Article

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Therapeutic Effect and Mechanism of Emodin on Migraine via Inhibition of TRPV4/p38 Signaling Pathway in a Nitroglycerin-Induced Rat Model

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Background: Migraine is known as a persistent neurological condition marked by recurring bouts of head pain and diverse neurological manifestations. Emodin exhibits a wide range of pharmacological activities, particularly its neuroprotective effects on neurodegenerative diseases. Emodin is capable to alleviate nitroglycerin (NTG)-induced migraine in rats. Furthermore, it has been witnessed that transient receptor potential vanilloid-4 (TRPV4)/p38 signaling pathway is involved in the development of migraine pathogenesis.

Methods: Rats subjected to repetitive NTG administration were considered a model replicating clinical manifestations of migraine. Three different Emodin dosage groups (high, medium, and low) and control group were used to observe the effects of different doses on the behavior and other related indicators of migraine rats.

Results: The results showed that high and medium doses of Emodin significantly delayed the appearance time of redness and scratching in the ears of rats, shortened their disappearance time, and increased the mechanical pain threshold of rats, indicating that Emodin can remarkably improve the behavior of migraine rats and increase their mechanical pain threshold. At the same time, high and medium doses of Emodin significantly increased the content of peripheral blood 5-hydroxytryptamine (5-HT) in migraine rats, indicating that Emodin can treat migraine headaches by increasing the peripheral blood 5-HT content. In addition, high and medium doses of Emodin can reduce the expression of TRPV4 protein and p38 mRNA in the trigeminal ganglion of migraine rats, indicating that Emodin can negatively regulate the expression and gene transcription of TRPV4/p38 signaling pathway-related proteins. Therefore, it inhibits neuroinflammation, reduces pain-induced sensitization, and exerts a therapeutic effect on migraine.

Conclusions: Our findings demonstrate that Emodin is capable to mitigate headaches, which are associated symptoms in migraine-afflicted rats. This effect is likely attributed to the elevation of peripheral blood 5-HT content and the suppression of expression and gene transcription related to the TRPV4/p38 signaling pathway.

Keywords: migraine; Emodin; 5-HT; TRPV4/p38 signaling pathway; analgesia

Introduction

Migraine, a persistent neurological condition, is characterized by repeated occurrences of head pain and assorted neurological manifestations, occasionally accompanied by early warning signs. It is more common in women and is regarded as one of the major causes of disability for women [1]. Migraine patients may face the risk of placental dysfunction during pregnancy [2]. Similarly, men may also face many challenges of migraine, such as erectile dysfunction, and are associated with cognitive impairment and cardiovascular disease [3]. Currently, western medicine is used for relieving headaches and related symptoms to improve the quality of daily life. Several new drugs such as Lasmiditan, Gepants, and CGRP monoclonal antibodies have emerged as potentially effective methods for treating migraine, but there are still certain limitations in indications, contraindications, and adverse reactions of the drugs [4–6]. Therefore, further research is imperative to explore their pharmacological and clinical effects. Long-term observations are essential to understand their impacts and potential implications.

There are several theories about the origin of migraine, however, its pathogenesis is still unclear. The vascular origin theory suggests that migraine might be caused by ab-

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normal constriction and dilation of intracranial blood vessels [7]. The cortical spreading depression theory suggests that the pathogenesis of migraine is due to cortical spreading depression which is associated with depolarization [8]. The trigeminal neurovascular theory considers the trigeminal vascular pain pathway as the final common pathway leading to migraine, in which meningeal vascular dilation and neurogenic inflammation are the major pathological changes, and neurogenic inflammation plays a key role [9]. Peripheral nociceptive sensory nerve endings are activated to release calcitonin gene-related peptide (CGRP), causing a series of inflammatory cascade reactions [10]. Inflammatory cytokines enter the nociceptive receptors and diffuse in the dura mater and meningeal blood vessels, transmitting pain signals through the trigeminal sensory fibers, and ultimately triggering migraine [10]. However, the specific molecular mechanism of the trigeminal neurovascular theory to elucidate the pathogenesis of migraine still needs to be explored.

The neurotransmitter, 5-hydroxytryptamine (5-HT) is closely related to pain and plays diverse roles in different receptors, and an imbalance in 5-HT system can lead to the occurrence of pain [11]. It has been reported that four types of receptors, namely 5-HT1, 5-HT2, 5-HT3, and 5-HT7, play key roles in the pathogenesis of migraine [11]. Transient receptor potential vanilloid-4 (TRPV4) expressed in multiple organ tissues is involved in various pro-inflammatory or anti-inflammatory processes, making it an attractive drug target for migraine treatment [12]. During the progression of pain-related pathology, the pivotal involvement of P38 protein kinase emerges, and the TRPV4/p38 signaling pathway assumes significance in the onset of migraines. Earlier research has established a correlation between the expression of TRPV4 and p38 protein and the pain threshold in rat models [13]. On overall basis, the TRPV4/p38 signaling pathway may play a significant role in the pathogenesis of migraines.

Research has reported extensive pharmacological activities of Emodin, particularly its neuroprotective effects on neurodegenerative diseases, such as anxiety, depression, Parkinson's and Alzheimer's disease, and cerebral ischemia, etc. [14]. Sun *et al*. [15] found that Emodin alleviates NTG-induced migraine in rats through the cGMP-PKG signaling pathway. This investigation postulated that Emodin may potentially alleviate migraines by modulating the expression of proteins associated with the TRPV4/p38 pathway. In the scope of this investigation, we have verified the effects of Emodin by subjecting rats to migraine-induced conditions in experimental settings. Our objective was to delve into the pain-relieving potential of Emodin as it relates to the intricate mechanisms underlying migraines, aiming to establish a solid scientific foundation for its therapeutic application.

Materials and Methods

Drugs

The nitroglycerin injection was manufactured by Henan Runhong Pharmaceutical Co., Ltd., with product batch number 2009142 (Zhengzhou, China). Rizatriptan benzoate tablets (Eulitidone) were manufactured by Hubei Euli Pharmaceutical Co., Ltd., with product batch number 201204 (Wuhan, China). Eulitidone was used as a positive control. Based on the daily dose of 5 mg for a 70 kg adult, the equivalent dose for rats was calculated as 0.45 mg/kg. To prepare a drug concentration of 0.045 mg/mL, 5 mg of Eulitidone was ground into fine powder and fully dissolved in 112 mL of warm water. Emodin was obtained from Solaibao (E8390, Specification:1 g, Beijing Solaibao Technology Co., Ltd., Beijing, China).

Animal Modeling

We procured 66 healthy SPF-grade male SD rats (weighing 180 g ± 20 g) from Liaoning Changsheng Biotechnology Co., Ltd., with the animal certificate number SCXK (Liao) 2020-0001 (Shenyang, China). All experimental protocols and animal welfare practices were strictly adhered to the "Guidelines for the Care and Use of Laboratory Animals" and received approval from Guizhou University of Traditional Chinese Medicine, with Ethical review No. 20210137. After quarantine, the rats were housed in the laboratory of the First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine. The animal room was well-ventilated, with a humidity of (40% ± 5%) and a temperature of (25 °C ± 2 °C). Rats were supplied with standard feed and unrestricted access to water, following a daily 12 h light-dark cycle. Allocation of rats involved random division into six distinct groups, including the blank control group (n = 11), the model control group (n = 11), the positive drug control group (n = 11), the high-dose Emodin group (n = 11), the medium-dose Emodin group (n = 11), and the low-dose Emodin group (n = 11). The procedures listed below were improved according to Tassorelli *et al*. [16]. After one week of appropriate feeding, gavage administration was started with a dosage of 1 mL/100g. Emodin was orally administered at high (60 mg/kg), medium (40 mg/kg), and low doses (20 mg/kg), while the positive drug control group was orally administered with Rizatriptan benzoate at a dose of 0.45 mg/kg. Both the blank control group and the model control group received oral administration of physiological saline. This administration was conducted once daily for seven consecutive days. One hour after the last administration, except for the blank control group, the rats in the remaining groups underwent subcutaneous injection of nitroglycerin at a dosage of 10 mg/kg into the neck to induce an experimental migraine rat model [17].

Sample Collection

Three hours (h) after injecting nitroglycerin, 3% pentobarbital sodium (45 mg/kg) was injected into the abdominal cavity for anesthesia. After anesthesia, 4–5 mL of blood samples were taken from the abdominal aorta, centrifuged at 3000 r/min for 10 minutes (min), and the separated serum samples were stored in a refrigerator at –20 °C for further analysis. Three randomly selected rats from each group were fixed by perfusion with paraformaldehyde through the heart. The experimental rats in each group were euthanized using the method of cervical dislocation. Subsequently, the brain tissue was quickly removed after decapitation, and the bilateral trigeminal ganglia were separated on ice according to the "Rat Brain Stereotaxic Map" [18]. These specimens were stored in a refrigerator at –80 °C for HE (hematoxylin-eosin) staining. Furthermore, fresh brain tissue was quickly taken out from eight other rats after decapitation, and the trigeminal ganglia were separated on ice according to the "Rat Brain Stereotaxic Map" [18]. After washing the bloodstains on the surface of the trigeminal ganglia with pre-cooled 0.9% saline solution, the ganglia were placed in pre-numbered EP tubes and stored in a –80 °C refrigerator. One side of the trigeminal ganglia was used for western blot, while the other side was used for qRT-PCR.

Behavioral Assessment

Previous studies have shown that after injecting nitroglycerin within 3–5 min, migraine model rats exhibited significant ears redness and frequent head scratching with the forelimbs, accompanied by a decrease in mechanical pain thresholds, after which the rats returned to calm after 3 h [19,20]. Therefore, we measured the appearance and disappearance time of ear redness and ear scratching in different groups of rats following the previous research [19]. According to the research of Yang *et al*. [20] the mechanical pain thresholds at 1 h, 2 h, and 3 h after modeling were determined. The specific procedures are as follows: the middle part of the rat's foot was gently stimulated using Von-frey fibers for a few seconds, the foot-shrinking reaction was observed and the minimum mechanical pain threshold for paw retraction in rats was recorded. All animal behavioral assessments were conducted in a quiet environment between 9:00 am and 17:00 pm. All behavioral tests were conducted using the same batch of rats, and each measurement was performed at least three times.

HE Staining

HE staining procedures were conducted following the standard protocol. After deparaffinization and rehydration, the trigeminal ganglion tissue was sliced and stained with hematoxylin solution for 5 min. It was then immersed in 1% acidified ethanol (1% HCl in 75% ethanol) for five cycles, followed by thorough washing with distilled water. The tissue was further subjected to eosin solution staining for 3 min and subsequently processed through dehydration with graded alcohol and clearing with xylene. Ultimately, the mounted slides were scrutinized and captured using a Leica DM3000 LED microscope (Leica, Baden-Württemberg, Germany). Specific details regarding the reagents employed are listed in **Supplementary Table 1**.

ELISA Testing

Serum 5-HT levels were quantified through enzyme-linked immunosorbent assay (ELISA) following the guidelines provided by the ELISA kit. In summary, the sample was diluted and introduced to an enzyme-labeled plate in combination with the antibody. The plate was washed five times with a dedicated washing solution, followed by incubation with a coloring solution and a subsequent stop solution. Ultimately, the absorbance at 450 nm was measured using a microplate reader (ELx800, BioTek, Winooski, VT, USA). Comprehensive details regarding the reagents utilized are shown in **Supplementary Table 1**.

Western Blot

The western blot process was initiated by lysing the trigeminal ganglion tissue in RIPA buffer to extract total protein, which was then separated on an SDS-PAGE gel and transferred to a PVDF membrane. The membrane was then incubated with a primary antibody and blocked with 5% skim milk at 4 °C overnight. After five wash cycles, an appropriate secondary antibody was added and the cells were incubated at 37 °C for 2 h. All the antibodies were diluted with a special diluent, and the dilution ratio of both the anti-β-actin and anti-TRPV4 was 1:1000. Finally, the sample was characterized through an ECL system and quantified using imaging. Details of the antibodies used are listed in **Supplementary Table 1**. Normalization of target protein expressions across various groups was achieved by utilizing harnessing β-actin as a reference. Each western blot was replicated at least six times for accuracy and reliability.

qRT-PCR

Total RNA from trigeminal ganglion tissue was extracted through the application of Trizol reagent. Subsequently, mRNA cDNA was synthesized, and qRT-PCR was conducted on the cDNA-SYBR Green mixture utilizing a real-time fluorescence quantitative PCR system. Results were analyzed using the $2^{-\Delta\Delta Ct}$ algorithm, employing β-actin as an internal reference for mRNA normalization across all samples. Specific primers utilized for qRT-PCR are detailed in Table 1.

Statistical Analysis

Statistical analyses were carried out through SPSS software (version 26.0, IBM Corporation, Armonk, NY, USA). Measured data were presented as means ± standard deviation (SD). Intergroup comparisons were con-

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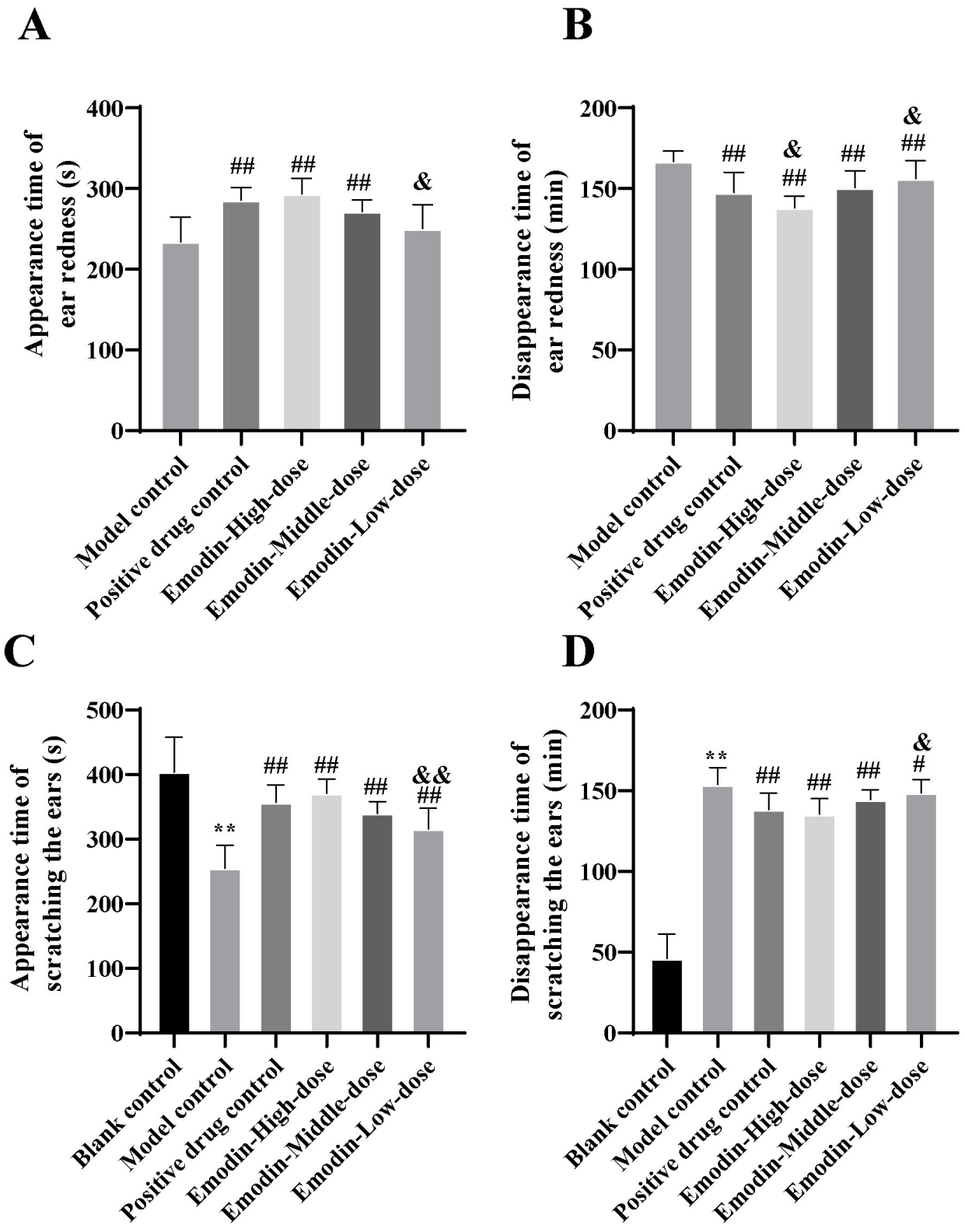

Fig. 1. Comparison of the appearance and disappearance time of ear redness and ear scratching in rats in each group. Each experimental treatment group contains 11 rat samples (n = 11). (A) Onset time of ear redness in different groups of rats. (B) Disappearance time of ear redness in different groups of rats. (C) Onset time of scratching the ears in different groups of rats. (D) Disappearance time of scratching the ears in different groups of rats. Note: Compared with the blank control group, $^{**}p < 0.01$; compared with the model control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$; compared with the positive drug control group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$.

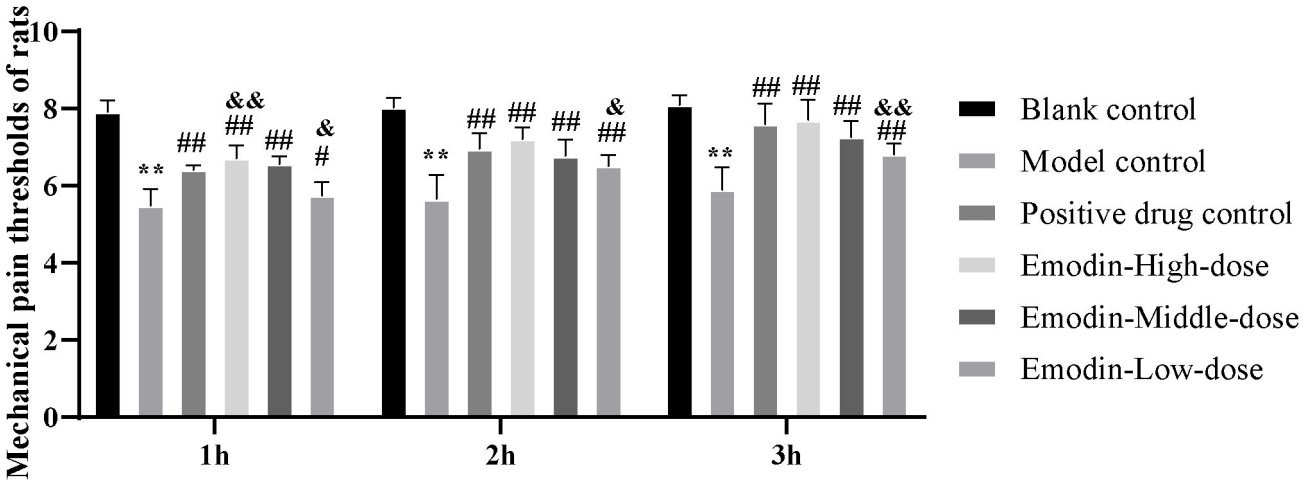

Fig. 2. Mechanical pain thresholds of rats in each group at 1 h, 2 h, and 3 h after modeling. Each experimental treatment group contains 11 rat samples (n = 11). Note: Compared with the blank control group, $^{**}p < 0.01$; compared with the model control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$; compared with the positive drug control group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$.

Table 1. Primer sequence.

Name	Primer	Sequence	Size
Rat β-actin	Forward	CACGATGGAGGGGCCGGACTCATC	240 bp
	Reverse	TAAAGACCTCTATGCCAACACAGT	
Rat P38	Forward	ATGTCGCAGGAGAGGCCACGTTCT	188 bp
	Reverse	AGAACGTGGGCCTCTCCTGCGACAT	

ducted through one-way ANOVA. Dunnett's multiple comparison was applied to identify pairwise differences between groups, while LSD-*t* test was employed for specific pairwise comparisons. The statistical significance was set at $p < 0.05$ and high significance at $p < 0.01$.

Results

Behavioral Observation

After the modeling process, no ear redness was observed in the blank control group. In comparison to the model control group, the appearance time of ear redness was significantly delayed ($p < 0.01$) in all dosing groups except for the Emodin low-dose group, while the disappearance of ear redness occurred significantly earlier ($p < 0.01$) in all dosing groups. Compared to the positive drug control group, the disappearance time of ear redness was remarkably earlier ($p < 0.05$) in the Emodin high-dose group. Additionally, in contrast to the blank control group, the appearance of ear scratching was significantly earlier in the model control group ($p < 0.01$). Relative to the model control group, each administration group exhibited a significant delay in the appearance of ear scratching ($p < 0.01$), and the disappearance time of ear scratching was significantly earlier in each administration group except for the Emodin low-dose group ($p < 0.01$, $p < 0.05$). There were no significant differences in the appearance and disappearance time of ear scratching in the Emodin high- and medium-dose groups compared to the positive drug control group ($p > 0.05$). Further details are shown in Fig. 1.

In comparison to the blank control group, the mechanical pain threshold significantly decreased in the model control group at 1 h, 2 h, and 3 h after modeling ($p < 0.01$). Compared to the model control group, each administration group exhibited a significant increase in the mechanical pain threshold at 1 h, 2 h, and 3 h after modeling ($p < 0.01$, $p < 0.05$). In comparison to the positive drug control group, the Emodin high-dose group showed a significantly higher mechanical pain threshold at 1 h after modeling ($p < 0.01$). Mechanical pain threshold in the Emodin medium-dose group was comparable ($p > 0.05$). However, at 2 h and 3 h post-modeling, mechanical pain threshold in both Emodin high- and medium-dose groups was comparable ($p > 0.05$). Specific details are presented in Fig. 2.

Effects of Different Doses of Emodin on the Morphological Structure of the Trigeminal Ganglion in Rats with a Model

After HE staining, the cells and their boundaries in the small field of view in the model control group were blurred compared to the blank control group (Fig. 3A,B), indicating the obvious damage to the trigeminal ganglion tissue nerve cells. After the intervention of Emodin high-dose, the number of damaged nerve cells in the trigeminal ganglion of rats was reduced (Fig. 3C,D). Individual cell in the Emodin

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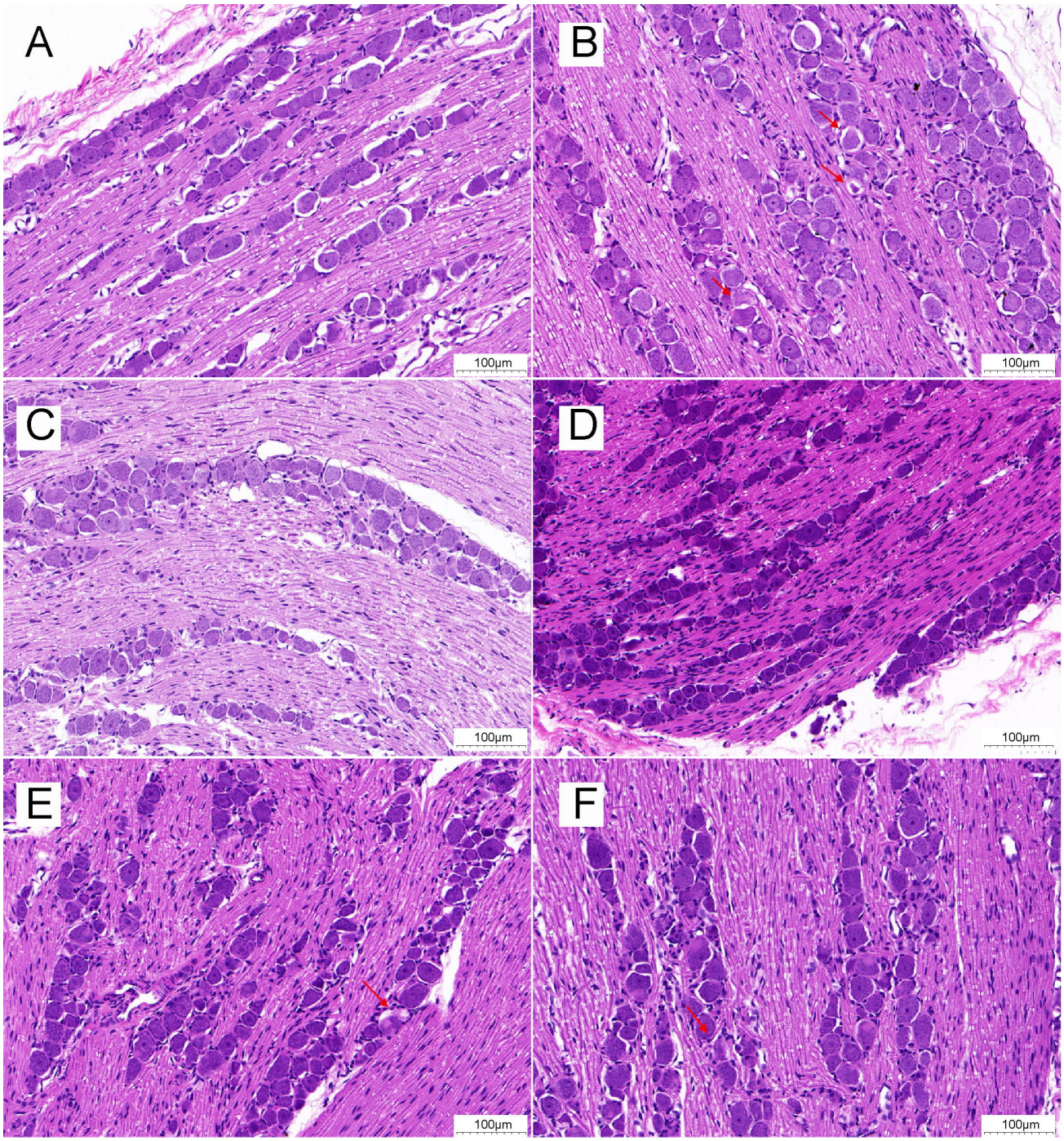

Fig. 3. HE (hematoxylin-eosin) staining of the trigeminal ganglion in different groups of rats. Arrows represent the localization of blurred boundaries of the damaged nerve cells. The magnification is 200×. (A) Blank control group. (B) Model control group. (C) Positive drug control group. (D) Emodin high-dose group. (E) Emodin middle-dose group. (F) Emodin low-dose group.

medium-dose group showed structural blurring (Fig. 3E), and local boundary blurring was observed in the Emodin low-dose group (Fig. 3F), suggesting that the nerve cells in the trigeminal ganglion tissue were damaged. Combined with the behavioral changes and mechanical pain threshold reduction in each group of rats, it was suggested that the migraine rat model was successfully established.

Emodin Effects on Peripheral Blood 5-HT in Rats with Different Doses

As depicted in Fig. 4, peripheral blood 5-HT levels showed a significant decrease in the model control group compared to the blank control group ($p < 0.01$). Conversely, when compared to the model control group, all dosing groups, excluding the Emodin low-dose group, displayed a significant increase in peripheral blood 5-HT lev-

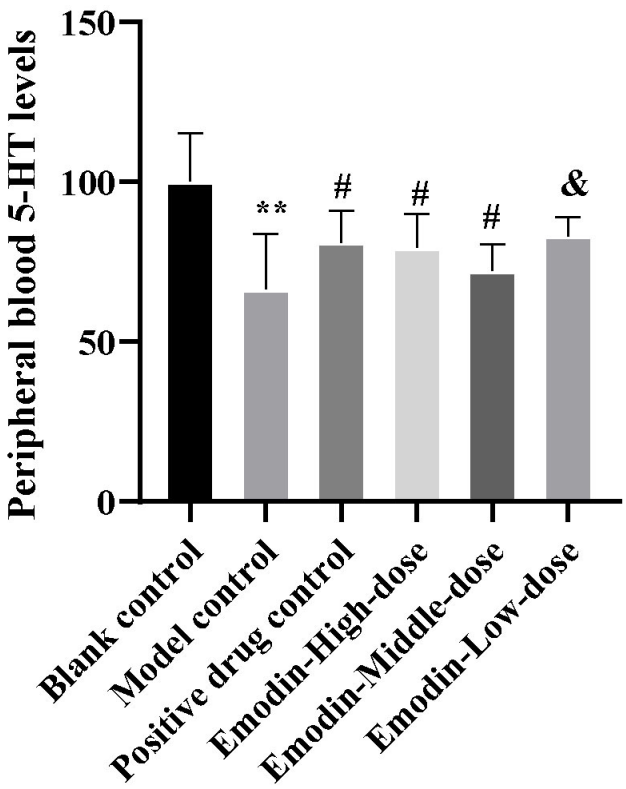

Fig. 4. Comparison of peripheral blood 5-hydroxytryptamine (5-HT) levels among different groups. Blank and positive drug control groups contain 8 rat samples (n = 8), and model control and Emodin doses groups contain 7 rat samples (n = 7). Note: Compared with the blank control group, $^{**}p < 0.01$; compared with the model control group, $^{\#}p < 0.05$; compared with the positive drug control group, $^{\&}p < 0.05$.

els ($p < 0.05$). Furthermore, in comparison to the positive drug control group, peripheral blood 5-HT levels were comparable in both Emodin high- and medium-dose groups ($p > 0.05$).

Emodin Effects on TRPV4 and p38 mRNA and Protein Levels in the Trigeminal Ganglion

Compared to the blank control group, the model control group had a notable escalation in TRPV4 protein and p38 mRNA expression within the trigeminal ganglion ($p < 0.01$). Moreover, a substantial reduction in the expression of TRPV4 protein and p38 mRNA within the trigeminal ganglion was observed in all drug administration groups, except for the Emodin low-dose group, as compared to the model control group ($p < 0.01$, $p < 0.05$). Compared to the positive drug control group, Emodin high- and medium-dose groups displayed comparable levels of TRPV4 protein and p38 mRNA expression in the trigeminal ganglion, showing no statistically significant differences ($p > 0.05$). These results are shown in Fig. 5.

Discussion

Migraine is recognized as a neurovascular disorder in modern medicine, characterized by unilateral or sometimes bilateral severe pulsating headache, which might be accompanied with aura symptoms such as nausea, vomiting, photophobia, and phonophobia [1]. In current studies, there are three widely accepted hypotheses, including the vascular origin theory [7], the cortical spreading depression theory [8], and the trigeminal neurovascular theory [9]. However, because of the complex complications in clinical manifestations, the pathogenesis of migraine has not been fully clarified so far, which has seriously hampered the development of therapeutic drugs. In this study, we constructed a migraine rat model induced by nitroglycerin to observe the therapeutic effects of different doses of Emodin on the behaviors and related indicators of migraine rats. Our research explores the therapeutic effect and mechanism of Emodin on migraine in rats.

HT is a neurotransmitter present in the central nervous system and peripheral structures, and acts as a hormone in platelet [21]. It does not only regulate central nervous system neurons but also regulate pain transmission and platelet aggregation [21]. 5-HT stored in the C fiber subpopulation is widely present in the trigeminal ganglion [22]. As a neurotransmitter, 5-HT can bind to different types of receptors, which participate in the physiological and pathological processes of pain [23]. Studies have demonstrated that 5-HT neurons exhibit an analgesic effect in young mice and become analgesics in experimental models of neuropathic pain [24]. Up-regulation of peripheral blood 5-HT levels can reduce the frequency and duration of migraine, improve pain intensity, and cerebral blood flow velocity [25]. Previous research highlighted the potential that pain sensitization in migraine rats can be alleviated by affecting the expression of peripheral blood 5-HT, thereby exerting an analgesic effect [26]. Presently, 14 subtypes of 5-HT receptors have been discovered. 5-HT1, 5-HT2, 5-HT3, and 5-HT7 receptors are involved in the pathological process of migraine [25]. The 5-HT7 receptor can affect the phosphorylation of signal pathways mediated by protein kinase A or extracellular signal-regulated kinases 1/2 and interact with cyclic adenosine monophosphate to mediate pain sensitization of nociceptive neurons [27]. 5-HT1B/1D receptor agonists and 5-HT1F receptor agonists are among the four major specific drugs for migraine treatment [28]. Therefore, the balance of 5-HT system plays an important role in the pathological process of headache, and the up-regulation of 5-HT activity can effectively treat and alleviate migraine.

Transient receptor potential (TRP) channels convert noxious stimuli into pain signals, which are widely expressed in trigeminal ganglion neurons and are closely related to the specific symptoms of migraine, such as hyperalgesia and allodynia, etc. [29,30]. TRPV4 is a member of the TRP family, expressed in nociceptive neurons and primary afferent nociceptors [31]. It is considered as a multi-modal

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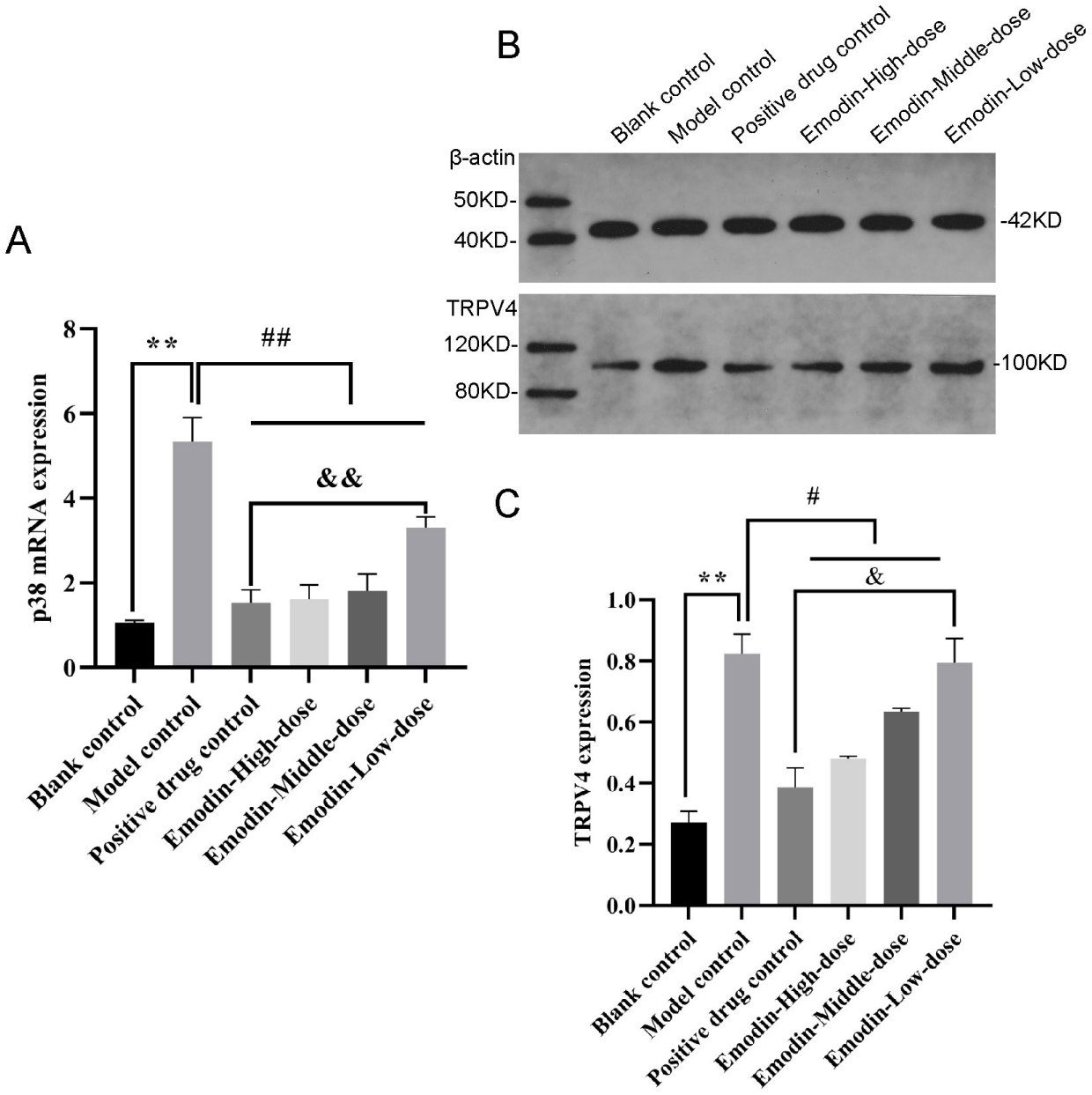

Fig. 5. Comparison of transient receptor potential vanilloid-4 (TRPV4) protein and p38 mRNA expression in trigeminal ganglia. (A) The p38 mRNA expression in trigeminal ganglia. (B) Representative western blot of TRPV4 protein in trigeminal ganglia, β-actin was used as the control and band density normalization. (C) Quantification of TRPV4 protein expression in trigeminal ganglia. Blank, positive drug, and model control groups contained at least 3 rats samples ($n \geq 3$). Note: Compared with the blank control group, $^{**}p < 0.01$; compared with the model control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$; compared with the positive drug control group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$.

sensor for chemical, thermal, and mechanical-induced pain and plays an important role in the pathological process of pain [32]. The activation of TRPV4 in trigeminal ganglion sensory neurons can promote the release of pain mediator, CGRP, and boost the dilation of meningeal blood vessels, thereby inducing pain occurrence [33]. TRPV4 antagonists can inhibit the release of CGRP, which provides new therapeutic strategies in the upstream area of migraine treatment [34].

p38 is a protein kinase known to further promote pain hypersensitivity in animal models [35]. Previous study demonstrated that the down-regulation of (p)-p38 in dorsal root ganglion neurons can improve systemic pain hypersensitivity [36]. It has been reported that the inhibition of dorsal root ganglion cell apoptosis can relieve neuropathic pain by regulating the p38 MAPK/CREB signaling pathway [37]. p38 is highly expressed in trigeminal ganglion neurons of migraine rats [38], and inhibiting the phospho-

rylation of p38 in the trigeminal spinal nucleus has a significant analgesic effect on migraine [39], and intervening in the gene expression of p38 can prevent migraine [40]. It suggests that p38 might be a potential therapeutic target for clinical migraine treatment. Moreover, intervening the expression of p38 effectively inhibits neurogenic inflammation and neurotransmitter production, alleviates the hypersensitivity of trigeminal nerve receptors to harmful stimuli, offsets cortical spreading depression, and ultimately achieves the therapeutic effect on migraine [39]. In this way, p38 is a downstream convergence point of multiple pain signaling pathways, and neurogenic inflammation mediated by p38 activation plays a crucial role in the pathogenesis of migraine.

The activation of the TRPV4/p38 signaling pathway plays a crucial role in the pathogenesis of migraine. Multiple endogenous or exogenous stimuli can activate TRPV4 in the trigeminal vascular system [41,42], promote the release of neuropeptide and CGRP [33], subsequently stimulate the expression of p38, amplify the inflammatory cascade reactions, and release pro-inflammatory factors such as IL-1β and TNF-α [41]. These processes lead to neurogenic inflammation and promote peripheral sensitization [43], thereby causing migraine. Therefore, neurogenic inflammation mediated by the activation of the TRPV4/p38 signaling pathway is involved in the pathogenesis of migraine. In order to clarify the effect of Emodin on the expression of TRPV4 and p38 mRNA in the trigeminal ganglion of migraine rats, the experimental rats were injected with nitroglycerin by subcutaneous injection to establish a migraine rat model. Nitroglycerin can be decomposed in the body of animal models to produce oxidative compounds, including NO, therefore activating TRPV4 to induce migraine occurrence [17]. Animal behavioral assessment, HE staining, western blot, and qRT-PCR, etc. were performed in this study. It was found that high and medium doses of Emodin can significantly delay the appearance time of ear redness and ear scratching, shorten the disappearance time of ear redness and ear scratching, and increase the mechanical pain threshold of migraine rats. Similar patterns appeared in the previous research of Jiang *et al*. [19]. Meanwhile, high and medium doses of Emodin remarkably increased the content of peripheral blood 5-HT of migraine rats, indicating that Emodin alleviates migraine by increasing the peripheral blood 5-HT content. Similar results have been discovered in the research of Ke *et al*. [25]. In addition, high and medium doses of Emodin can decrease the expression of TRPV4 protein and p38 mRNA in the trigeminal ganglion of migraine rats, negatively regulating the expression of TRPV4/p38 signaling pathway-related proteins to suppress migraine, which is consistent with previous findings [37,43]. There are certain limitations of this study including a restricted range of detection indicators, insufficient coverage of upstream and downstream of the TRPV4/p38 signaling pathway, the small sample size, and relatively simple grouping. Notably, the TRPV4/p38 inhibitor group or gene knockout group was not set as a control. Therefore, it is necessary to further improve and strengthen the group setting and reveal the therapeutic mechanism of Emodin on migraine in future studies, to provide more scientific basis for the utilization of Emodin in migraine treatment.

Conclusions

The study showed that high and medium doses of Emodin significantly delayed the appearance time and shortened the disappearance time of redness and scratching in the ear of rats, and increased the mechanical pain threshold of rats. In addition, high and medium doses of Emodin remarkably increased the peripheral blood 5-HT content of rats and reduced the expression of TRPV4 and p38 mRNA in the trigeminal ganglion of migraine rats. These results concluded that Emodin can significantly alleviate headaches and related symptoms in migraine rats, possibly by increasing the content of peripheral blood 5-HT and inhibiting the expression and gene transcription of TRPV4/p38 signaling pathway-related proteins. Collectively, these findings will lay a foundation for further exploration of Emodin on migraine through TRPV4/p38 signaling pathway, and provide potential therapeutic targets for migraine prevention.

Availability of Data and Materials

The simulation experiment data used to support the findings of this study is available from the corresponding authors upon request.

Author Contributions

YHW and DDL designed the research study; YLL, WL, SSW, QSH, PC and YH performed the research; SSW, QSH, PC and YH collected and analyzed the data. YLL and WL have been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

All experimental protocols and animal welfare practices were strictly adhered to the “Guidelines for the Care and Use of Laboratory Animals” and received approval from Guizhou University of Traditional Chinese Medicine, with Ethical review No. 20210137.

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Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243805.297.

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