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Original Article

Therapeutic mechanism of steaming umbilical cord therapy with Chinese herbal medicine on a rat model of IBS-D via the PAR-2/TRVP1 pathway

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Abstract: Objective: This study aimed to investigate the PAR-2/TRVP1-based mechanism of steaming umbilical cord therapy with Chinese Herbal Medicine (SUCT-CHM) in IBS-D rat models. Methods: Sixty-two IBS-D modeled rats were established, and were randomly assigned to the control group (n = 31) and the experimental group (n = 31). The model group did not receive intervention measures, and the experimental group was treated with SUCT-CHM. After 14 days of intervention, the two groups of rats were compared in terms of body weight, gastrointestinal function, Bristol stool score, wet/dry weight ratio of rat stool, and abdominal withdrawal reflex scores. The transient receptor potential vanilloid receptor 1 (TRPV1), protease-activated receptors-2 (PAR-2), calcitonin gene related peptide (CGRP) and Substance P (SP) protein expression were detected using ELISA. Results: After 14 d of intervention, compared to the control group, the rats in the experimental group showed a significant increase in body mass indexes ($P < 0.05$); decreased Bristol stool scores ($P < 0.05$); less stagnation of the intestinal contents and greater intestine propulsion rate ($P < 0.05$), lower wet/dry weight ratio of rat stool ($P < 0.05$), abdominal withdrawal reflex scores ($P < 0.05$) as well as PAR-2, TRVP1, CGRP and SP expression levels ($P < 0.05$). Conclusion: SUCT-CHM was effective in treating IBS-D in rats. It improved gastrointestinal function and reduced visceral hypersensitivity in rats possibly via the PAR-2/TRVP1 pathway.

Keywords: PAR-2/TRVP1, steaming umbilical cord therapy with Chinese herbal medicine, IBS-D model of rats, gastrointestinal function

Introduction

Irritable bowel syndrome (IBS) is a non-specific gastrointestinal neurological disorder of the digestive system that manifests clinically as a gastrointestinal stress response causing pain, dry stool or increased bowel movements, and may be accompanied by mixed or alternating constipation and diarrhea, with a lack of biomarkers and no significant abnormal changes in serum biochemical markers [1]. IBS is a common disease of the digestive system, susceptible to multiple external and internal factors, with an incidence of 10-20%. It has been found that the predisposing factors of IBS are closely related to living conditions, gender, lifestyle, psychology and genetics [2]. IBS is more prevalent in the female population and IBS patients account for 30% to 50% of the total number of patients with digestive disease. The symptoms of IBS are often non-specific, causing great physical and psychological distress, and are prone to persistence or recurrence, causing many difficulties in people's life and work. Statistics show that IBS ranks second in the incidence of common diseases and first in acute upper respiratory infections [3]. Therefore, exploration of its pathogenesis can effectively guide its prevention and treatment.

Steaming umbilical cord therapy stimulates the acupuncture point of the umbilicus, *ShenQue*, with Chinese medicine combined with moxibustion, which can effectively relieve the intestinal stress response by stimulating the meridians, regulate gastrointestinal function as well as bowel movements, and enhance gastrointestinal absorption [4]. This therapy is simple in operation and inexpensive, and can act directly on the gastrointestinal tract with few side effects, showing positive results for a variety of gastrointestinal functional disorders. Neurotransmitters, dopamine receptors, inflammatory factors, and signaling pathways play an important part in the hypersensitivity response in irritable bowel syndrome. Transient receptor potential vanilloid receptor 1 (TRPV1) is involved in the pathogenesis of nociceptive hypersensitivity [5]. In contrast, protease receptor 2 (PAR-2) and TRPV1 mediate the pain hypersensitivity response by stimulating sensory transduction nerves on the gastrointestinal tract, where TRPV1 receptors are sensitized and phosphorylated. PAR-2/TRVP1 is induced by proteases and may stimulate other proteins, such as CGRP and SP, which play important roles in gastrointestinal dynamics and sensation, and the release of isozymes from primary afferent sensory nerve fibers may increase neuronal excitability, inhibit gastrointestinal dynamics, and increase visceral pain sensations.

SUCT-CHM showed good efficacy in the treatment of irritable bowel syndrome. However, there are few studies on the mechanism of SUCT-CHM via PAR-2/TRVP1 in irritable bowel syndrome. In this study, we investigated the efficacy of SUCT-CHM in a rat model of IBS-D through the PAR-2/TRVP1 pathway.

Material and methods

Animal sourcing and grouping

Sixty-two SD Male rats, housed in SPF class cages, with a weight range of (190±45) g, were purchased from the Animal Experimentation Center to establish the rat model of IBS-D. These rats were randomly allocated to control and experimental groups, with 31 rats in each group. No interventions or treatment were given in control group, and the experimental group was treated with SUCT-CHM every other day for 2 weeks. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study was approved by The Affiliated Hospital of Jiangxi University of TCM.

Main reagents and apparatus

The Chinese herbs (Magnolia, Immature Orange Fruit, Alisma plantago-aquatica, Yam, Peach kernel and Exrementum Vespetilionis Murini, Moxa, and Senna glycosides); Saline; Rat fixation plate; Analytical balance; Low temperature refrigerator; high speed frozen centrifuge, ELISA assay kit for detection of PAR-2 antibody, TRPV1 antibody, SP antibody, CGRP antibody) (R&D, USA).

Modeling method

Rats were gavaged with an aqueous solution of Senna glycosides which had been boiled in water for 5 minutes to prepare an aqueous decoction at a concentration of 2 g/mL and administered intragastrically (10 mL/kg). The gastrointestinal function of the rats was impaired by solitary confinement and restraints. The following operations were randomly performed: 1. Binding the rats' limbs through strips of gauze so that they could not move for several hours. 2. The rats' tails were clamped for about 60 s, 3. Food feedings every other day. 4. Disrupting the rats' circadian rhythms. 5. Placing them in cold water for 180 s. 6. Incarcerating them at 50 degrees for 5 min. 7. Shaking water bath at 150 times/min for 45 min, 3 times/day. The above operations were performed in an unordered cycle for 14 consecutive days. The mental status, food intake, body weight and rat feces were observed. Significant differences in these indicators indicate that the IBS-D model was established successfully.

Treatment method

The Shen Que acupoint around the umbilicus was selected, positioned at the junction of the upper 3/4 and lower 1/4 of the external genital line from the upper edge of the sternum to the mid-thoracic line. The composition of the herbal medicine used for fumigation were mixed, followed by ultra-fine grinding, which was stored in a dry place. The hair around the umbilicus was removed and the rats were fixed on the plate. The Shen Que acupoint was disinfected with 75% ethanol. The flour was mixed with hot boiling water to form a ring (thickness of ring: 1 cm, and diameter of the hollow inside the ring: 1 cm). The ring on the Shen Que was filled with the prepared herbal powder (about 5 g). On top of herbal medicine laid a tower-shaped *Artemisia argyi* with a diameter of about 1 cm and a height of 1 cm. Three consecutive moxibustions were performed with 20 minutes each time. After moxibustion, the Chinese medicine was wrapped with non-woven fabric and applied externally to the umbilical points for 24 hours and then discarded. The therapy was performed once every other day for 14 days in total.

Outcome measurements

(1) Comparison of rat body weight: The body weight of rats in each group was weighed using an analytical balance before modeling, after modeling and after 14 d of treatment.

(2) Score for stool characteristics: Bristo stool sale was used to described the stool features: Type 1: Separate hard lumps, like nuts (difficult to pass and can be black); Type 2: Sausage-shaped, but lumpy; Type 3: Like a sausage but with cracks on its surface (can be black); Type 4: Like a sausage or snake, smooth and soft (average stool); Type 5: Soft blobs with clear cut edges; Type 6: Fluffy pieces with ragged edges, a mushy stool (diarrhoea); Type 7: Watery, no solid pieces, entirely liquid (diarrhoea). Types 1-7 corresponds to 1-7 points.

(3) Stagnation of the intestinal contents and intestine propulsion rate: On day 14, rats were fasted for 24 hours. Fresh toner suspension (10% gum arabic + 10% activated carbon) was perfused into the stomach orally. Rats were anesthetized by intraperitoneal injection of 0.4% sodium pentobarbital (0.01 mL/g) for 15 min. The pylorus of the stomach was ligated, and the total weight of the stomach was weighed.

The stagnation of the intestinal contents = (total weight of stomach - net weight of stomach)/total weight of charcoal powder × 100%. The total length of the small intestine and the length of toner suspension from the pylorus to the end of the small intestine were measured. The intestinal propulsion rate = length of charcoal powder/length of small intestine × 100%.

(4) The wet/dry weight ratio was calculated as (wet weight - dry weight)/wet weight × 100%. The change in wet/dry weight ratio before and after treatment was recorded.

(5) Abdominal wall reflex scores: Rectal saccule dilatation was performed to assess intestinal sensitivity. At 14 days after treatment, the body and tail of the rats were fixed with a wooden board, and the intestines of the rats could subsequently be dilated by ballooning. Different values of the balloon pressure were set to record the abdominal wall reflex score (AWR) of the rat intestine. 1 = brief head movement followed by immobility; 2 = contraction of abdominal muscles; 3 = lifting of abdominal wall; 4 = body arching and lifting of pelvic structures; AWR scores at each pressure value was tested 3 times at 4-minute intervals. The average of the AWR scores was used as the test result.

(6) ELISA test: 14 d after treatment, rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (4 mL/kg^{-1}). Colon tissue samples were rapidly excised on ice and quickly placed in at -80°C. PAR-2, TRVP1, CGRP and SP protein expression levels were calculated by strictly following ELISA instructions.

Statistical analysis

Statistical analysis was performed by SPSS 26.0 software. Measurement data were expressed as ($\overline{x}$ ± s) and analyzed by t test. Count data were expressed as a percentage and analyzed by chi-squared test. $P < 0.05$ indicated a significant statistical difference between the two groups.

Results

After 14 d of intervention, the body weight of the rats in experimental group showed a significant trend of increase compared with the control group ($P < 0.05$), suggesting SUCT-CHM could increase the body weight of IBS-D rats more effectively (**Figure 1**).

Assessment of the stool characteristic scores

Compared with the control group, there was a significant reduction in stool characteristic scores of the experimental group ($P < 0.05$), indicating that SUCT-CHM could more significantly reduce the stool characteristic scores of IBS-D rats (**Figure 2**).

Comparison of stagnation of the intestinal contents and intestine propulsion rate

The experimental group exhibited lower residual rate of intestinal contents and higher small

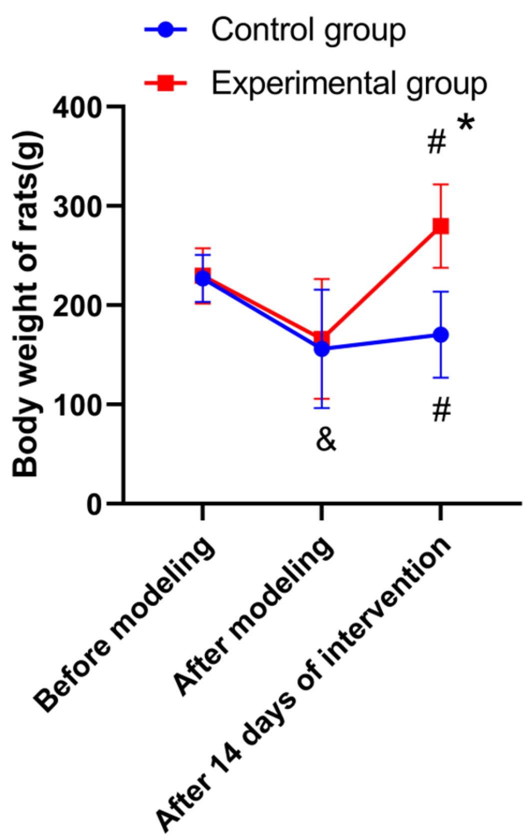

Figure 1. Comparison of body weight. Compared with post-modeling, $^{\#}P < 0.05$; compared with control group, $^{*}P < 0.05$, compared with pre-modeling, $^{\&}P < 0.05$.

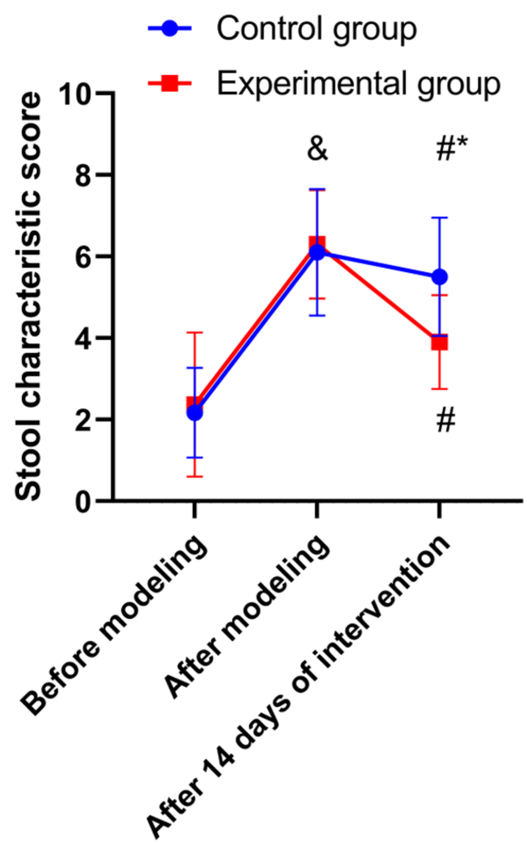

Figure 2. Comparison of stool characteristics scores. Compared with post-modeling, $^{\#}P < 0.05$; compared with control group, $^{*}P < 0.05$, compared with pre-modeling, $^{\&}P < 0.05$.

intestinal propulsion rate than the control group ($P < 0.05$). The results of this study showed that SUCT-CHM could more significantly reduce the residual rate of intestinal contents and increase the small intestinal propulsion rate in IBS-D rats (**Figure 3**).

Evaluation of wet/dry weight ratio

The wet/dry weight ratio of the experimental group was significantly reduced compared with that of the control group, with significant difference ($P < 0.05$). The results of this study found that SUCT-CHM could more significantly improve the wet/dry weight ratio in IBS-D rats (**Figure 4**).

Comparison of AWR scores

Compared with the control group, AWR scores of rats in the experimental group were more significantly reduced ($P < 0.05$), showing that SUCT-CHM could more significantly decrease the AWR scores in IBS-D rats (**Figure 5**).

Comparison of protein levels

ELISA results yielded that expression level of PAR-2, TRVP1, CGRP and SP were lower in the experimental group than in control group ($P < 0.05$). The results showed that SUCT-CHM could more significantly reduce the protein expression levels of PAR-2, TRVP1, CGRP and SP (**Figure 6**).

Discussion

IBS is a stress-responsive syndrome occurring in the gastrointestinal tract with non-specific symptoms, including gastrointestinal pain, difficult defecation or gastrointestinal diarrhea, without clear clinical diagnostic criteria [6]. IBS includes alternating or mixed constipation and diarrhea, or predominantly one of these mani-

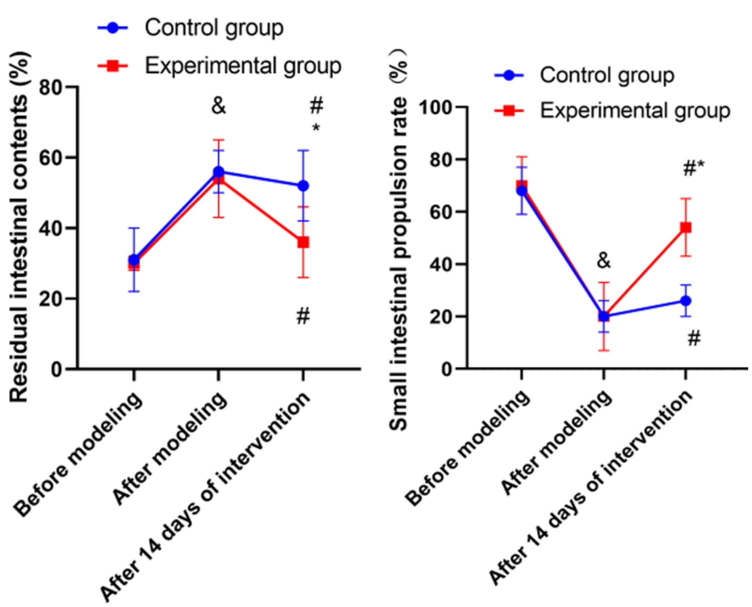

Figure 3. Comparison of residual rate of intestinal contents and intestine propulsion rate in rats. Compared with post-modeling, $^{\#}P < 0.05$; compared with control group, $^{*}P < 0.05$, compared with pre-modeling, $^{\&}P < 0.05$.

festations, and irritable bowel syndrome with diarrhea is defined as the IBS-D type [7]. The incidence of IBS ranges 8-25% worldwide, compared to 7.4% in China [8]. It has been found that more than half of the patients with IBS were comorbid with other digestive disorders [9, 10]. Evidence has shown that it is associated with intestinal motility, abnormal visceral sensation, inflammatory infections, and neuroendocrine disorders, as well as showing strong links with genetic, dietary, and other factors [11]. IBS is self-limiting, and some patients can recover without treatment, so the treatment is not given enough attention clinically. Patients with IBS are usually treated with drugs. However, the efficacy is not ideal, and there are also many side effects with high costs. Chinese herbal medicine has shown unique advantages and value in the treatment of IBS [12]. More and more clinical studies have shown that herbal steaming cord therapy is effective for IBS, and its curative effect has been recognized by most patients [13].

In this study, SUCT-CHM significantly improved the indexes related to gastrointestinal function in the experimental group than in control group ($P < 0.05$). Traditional Chinese Medicine is based on the pricinpal that the human body consists of twelve meridians and hundreds of acupuncture points, and the acupuncture point of umbilicus are closely related to the gastrointestinal function, thus moxibustion and administration of herbal medicines on the umbilicus could regulate the function of the intestinal tract [14]. The umbilical cord is thin, with the weakest barrier function and is highly permeable, facilitating the penetration and absorption of drugs. SUCT-CHM continuously stimulate the umbilicus and the corresponding acupuncture points with Chinese herbal medicines, which can enhance the function of the spleen and regulate the balance of yin and yang by stimulating the meridians, eventually improve symptoms [15]. SUCT-CHM is also characterized by simple manipulation, low cost, and few side effects [16]. The body weight of the experimental group after 14 days of intervention was significantly increased compared with that before modeling. This shall be attributed to the fact that SUCT-CHM can improve the regulatory function of digestive system, reduce gastrointestinal irritation and promote the digestion and absorption of food. Therefore, it can not only effectively cure irritable bowel syndrome, but also promote good metabolism and weight gain. SUCT-CHM activates the body's nerve conduction system and immune system continuously by stimulating the skin around the umbilicus, which could regulate the secretion and release of brain and intestinal peptides, improve immunity, increase body weight, improve bowl movement and intestinal residual rates, relieve bowel discomfort and fecal dryness, and reduce gastrointestinal irritation reaction; thus has shown more pronounced therapeutic effects [17-20].

In the present study, the expression levels of PAR-2, TRVP1, CGRP, and SP in the experimental group was significantly lower than in the control group ($P < 0.05$). Therefore, the efficacy of SUCT-CHM in the treatment of IBS-D may be achieved by down-regulating PAR-2, TRVP1, CGRP and SP protein expression. TRVP1 enhances calcium ion uptake through non-spe-

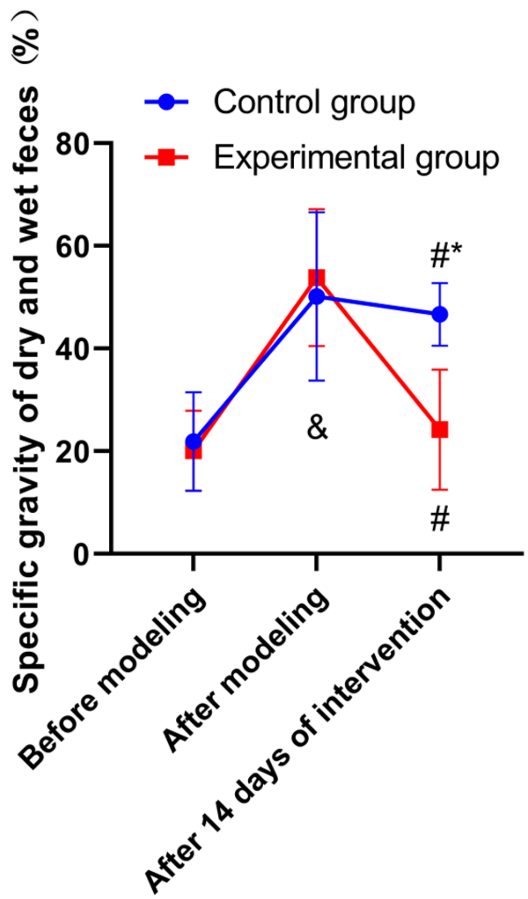

Figure 4. Comparison of wet/dry weight ratio. Compared with post-modeling, $^{\#}P < 0.05$; compared with control group, $^{*}P < 0.05$, compared with pre-modeling, $^{\&}P < 0.05$.

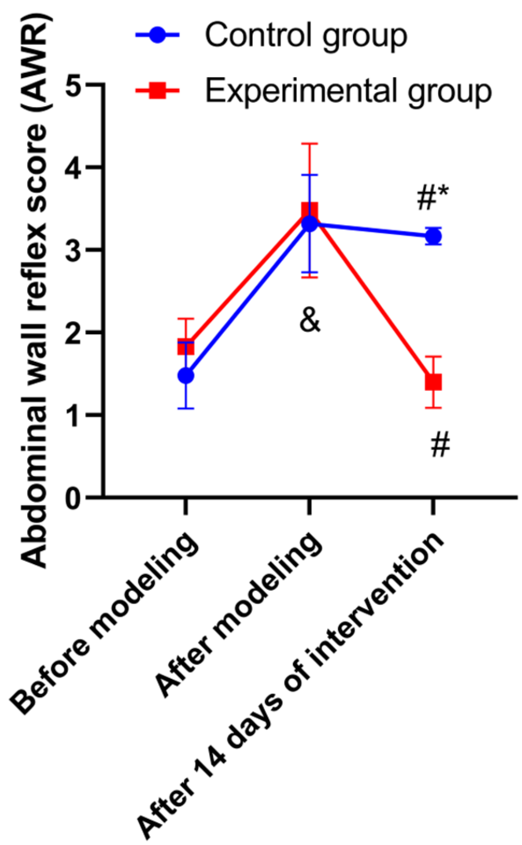

Figure 5. Comparison of AWR scores in rats. Compared with post-modeling, $^{\#}P < 0.05$; compared with control group, $^{*}P < 0.05$, compared with pre-modeling, $^{\&}P < 0.05$.

cific cation channels and may be activated by stimulation of other external factors, leading to different levels of pain in the body, as well as the release of various nociceptive proteins, neuropeptides and substance P through nerve cells [21]. PAR-2 can bind to nociceptive protein receptors through G-protein channels, PAR-2 is widely expressed and involved in pain sensitivity in gastrointestinal peripheral nerves. IBS stimulates the release of large amounts of trypsin from mast cells and macrophages and binds to PAR-2 to activate downstream phosphatases. It synergizes with Ca^{2+} to activate protein kinase C and mediate TRPV1 phosphorylation, leading to a decrease in nociceptive thresholds and an increase in Ca^{2+} inward flow, enhancing a prolonged or exaggerated response to pain. It also transmits corresponding pain signals through sensory transduction nerves on the gastrointestinal tract and stressfully controls the bowl movement [22]. SP proteins are released extracellularly in colonic tissues, activating cytokines and platelets, promoting macrophage activation and proliferation, and releasing neurotransmitters such as 5-HT and histamine to regulate peristalsis and visceral sensitization. CGRP regulates gastrointestinal secretion and motor function, accelerates colonic motility, and leads to clinical symptoms such as abdominal pain, bowel sounds and diarrhea, suggesting that elevated CGRP is closely related to visceral hypersensitivity [15, 23-25]. However, it is worth noting that since the present study was limited to animal subjects, data regarding clinical studies on humans need to be conducted in further.

In conclusion, SUCT-CHM is effective in promoting gastrointestinal function and reducing visceral hypersensitivity in rats, which may be achieved through the PAR-2/TRVP1 signaling pathway.

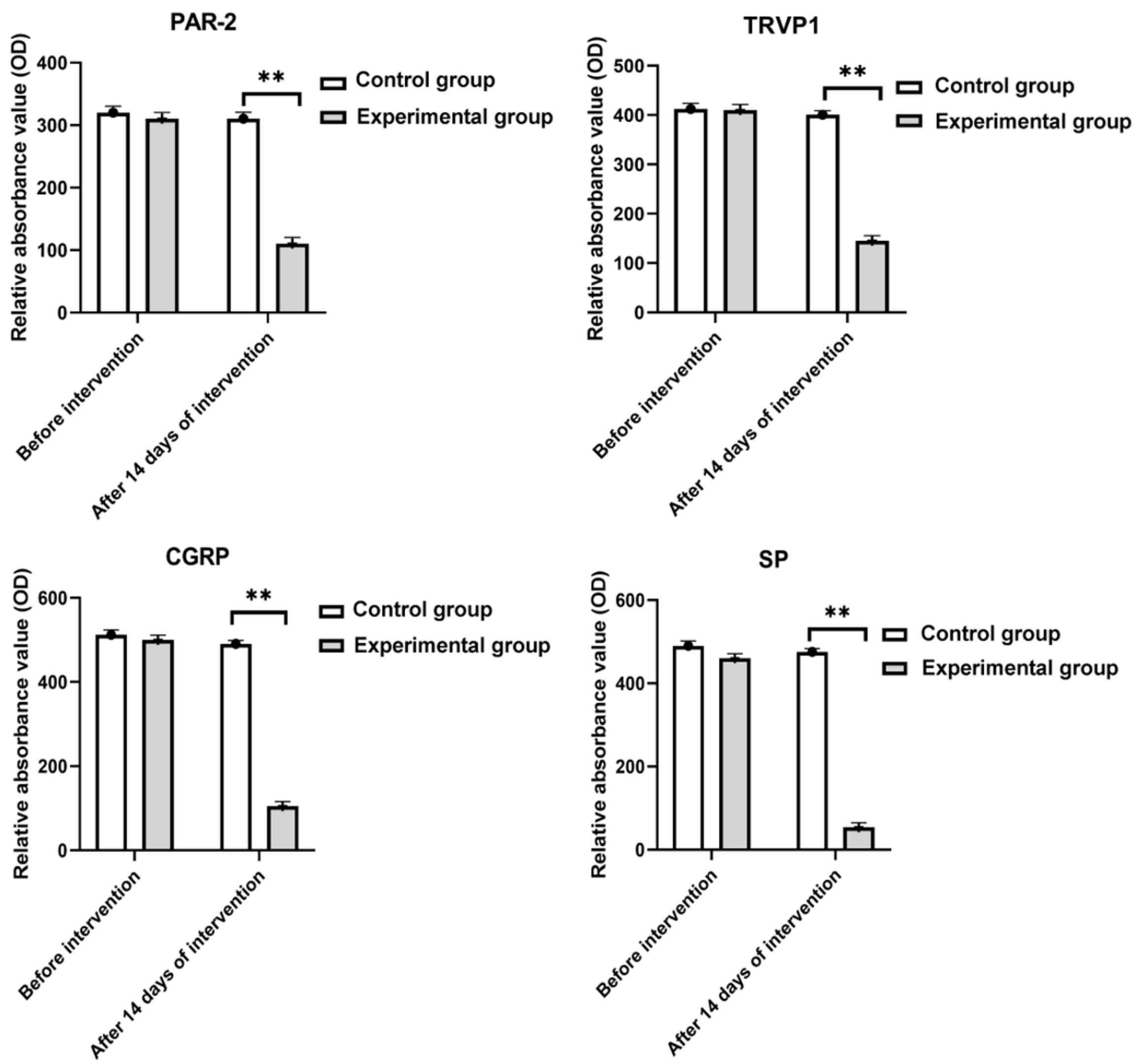

Figure 6. Comparison of PAR-2, TRVP1, CGRP and SP protein expression levels by ELISA. After 14 days of intervention, PAR-2, TRVP1, CGRP and SP protein expression levels in the experimental group were significantly lower than those in the control group. $^{**}P < 0.01$ compared to pre-intervention.

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Disclosure of conflict of interest

None.

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