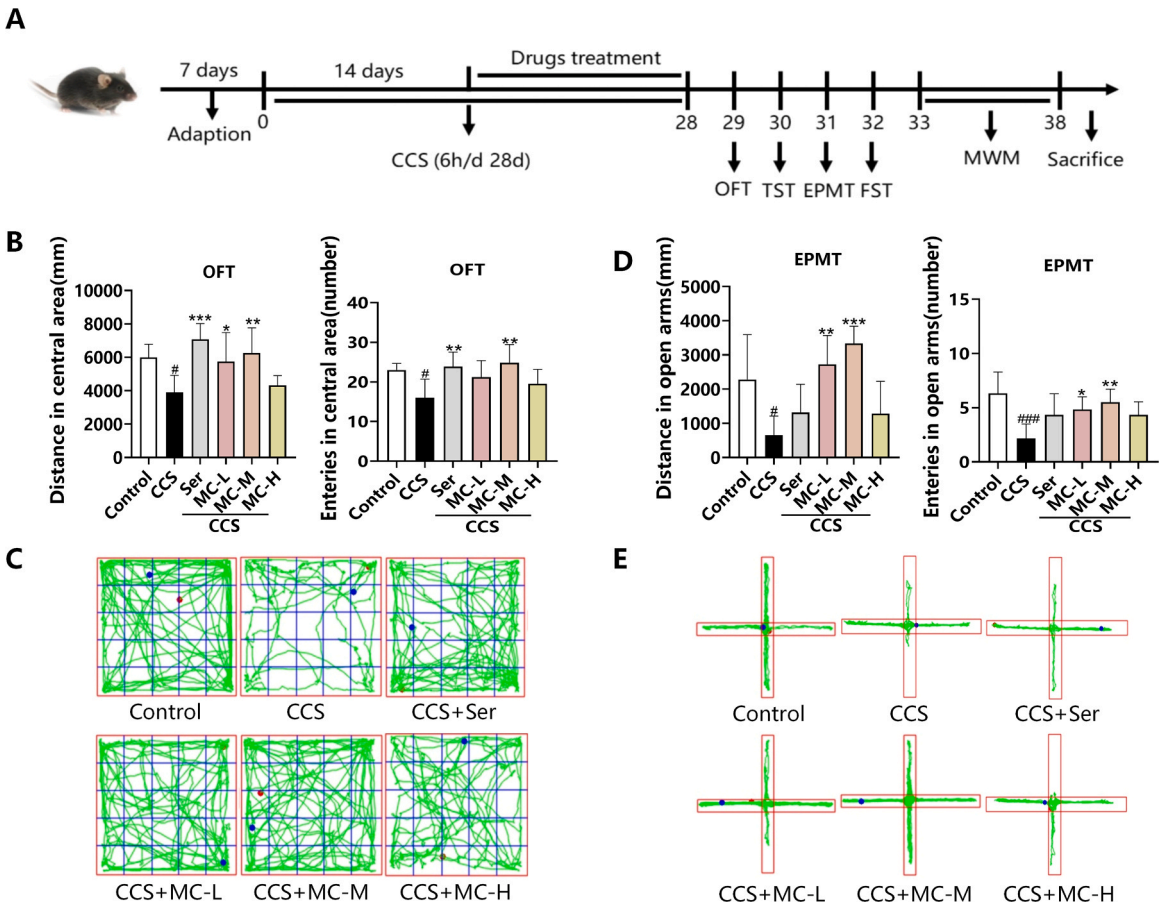

Fig. 1. Medicinal cannabis oil treatment significantly ameliorated anxiety-like behaviors in CCS mice. (A) The timeline of the experimental process and the events that occur at important points in time. (B) Medicinal cannabis oil significantly increases the distance and number of entries into the central region in CCS mice. (C) Trajectory map of mice in OFT. (D) Medicinal cannabis oil significantly increased the distance and number of entries into the open arm in CCS mice. (E) Trajectory map of mice in EPMT. n=8, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared to Control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared to CCS group.

square consisting of 9 small compartments right in the middle is defined as the central area. Each mouse was placed in the center of the box, and a video imaging system was used to record the activity trajectories of the mice over 5 min, and statistical analyses were performed on the duration and number of stays of the mice into the central area.

2.3.2. Tail suspension test (TST)

On day 30, in a quiet environment, the mice were fixed at the top of the test box (Shanghai Xinruan Information Technology CO., Ltd, Shanghai, China) about 1 cm above their tails with their heads naturally downward. Mouse behavior was recorded using a video imaging system, observing the activity of the mice over a 6 min period and recording the cumulative immobility time of the mice over the latter 4 min [26].

2.3.3. Elevated plus maze test (EPMT)

On day 31, the mice's anxiety-like behavior was examined using EPMT. The maze (35 cm × 5 cm × 10 cm, Shanghai Xinruan Information Technology CO., Ltd, Shanghai, China) consists of two open arms, two closed arms, and a central area. Mice were placed in the central area with their heads facing the open arm and allowed to explore for 5 min, analyzing the distance and number of times that mice traveled into the open arm area.

2.3.4. Forced swimming test (FST)

On day 32, in a quiet environment, mice were placed in a glass cylinder (Shanghai Xinruan Information Technology CO., Ltd, Shanghai, China) for a forced swimming test, which was filled with 2/3 of water, and the water temperature was about 25±1℃. The activity of the mice was observed for 6 min by the Behavioural Video Acquisition and Analysis System, and the cumulative immobility time of the mice was recorded for the latter 4 min to assess the depressive-like behavior of the mice [27,28].

2.3.5. Morris water maze (MWM)

Before the start of the experiment, the black water maze (Shanghai Xinruan Information Technology CO., Ltd, Shanghai, China) was filled with water, according to the experimental needs, the height of the water surface was 2 cm below the station/ 2 cm above the station, and the water temperature was maintained at 25℃ ± 1℃. The skimmed milk powder was sufficiently dissolved in the water so that the mice could be positioned. The water maze was divided equally into 4 quadrants, and a white transparent circle-shaped station with a diameter of 9 cm was placed in the target quadrant (set as Area 5). The specific experimental steps are as follows: (1) Platform visualization period: the station was exposed to the water surface for 2 cm and placed in Area 1. Mice were randomly placed into the pool with their heads towards the pool wall from three quadrants, Area 2, Area 3, and Area 4, and were free to swim for 60 seconds. Mice find the station within 60 seconds, make them stay on the station for 10 seconds, and remove them. If the station cannot be found within 60 seconds, lead the mouse to the station to stay for 10 seconds. (2) Localisation navigation period: On the 2nd day of the end of the platform visualization period, the platform was hidden 2 cm underwater. Mice were placed into the pool for 60 seconds of free swimming with their heads facing the wall of the pool randomly from

three directions: Area 2, Area 3, and Area 4. If the mice found the station within 60 seconds, the time used was recorded as the escape latency, and they were removed after remaining on the station for 10 seconds. If the mice could not find the station within 60 seconds, they would be led to the station for 10 seconds and the escape latency was recorded as 60 seconds. The experiment lasted for 4 days. (3) Space exploration period: After the localization navigation experiment, the underwater station was removed. Mice were placed into the pool with their heads facing the wall from Area 2, Area 3, and Area 4. The movement trajectory and target quadrant residence time of the mice in 60 seconds were recorded, Counted the number of times that the mice entered and visited the target quadrant area in 60 seconds.

2.4. Brain tissue factor

At the end of the behavior test, the body mass of the mice was recorded and the mice were sampled to calculate the brain tissue factor of each group of mice.

2.5. Hematoxylin-eosin staining (HE)

Mouse brain tissues were fixed in 4 % paraformaldehyde for 24 h before HE staining. Afterward, the tissues were dehydrated in different concentrations of alcohol, embedded, and sectioned. Next, paraffin sections were dewaxed to water and stained with hematoxylin for nuclei and eosin for cytoplasm, and finally dehydrated and sealed. These sections were observed using Cytation5 (BioTek, USA) to assess morphological changes of neuronal cells in the hippocampus of mouse brain tissue.

2.6. Western blot (WB)

Mouse hippocampal tissues from each group were homogenized by adding 200 μL of lysate (RIPA lysate buffer: phosphatase (PPLYGEN, Beijing, China) and protein inhibitor mixture (NCM, Suzhou, China) = 1:100). The supernatant was subsequently centrifuged at 12,000 rpm, 4℃, 15 min and quantified according to the Protein Quantification Kit instructions, with the addition of 1/4 of the protein volume of the loading buffer and at 100℃ for 10 min. 30 μg of protein was separated by electrophoresis using a 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then blotted onto polyvinylidene fluoride (PVDF) membranes. Membranes were closed for 1 h at room temperature by applying 5 % skimmed milk powder (dissolved in TBST), followed by overnight incubation with primary antibodies at 4℃. The next day, membranes were then rinsed with TBST and incubated with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature.β-actin was used as an internal reference protein and chemiluminescent (ECL, NCM, Suzhou, China) as a luminescent reagent, the bands were visualized using a 5200Multi (Tanon, Shanghai, China) to detect the effect of medicinal cannabis on the expression levels of TRPC6, BDNF, Bax and Bcl2 proteins in the hippocampal tissues of CCS mice. The flowing antibodies: BDNF (ab108319, 1:1000, 15 kDa, abcam, USA); TRPC6 (ab228771, 1:1000, 92 kDa, abcam, USA); Bax (50599-2-Ig, 1:2000, 21 kDa, Proteintech, China); Bcl2 (ab196495, 1:1000, 21 kDa, abcam, USA); horseradish peroxidase (HRP)-conjugated secondary antibodie (1:5000, Proteintech).

2.7. Real-time quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from the hippocampal tissues of mice in each group according to the instructions (Transfection Reagent, Polyplus, France) and cDNA (cDNA synthesis kit, TransGen Biotech, China) was synthesized by reverse transcription. A real-time quantitative reverse transcription PCR instrument was used to detect the mRNA expression levels of TRPC6 and BDNF in the hippocampal tissues of mice in each group, and β-actin was used as the endogenous control. The primer sequences used are as follows: TRPC6 primers: forward: 5'-GATATCTTCAA ATTCATGGTC-3' and reverse: 5'-CTCAATTTCCTG-GAATGAA-3' ; BDNF primers: forward: 5'-AACCA-TAAGGACGCGGACTT-3' and reverse: 5'-TGCAGTCTTTTTATCTGCCG-3' ; β-actin primers: forward: 5'-AGGCCAACCGTGAAAAGATG-3' and reverse: 5'-TGGCGTGAGG GAGAGCATAG-3'. The reaction thermal profile consisted of polymerase activation (94℃, 30 s), and 40 cycles including denaturation (94℃, 5 s), annealing (55℃, 15 s), and elongation (72℃, 10 s).

2.8. Statistical analysis

All data in this study were presented as mean ± standard deviation. The data were estimated by one-way analysis of variance (ANOVA) followed by a post hoc Tukey test to compare the various groups. GraphPad Prism 8.0.2 Software was used for graphing. $p < 0.05$ indicated that the differences were statistically significant.

3. Results

3.1. Medicinal cannabis oil treatment significantly ameliorated anxiety-like behaviors in CCS mice

To investigate the PTSD-like symptoms in the CCS model, behavioral responses were measured (Fig. 1A). The anxiety-like behaviors were evaluated using the OFT and EPMT. For the OFT, the CCS mice exhibited shorter distance ($p < 0.05$) and decreased entry times ($p < 0.05$) in the central area compared to the Control group (Fig. 1B, C). The distance ($p < 0.05$) and the entries ($p < 0.001$) in the open arms were significantly decreased in CCS mice compared to the Control group on the EMPT (Fig. 1D, E). These indicated that CCS mice show notable anxiety-like behaviors. To investigate the therapeutic potential of medicinal cannabis oil on PTSD induced by CCS, the mice were intragastric administered with medicinal cannabis oil. In the OFT, the traveled distance ($p < 0.05$) and the number of entries ($p < 0.01$) in the central area were significantly increased in the medicinal cannabis oil groups compared to the CCS group, with an effect comparable to that of sertraline ($p < 0.01$; Fig. 1B, C). Meanwhile, medicinal cannabis oil administration intervention significantly increased the distance ($p < 0.01$) and number of traveled ($p < 0.05$) to the open arm area compared to the CCS group (Fig. 1D, E). Taken together, these results suggested that CCS could induce anxiety-like behaviors in mice while medicinal cannabis oil administration improved the abnormal behaviors induced by CCS.

3.2. Medicinal cannabis oil treatment significantly improved depression-like behaviors in CCS mice

TST and FST are commonly used to examine the therapeutic effects of drugs on depression-like behavior in mice. As shown in Fig. 2, the immobility time was significantly increased in the CCS group compared to the Control group in the TST ($p < 0.05$; Fig. 2A) and FST ($p < 0.05$; Fig. 2B), indicating that the CCS mice showed depression-like behaviors. On the contrary, medicinal cannabis oil treatment significantly reverse these behavioral changes, including significantly decreased the immobility time in the TST ($p < 0.01$; Fig. 2A) and FST ($p < 0.05$; Fig. 2B). Collectively, these data implicated that medicinal cannabis oil treatment ameliorated PTSD-like symptoms in CCS mice.

3.3. Effects of medicinal cannabis oil on learning and cognitive performance in CCS mice

In this experiment, we found that CCS did not significantly reduce the number of target quadrant crossing ($p > 0.05$) and the time spent in the target quadrant ($p > 0.05$) in mice, indicating that CCS may not

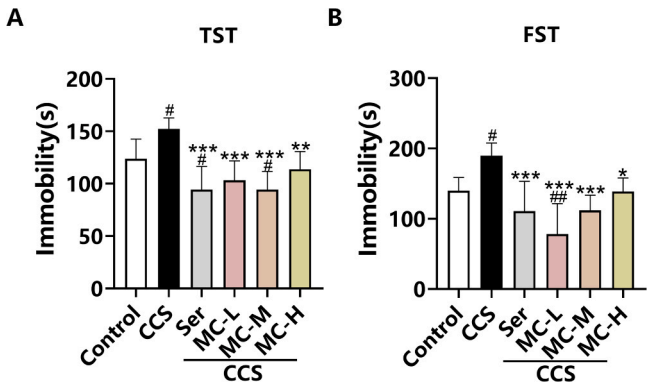

Fig. 2. Medicinal cannabis oil treatment significantly improved depression-like behaviors in CCS mice. (A) Medicinal cannabis oil treatment significantly decreased the freezing time in the TST. (B) Medicinal cannabis oil treatment significantly decreased the immobility time in the FST. n=8, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared to Control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared to CCS group.

affect mouse cognition (Figure S1A-C). The number of target quadrant crossing ($p > 0.05$) and the time spent in the target quadrant ($p > 0.05$) in mice showed an upward trend, but there was no statistical significance after the medicinal cannabis oil administration intervention (Figure S1A-C).

3.4. Medicinal cannabis oil prevented CCS-induced brain histomorphological abnormalities

After behavioral testing, the brain tissues of mice were isolated and the organ index was calculated, but there was no significant difference in brain tissue indices between the groups (Figure S2, $p > 0.05$). Subsequently, the pathological changes in the hippocampus were detected using HE staining. We found loosely and disordered arrangement neurons in the hippocampal CA2 region, and markedly increased nuclear consolidation in the CA3 region, and DG region of the mouse hippocampus in the CCS group (Fig. 3A). In the brain tissue of mice in the CCS group, a large amount of necrosis in nerve cells and deep staining of nuclear consolidation were observed in the hippocampal CA3 region ($p < 0.001$). Compared with the CCS group, hippocampal tissue in medicinal cannabis oil treated mice tended to be normal, including having increased neurons numbers and a normal structure arranged more neatly, with regular cell morphology and clear nucleus in hippocampal CA2 area and DG (Fig. 3A); and necrosis of nerve cells in the hippocampal CA3 area was significantly reduced ($p < 0.01$; Fig. 3B). Thus, the data indicated a key neuroprotective role of medicinal cannabis oil in CCS mice.

3.5. Medicinal cannabis oil reduced CCS-induced apoptosis through BDNF/TRPC6 signaling pathway

Previous studies have shown that the developmental process of PTSD pathophysiology was related to apoptosis [29,30]. We examined indicators of cell apoptosis in the mice hippocampus, including the expression levels of Bax and Bcl2. The results showed that, after CCS modeling, the protein levels of Bax were significantly increased ($p < 0.05$), accompanied by a significantly reduced protein level of Bcl2 ($p < 0.01$; Fig. 4A-C), indicated that CCS induced cell apoptosis in the mice hippocampus. Then, we assessed the effects of medicinal cannabis oil on hippocampus apoptosis indicators and found that the expression levels of Bax in the hippocampus of the medicinal cannabis oil group were significantly lower than those of the CCS mice ($p < 0.05$), and the expression level of Bcl2 were significantly increased compared with CCS group ($p < 0.01$; Fig. 4A-C), suggesting that medicinal cannabis oil reduced CCS-induced apoptosis.

It is reported that the BDNF/TRPC6 signaling pathway plays a key role in neuroprotective [22]. As shown in Fig. 4, the protein levels of TRPC6 ($p < 0.05$) and BDNF ($p < 0.05$) were significantly reduced in hippocampal tissues in CCS group compared with the control group (Fig. 4D-F). The qRT-PCR results showed that CCS resulted in a significant reduction in the mRNA expression level of TRPC6 and BDNF in mouse hippocampal tissues (Fig. 5A, B), which were consistent with the WB results. Similarly, we assessed the effect of medicinal cannabis oil on the BDNF/TRPC6 signaling activation after CCS. The results showed that medicinal cannabis oil significantly increased the expression levels of hippocampal TRPC6 ($p < 0.05$) and BDNF ($p < 0.05$) in CCS mice (Fig. 4D-F). These results suggest that medicinal cannabis oil may ameliorate the CCS-induced cell apoptosis via BDNF/TRPC6 signaling.

4. Discussion

In this study, we established the PTSD mice model induced by CCS simulating long voyage stress and found that medicinal cannabis oil alleviates CCS-induced PTSD-like behaviors. Meanwhile, medicinal cannabis oil may improve hippocampal damage in CCS mice and reduce

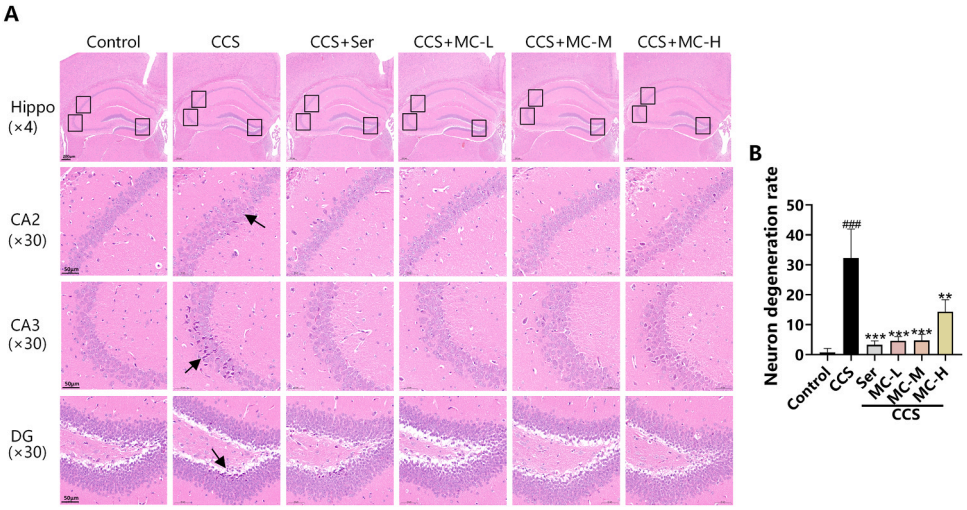

Fig. 3. Medicinal cannabis oil prevented CCS-induced brain histomorphological abnormalities. (A) Histological analysis of Hippocampus tissue via HE staining. (B) Medicinal cannabis oil treatment significantly reduced the rate of neuronal degeneration in the hippocampal CA3 region of CCS mice. n=3, $^{\#\#\#}p < 0.001$, compared to Control group; $^{**}p < 0.01$, $^{***}p < 0.001$, compared to CCS group.

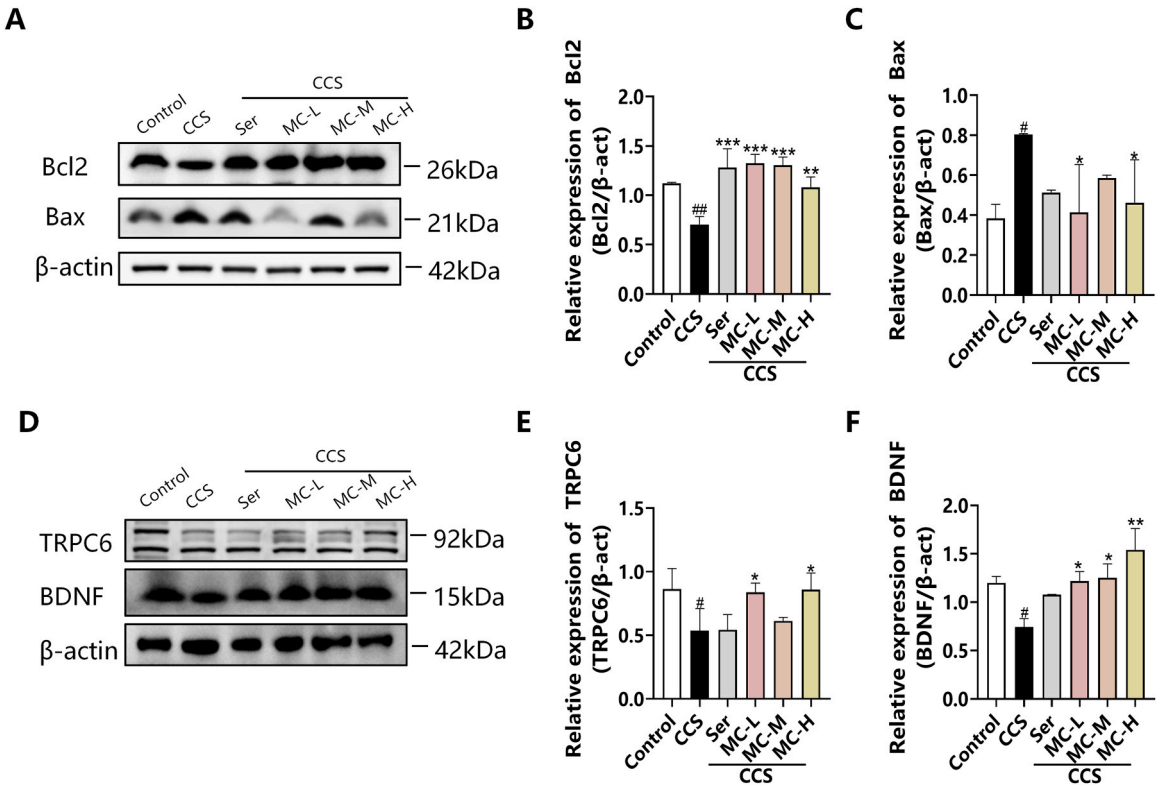

Fig. 4. Medicinal cannabis oil ameliorated CCS-induced cell apoptosis through BDNF/TRPC6 signaling pathway. (A, D) Western blot analysis for Bcl2, Bax, TRPC6, and BDNF expression in the Control, CCS, Ser, MC-L, MC-M, and MC-H. β-action was used as an internal control to measure the quality of protein. (B) Relative intensity of Bcl2 was assessed by densitometry. (C) Relative intensity of Bax was assessed by densitometry. (E) Relative intensity of TRPC6 was assessed by densitometry. (F) Relative intensity of BDNF was assessed by densitometry. n=3, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared to Control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared to CCS group.

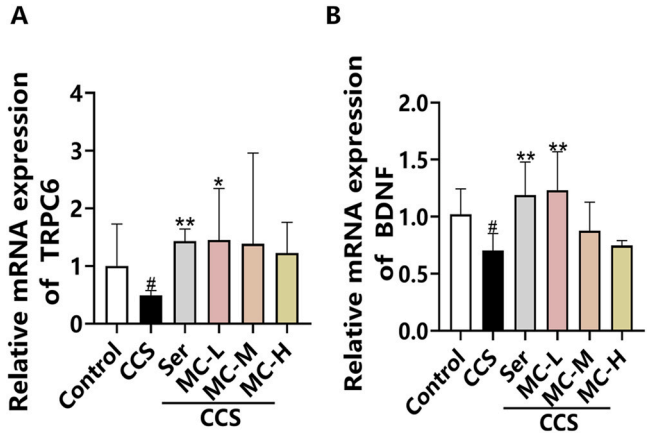

Fig. 5. Medicinal cannabis oil expressed CCS-induced BDNF/TRPC6 signaling pathway. (A) Relative mRNA expression of TRPC6 was assessed. (B) Relative mRNA expression of BDNF was assessed. n=3, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared to Control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared to CCS group.

CCS-induced cell apoptosis through BDNF/TRPC6 signaling pathway.

PTSD is not a well-defined disorder, without any objective parameters, and there is a large overlap with other disorders, including mood disorders [31]. W Leksowski [32] published research showing that people on long voyages are vulnerable to mood disorders such as depressive syndromes, neurosis syndromes, and perceptual disorders. Ship noise will affect seafarers during long voyages, and the shipboard environment correlates with neurotic symptoms in seafarers [33,34]. However, the lack of available animal models has limited research related to PTSD due to long voyages. We used multiple stressors such as restraint, shaking, noise, and unnatural light to establish a CCS mice model to simulate long voyage stress. Behavioral tests were then used to evaluate the mood disorders of the mice.

Medical cannabis has been reported to have potential efficacy in reducing pain, muscle spasms, chemotherapy-induced nausea and vomiting, and refractory childhood epilepsy [35]. Usage analysis found that cannabis or derivative products are available to control PTSD symptoms, especially among veterans [36]. It has also been clinically shown that oral CBD significantly improves patients' PTSD symptoms [37]. We constructed a long voyages model through CCS and behaviorally clarified that medical cannabis oil was effective in ameliorating PTSD symptoms due to CCS.

Most studies have shown that PTSD leads to changes in the function and structure of the patient's brain [38]. In addition, it has been shown that patients with PTSD have reduced hippocampal volume or reduced hippocampal NAA levels, and that the higher severity of PTSD symptoms is associated with lower hippocampal volume and higher hippocampal/parahippocampal blood flow, but the correlation between hippocampal volume and hippocampal blood flow was not found to be significant [39,40]. We found that CCS did not cause changes in the volume of mouse brain tissue, but the HE staining results showed that CCS caused abnormalities in mouse hippocampal tissue, which showed increased nuclear consolidation, decreased cell number, and disordered arrangement. In contrast, medicinal cannabis oil administration intervention significantly ameliorated hippocampal tissue damage in mice caused by CCS.

Apoptosis plays an important role in PTSD. PTSD can cause hippocampal neuronal apoptosis, and apoptosis may be one of the causes of hippocampal atrophy. Studies have shown that abnormalities in Bcl2

and Bax apoptosis-related factors may be related to the pathogenesis of apoptosis in PTSD [41,42]. In this study, we found that medicinal cannabis oil significantly reversed CCS-induced abnormalities in Bcl2 and Bax protein expression. Studies have shown that the binding of BDNF to TrkB results in the PLC-dependent activation of TRPC6, and TRPC6 plays a very important role in BDNF-mediated neuronal protection, BDNF-triggered intracellular Ca^{2+} elevation, and BDNF-induced CREB activation [43,44]. It was shown that BDNF/TrkB inhibits apoptosis by regulating TRPC3/6 channels [45]. We found experimentally that CCS led to a significant reduction in TRPC6 and BDNF levels in mouse hippocampal tissue, which was reversed by medicinal cannabis oil treatment. However how medicinal cannabis oil affects apoptosis in the hippocampal of CCS mice through the BDNF/TRPC6 signaling pathway, and its specific mechanism needs further study.

5. Conclusions

We constructed the CCS model and clarified that medicinal cannabis oil has a significant ameliorating effect on PTSD-like behavior in CCS. Meanwhile, we found that medicinal cannabis oil may improved hippocampal apoptosis in CCS mice through BDNF/TRPC6, which provided an experimental basis for understanding the treatment of PTSD with medicinal cannabis oil.

CRediT authorship contribution statement

Xin Shen: Validation. **Lin Li:** Validation. **Yi Ru:** Formal analysis. **Chunqi Yang:** Formal analysis. **Huijing Yu:** Validation. **Baoying Shen:** Writing – original draft, Validation, Methodology, Formal analysis. **Zhixing Wang:** Methodology, Formal analysis. **Guangxu Du:** Validation. **Chengcai Lai:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Yue Gao:** Supervision, Project administration, Methodology.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2024.115005.

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