Article

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Exploring of the Analgesic Effect of Emodin on Migraine Rat Model via the CREB/BDNF/TrkB Signaling Pathway: A Preliminary Study

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Background: Migraine is a prevalent neurovascular headache characterized by recurring pain episodes. Previous research indicates that managing the expression of the cAMP response element-binding protein/Brain-derived neurotrophic factor/Tyrosine receptor kinase B (CREB/BDNF/TrkB) pain signaling pathway may enhance migraine conditions. This study delves into the pharmacological effects and analgesic mechanisms of emodin in treating nitroglycerin-induced migraines in animal models, focusing on the CREB/BDNF/TrkB signaling pathway.

Methods: Sixty-six male Sprague Dawley (SD) rats were randomly divided into six groups: Control, Model, positive control, and low, medium and high doses of emodin treatment groups. All groups, except the control, underwent the establishment of experimental migraine animal models and received treatment for seven consecutive days. Subsequently, behavioral evaluations and heat pain threshold assessments were conducted. Enzyme-linked immunosorbent assay (ELISA) was employed to measure the levels of Brain-derived neurotrophic factor (BDNF) and calcitonin gene-related peptide (CGRP) in rat serum. Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) was performed to detect the mRNA expression levels of *CGRP* and cAMP response element-binding protein (*CREB*). Western blot analysis was utilized to assess the protein expression levels of Tyrosine receptor kinase B (TrkB) and Cyclooxygenase-2 (COX-2).

Results: Behavioral assessment, measurement of thermal pain threshold, and mechanical pain thresholds indicated that, in comparison to the Model group, the emodin treatment group exhibited a significant improvement in abnormal behavior in migraine rats ($p < 0.05, p < 0.01, p < 0.001, p < 0.0001$). Moreover, there was an increase in thermal pain threshold and mechanical pain thresholds in the emodin treatment group ($p < 0.05, p < 0.01, p < 0.0001$). ELISA experiments revealed that, when compared to the Model group, the emodin-high-dose (emodin-H) treatment group exhibited reduced serum levels of BDNF and CGRP ($p < 0.01$). Additionally, RT-qPCR and Western blot (WB) experiments demonstrated the downregulation of *CGRP* ($p < 0.001$) and *CREB* ($p < 0.05$) mRNA expression levels. Furthermore, there were decreased expression levels of TrkB and COX-2 proteins in the rat brainstem ($p < 0.05, p < 0.01$).

Conclusion: This study confirms that emodin can markedly enhance abnormal behavioral activities and elevate the thermal pain threshold in the migraine rat model. Its effects appear to be mediated by the downregulation of upstream COX-2 and CGRP, along with the inhibition of the CREB/BDNF/TrkB pain signaling pathway.

Keywords: migraine; emodin; COX-2; CGRP; CREB; BDNF; TrkB

Introduction

Migraine stands as the most prevalent form of primary headache. Studies underscore its significance, ranking migraines as the sixth leading cause of disability-adjusted life years (DALYs) globally among all diseases and injuries [1,2]. Epidemiological data reveal a global migraine prevalence of approximately 15%, with China reporting a prevalence of around 9% [3]. Characterized by moderate to severe throbbing headache attacks lasting from minutes to days, migraines often come with accompanying symptoms such as nausea and vomiting, contributing to their high incidence and recurrence rates [4].

The impact of migraines on human health is profound, affecting normal work and life, diminishing the quality of life, and imposing a significant mental and economic burden on patients, families, and society at large. Despite extensive research, the causes and mechanisms of migraines remain subjects of debate. Current understanding suggests

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that the etiology may be linked to genetic factors, environmental influences, metabolism, hormones, and drug-related factors [5,6]. Meanwhile, various theories, including vascular theory [7], neuronal theory [8], and inflammatory mediator theory [9], contribute to the understanding of migraine pathogenesis. Clinical prevention and treatment of migraines face challenges due to the unclear etiology and pathogenesis. Mainstream western medicine relies on analgesics for migraine treatment, yet their toxic side effects and contraindications limit their suitability for many patients. Thus, the pressing research question revolves around identifying a safer and more effective method among the multitude of treatment options that can target the underlying etiology of migraines.

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is an ancient natural hydroxyanthraquinone sourced from the roots and rhizomes of various medicinal plants like *Platycodon grandiflorum*, *Rheum officinale*, *Lilium brownii*, and *Polygonaceae* [10,11]. Notably, emodin demonstrates protective effects on the central nervous system [12]. In recent years, researchers have conducted extensive studies elucidating the pharmacological mechanisms of emodin. It shows potential activity against cardiovascular diseases and various neurological disorders, in addition to possessing anti-inflammatory and smooth muscle contractile pharmacological effects [13–15]. Moreover, Xiong *et al*. [16] observed that emodin could reduce the release of calcitonin gene-related peptide (CGRP) in the trigeminal ganglion, thereby inhibiting orofacial pain. Another study by Sun *et al*. [17] discovered that emodin has the capability to alleviate nitroglycerin-induced migraines through the cyclic guanosine monophosphate-protein kinase G-dependent (cGMP-PKG) pathway.

The periaqueductal gray (PAG) is a pivotal structure implicated in the transmission and modulation of pain, sympathetic responses, as well as the learning and execution of defensive and aversive behaviors. Functionally, the PAG plays a substantial role in the descending regulation of pain perception and may act as an initiator of migraine attacks [18]. Research underscores the significance of the PAG in central endogenous pain modulation, housing a diverse array of inflammatory factors, neuropeptides, neurotransmitters, estrogen, and related receptors closely linked to migraine attacks. Additionally, it serves as a critical component of the descending inhibitory system, exerting inhibitory or excitatory control over the transmission of nociceptive sensations [19,20].

Moreover, the PAG serves as a vital neural center for autonomic regulation [21]. Within the PAG region, the dorsolateral area receives extensive input from other brain regions and sends descending neuronal projections to the medulla to regulate autonomic activity. Activation of the PAG contributes to the integration of sympathetic nerve activity, controlling vasoconstriction to regulate arterial blood pressure [22]. Accumulating evidence from human studies suggests a crucial role for the PAG in regulating migraines and medication-overuse headaches [23].

Furthermore, both domestic and foreign studies have reported that Cyclooxygenase-2 (COX-2) and calcitonin gene-related peptide (CGRP) are key proteins involved in migraine analgesia [24–26]. They mediate the expression of the cAMP response element-binding protein/Brain-derived neurotrophic factor/Tyrosine receptor kinase B (CREB/BDNF/TrkB) pain signaling pathway, thereby influencing migraine regulation. The severity of migraines is positively correlated with the serum levels of BDNF and CGRP in patients. Additionally, studies indicate a significant increase in COX-2-positive nerve cells in the PAG area during migraine attacks [27]. These clinical indicators hold substantial diagnostic and therapeutic value for evaluating the condition and efficacy of patients with neuropathic pain.

Hence, we hypothesize that the mechanism underlying the efficacy of rhein in treating migraines may entail the modulation of vasoactive peptides and neurogenic inflammation. This modulation, in turn, regulates the CREB/BDNF/TrkB pain signaling pathway, ultimately leading to analgesic effects in migraine model rats. Consequently, this experiment seeks to investigate the analgesic effects of rhein on migraine model rats from various angles, examining both tissue and molecular levels. The findings aim to establish a theoretical foundation for the effective treatment of migraines.

Materials and Methods

Experimental Materials

Experimental Animals

The experimental animals, comprising 66 healthy male rats of SPF grade, were procured from Liaoning Changsheng Biotechnology Co., Ltd. (Shenyang, Liaoning, China), with individual weights falling within the range of 160–210 g. The purchase license number is SCXK (Liao) 2020-0001. Throughout the experiment, the animals were housed in the Central Laboratory Animal Room at our university, situated on the 5th floor of the library building in the Jiaxiu Campus of Guizhou University of Traditional Chinese Medicine. The animal facility maintained an SPF-level environment with a temperature set at 23 ± 2 °C, and humidity ranged between 40% and 70%. The light cycle followed a 12-hour pattern of light and darkness, with each cage accommodating 3–4 rats. Daily, the bedding was replaced, and the provided food and water underwent sterilization.

Experimental Drugs

Emodin (E8390, Specification: 1 g, Beijing Solaibao Technology Co., Ltd., Beijing, China), Nitroglycerin injection (Henan Runhong Pharmaceutical Co., Ltd., Zhengzhou, Henan, China, Specification: 1 mL:5 mg,

Batch Number: 2009142), and Rizatriptan Monobenzoate Tablets (Olitrans) (Hubei Ouli Pharmaceutical Co., Ltd., Wuhan, Hubei, China, Specification: 5 mg (as pizotifen), Batch Number: 201204).

Animal Grouping and Drug Pretreatment

After 7 days of adaptive feeding, the rats were randomly allocated into six groups: (1) normal Control group (Control); (2) model control group (Model); (3) positive drug control group (Rizatriptan Monobenzoate Tablets (RMT)); (4) low-dose emodin group (emodin-L); (5) medium-dose emodin group (emodin-M); (6) high-dose emodin group (emodin-H). The grouping, modeling, and gavage timings adhered to the protocol outlined by Liang Wenlin *et al*. [28].

Methods

Model Preparation and Grouping

Sixty-six male rats were randomly allocated into six groups: control, model, positive control (Rizatriptan Monobenzoate Tablets (RMT), 0.001 g/kg), and three emodin treatment groups (low, medium, and high doses of 20, 40, and 60 mg/kg, respectively). All groups, excluding the Control, received treatment for seven consecutive days, followed by a subcutaneous injection of niter acid gansu oil (NTG) (10 mg/kg) in the neck 30 minutes after the last treatment.

The Control group received a subcutaneous injection of normal saline (NS). The experimental model for migraine induction in rats was established in all groups, except the Control group, following the method outlined by Tassorelli [29]. This involved subcutaneously injecting nitroglycerin at a dose of 10 mg/kg to induce the experimental migraine animal model.

Behavioral Observation

After the modeling process, each rat was placed in a cardboard box and divided into the following five-time intervals: 0–30 min, 30–60 min, 60–90 min, 90–120 min, and 120–150 min, with each interval lasting for 30 minutes. The observed behaviors included head scratching, crawling in the cage, and running back and forth. The number of scratching movements was recorded by counting the instances of rats scratching their heads with their forelimbs since modeling, with each 30 minutes as an observation window, and the count of scratching movements continuously recorded in segments [30].

The behavior of rats crawling on the cage cover with their forelimbs was observed, and the number of times was recorded in segments [31]. Running back and forth in the box in different directions was considered a back-and-forth movement, and the number of such movements was continuously recorded in segments. All animal experiments in this study adhered to the requirements and regulations outlined in the Guiding Opinions on the Treatment of Experimental Animals issued by the Ministry of Science and Technology in 2006.

Thermal Pain Threshold and Mechanical Pain Thresholds Determination

Following the modeling process, the rat's thermal withdrawal latency and mechanical pain thresholds were measured every 1 hour using a plantar thermal pain meter. Three repeated measurements were taken every 2 minutes, and the average value was recorded as the rat's thermal pain threshold [32]. This measurement was conducted for a duration of 3 hours. The plantar thermal pain meter operates on the principle that, upon initiation, the machine elevates the temperature to induce pain in the rat's hind foot center through high-transmittance glass. When the rat perceives the pain, it swiftly lifts its hind leg. At this point, the fiber optic sensor positioned at the center of the high-transmittance glass accurately detects the time interval from the initiation of irradiation to the lifting of the leg (i.e., pain threshold latency). This experiment effectively gauges the impact of analgesic drugs on the rat's reaction time to external thermal stimulation in an unrestrained state.

Tissue Collection and Sample Storage

The rats underwent a 12-hour fasting period before tissue collection. Anesthesia and euthanasia procedures involved intraperitoneal injection of 3% pentobarbital sodium (45 mg/kg) for anesthesia, followed by euthanasia. Blood samples were collected from the abdominal aorta, centrifuged at 3000 rpm and 4 °C for 10 minutes under sterile conditions, and the serum was carefully extracted using a pipette. The collected serum was stored in a –80 °C freezer for subsequent use.

From each group, three rats were randomly chosen for further analysis. Following blood collection, the midbrain tissue was extracted, immersed in a dehydration solution, and embedded in a 4% paraformaldehyde solution. The remaining 48 rats underwent immediate brain removal after decapitation. The midbrain was swiftly dissected on an ice bag and evenly divided into two parts, each placed in sterile centrifuge tubes containing 2 mL of liquid nitrogen. These tubes were labeled with group numbers and stored in a –80 °C freezer for subsequent use in Western blot and Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR).

ELISA Testing

The levels of BDNF and CGRP in the serum were assessed through enzyme-linked immunosorbent assay (ELISA) following the guidelines provided by the BDNF ELISA kit (E-EL-R1235, Elabscience, Wuhan, China) and the CGRP ELISA kit (E-EL-R0135, Elabscience, Wuhan, China). In summary, the sample was appropriately diluted and added to an enzyme-labeled plate along with the corre-

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Table 1. The specific information of the primary antibody and secondary antibody in Western blot experiment.

Antibody		Source Information
Primary antibody	β-actin (Mouse MAB)	T0022, Affinity Biosciences, Jiangsu, China
	TrkB (Rabbit PAB)	R25991, Zenbio, Chengdu, China
	COX-2 (Rabbit MAB)	R23969, Zenbio, Chengdu, China
Secondary antibody	Goat Anti-Rabbit IgG H&L (HRP)	A0208, Beyotime Biotechnology, Shanghai, China
	Goat Anti-Mouse IgG H&L (HRP)	SA00001-1, Proteintech, Wuhan, China

Note: MAB, Monoclonal Antibody; PAB, Polyclonal Antibody; TrkB, Tyrosine receptor kinase B; COX-2, Cyclooxygenase-2.

sponding antibody. The plate underwent five washes with a washing solution and subsequent incubation with a coloring solution and a stop solution. Finally, the absorbance at 450 nm was measured using a microplate reader.

Western Blot

The Western blot procedure involved lysing the midbrain tissue in RIPA buffer (P0013B, Beyotime, Shanghai, China) to extract total protein. Subsequently, the proteins were separated on an SDS-PAGE gel (JY300, Beijing Junyi Oriental electrophoresis Equipment Co., Ltd., Beijing, China) and transferred onto a PVDF membrane (IPVH00010, Millipore, Bedford, MA, USA). The membrane was then incubated with a primary antibody (concentration: 1:1000) and blocked with 5% skim milk at 4 °C overnight. Following five wash cycles, an appropriate secondary antibody (concentration: 1:600) was applied, and the cells were incubated at 37 °C for 2 hours. Finally, the sample was characterized through an ECL system (P1050, Applygen Technologies Inc., Beijing, China) and quantified using imaging (BX53, Olympus, Tokyo, Japan). Specific information regarding the primary and secondary antibodies is provided in the Table 1.

Reverse Transcription-Quantitative Polymerase Chain Reaction

The total RNA was extracted from the midbrain tissue using Trizol reagent (15596-026, Ambion, Austin, TX, USA). Subsequently, the cDNA of mRNA was synthesized, and a RT-qPCR was conducted on the cDNA-SYBR Green mixture utilizing a real-time fluorescence quantitative PCR system (QuantStudio6, Applied Biosystems, Foster City, CA, USA). The obtained results were analyzed using the $2^{-\Delta\Delta Ct}$ method, with *β-actin* serving as an internal reference for mRNA normalization in each sample. The specific primers employed for RT-qPCR are detailed in Table 2.

Statistical Analysis

Data analysis was performed using GraphPad Prism 8.0 statistical software (GraphPad Software, San Diego, CA, USA). The results are presented as mean ± standard error of the mean (Mean ± SEM). One-way analysis of variance (ANOVA) was employed, followed by Tukey's test for post hoc analysis. The significance level was set at $p < 0.05$ to indicate statistically significant differences.

Table 2. Primer sequence.

Name	Primer	Sequence
CGRP	Forward	5′-TGGTTGTCAGCATCTTGCTC-3′
	Reverse	3′-GCTCCCTGACTTTCATCTGC-5′
CREB	Forward	5′-AACATACCAGATTCGCACAGC-3′
	Reverse	3′-ACGACATTCTCTTGCTGCTTC-5′
β-actin	Forward	5′-AGATGACCCAGATCATGTTTGA-3′
	Reverse	3′-ATGAGGGAGCGCGTAACC-5′

Note: *CGRP*, calcitonin gene-related peptide; *CREB*, cAMP response element-binding protein.

Results

Emodin Improves Abnormal Behavior

The results analysis is depicted in Fig. 1A–C. In the migraine Model group, rats exhibited an increase in the instances of head-scratching, significant cage activity, and heightened back-and-forth movement within 30–150 minutes after nitroglycerin induction. These behaviors were significantly higher than those observed in the Control group at various time points ($p < 0.01$, $p < 0.0001$). Both the RMT and emodin-H groups demonstrated a significant improvement in abnormal behaviors such as head-scratching, cage activity, and back-and-forth movement in migraine rats, with statistically significant differences noted ($p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$).

Emodin Increases Thermal Pain Threshold and Mechanical Pain Thresholds

As per Fig. 2A, compared to the Control group, the pain thresholds of the Model group significantly increased after 1 hour, 2 hours, and 3 hours of modeling ($p < 0.01$, $p < 0.001$). The pain thresholds of rats in the RMT, emodin-M, and H groups increased significantly after 1 hour, 2 hours, and 3 hours of modeling ($p < 0.05$, $p < 0.01$, $p < 0.0001$). There was no significant difference in pain threshold between the emodin-L group and the Model group after 1 hour to 3 hours of modeling ($p > 0.05$).

In accordance with Fig. 2B, in comparison to the Control group, the mechanical pain threshold of rats in the Model group significantly decreased at 1 hour, 2 hours, and 3 hours ($p < 0.0001$). Relative to the Model group,

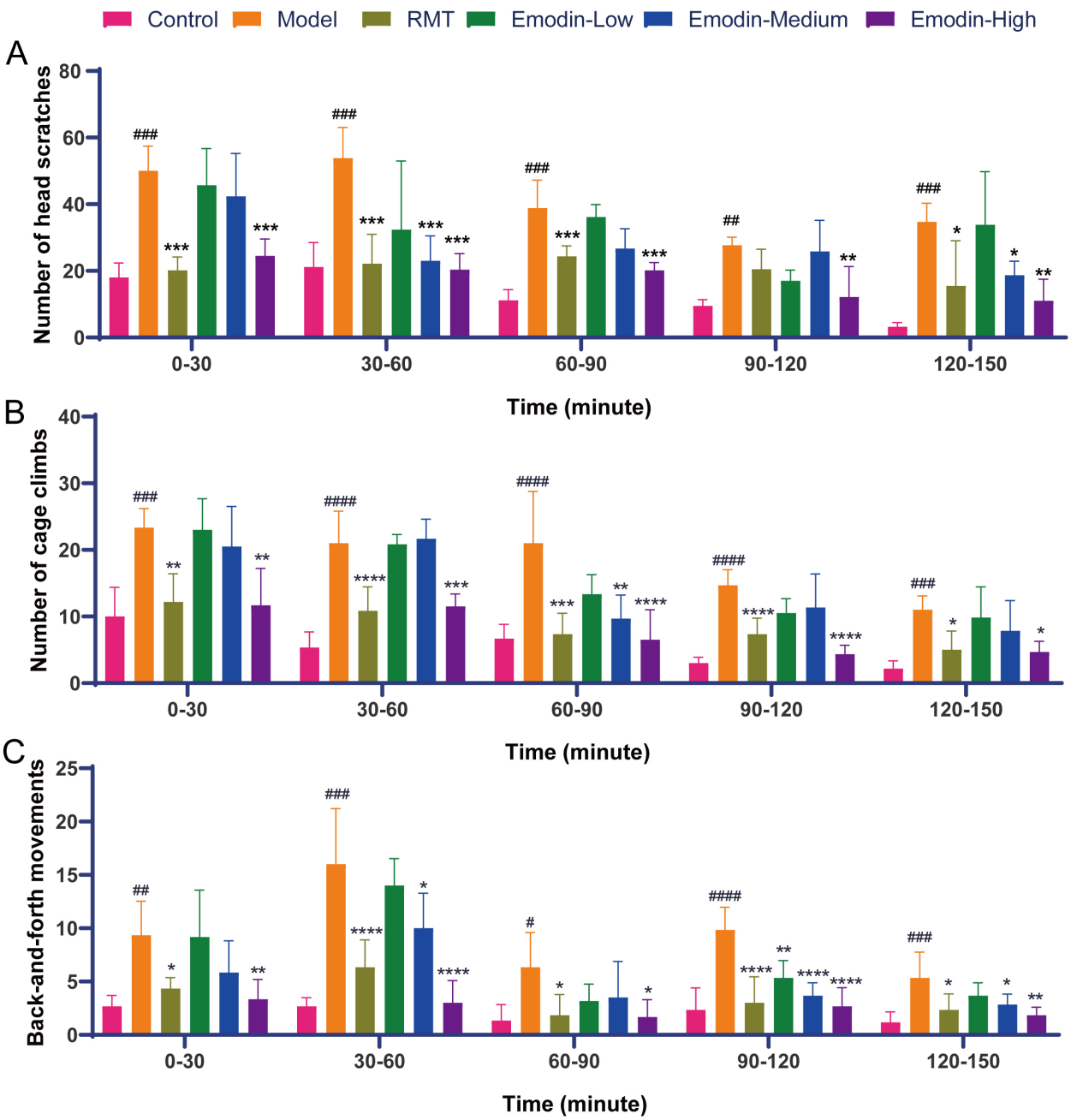

Fig. 1. Emodin improves abnormal behavior. (A) Comparison of the number of head-scratching in rats at different time points (x ± s, times/30 minutes). (B) Comparison of the number of cage activity times in rats at different time periods (x ± s, times/30 minutes). (C) Comparison of the number of back-and-forth movements in rats at different time points (x ± s, times/30 minutes). Compared with the Control group $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$. Compared with the Model group $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

the RMT, emodin-L, and emodin-H groups exhibited a significant increase in mechanical pain threshold at 1 hour, 2 hours, and 3 hours ($p < 0.01$, $p < 0.0001$).

Emodin Reduces Serum Levels of BDNF and CGRP in Migraine Rats

As depicted in Fig. 3A, following the subcutaneous injection of nitroglycerin to induce the migraine model for 4 hours, the BDNF level in the serum of the Model group rats was significantly higher than that in the Control group rats, showing a significant statistical difference ($p < 0.05$). The BDNF expression levels in the serum of RMT and emodin-H group rats were significantly lower than those in the Model group rats, exhibiting a significant statistical difference ($p < 0.05$). However, the BDNF expression levels in the serum of emodin-L and M group rats showed no significant statistical difference compared to the Model group rats ($p > 0.05$).

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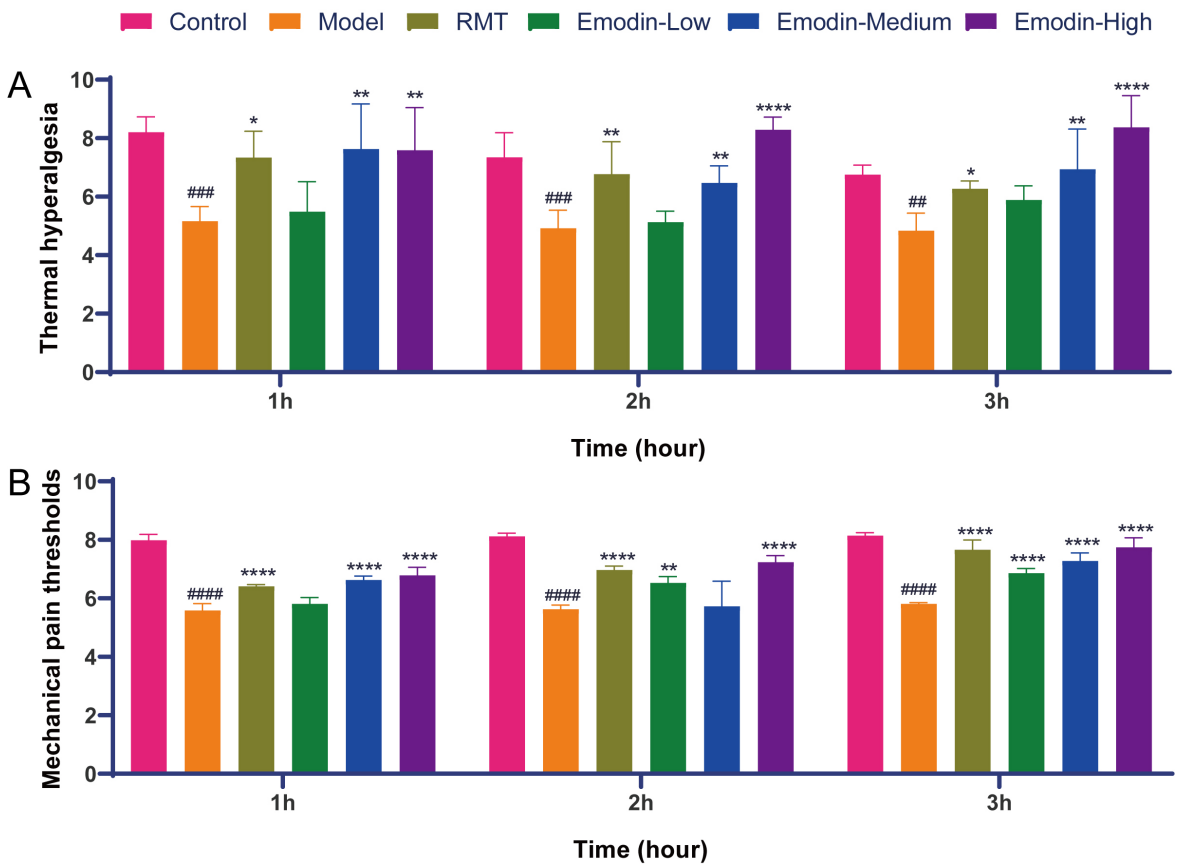

Fig. 2. Emodin increases thermal pain threshold and mechanical pain thresholds. (A) Comparison of thermal pain threshold in rats of each group at different time points after modeling. (B) Comparison of mechanical pain thresholds in rats of each group at different time points after modeling. Compared with the Control group $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$. Compared with the Model group $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$.

Furthermore, as shown in Fig. 3B, following the subcutaneous injection of nitroglycerin to induce the migraine model for 4 hours, the CGRP expression level in the serum of the Model group rats was significantly higher than that in the Control group rats, demonstrating a significant statistical difference ($p < 0.01$). The CGRP expression levels in the serum of RMT, emodin-L, M, and H group rats were significantly lower than those in the Model group rats, with a significant statistical difference ($p < 0.05$).

Emodin Downregulates mRNA Expression Levels of CGRP and CREB in the Brainstem Tissue of Migraine Rats

The quantitative analysis results are presented in Fig. 3C,D. In Fig. 3C, compared with the Control group, *CGRP* mRNA expression in the brainstem of rats in the Model group was significantly up-regulated, and the difference was statistically highly significant ($p < 0.0001$). The mRNA expression levels of *CGRP* were significantly down-regulated in the RMT, emodin-L, M, and H groups, with the differences being statistically significant ($p < 0.05$, $p < 0.01$, $p < 0.001$).

As illustrated in Fig. 3D, compared with the Model group, the mRNA expression of *CREB* in the brainstem tissue of the Control group was significantly downregulated, and the difference was statistically significant ($p < 0.01$). The mRNA expression levels of *CREB* were significantly down-regulated in the RMT, emodin-L, M, and H groups, with the differences being statistically significant ($p < 0.05$, $p < 0.01$).

Emodin Reduces Protein Expression Levels of TrkB and COX-2 in the Brainstem Tissue of Migraine Rats

As demonstrated in Fig. 4, the TrkB protein expression in the brainstem of Model group rats was significantly higher than that in the Control group, and the difference was statistically significant ($p < 0.01$). The TrkB protein expression in the brainstem of RMT and emodin-H group rats was significantly lower than that in the Model group, and the difference was statistically significant ($p < 0.05$). However, there was no significant difference in the TrkB protein expression in the brainstem of emodin-L and M group rats compared to the Model group ($p > 0.05$). Additionally, the COX-2 protein expression in the brainstem of Model group rats was significantly higher than that in the Control group,

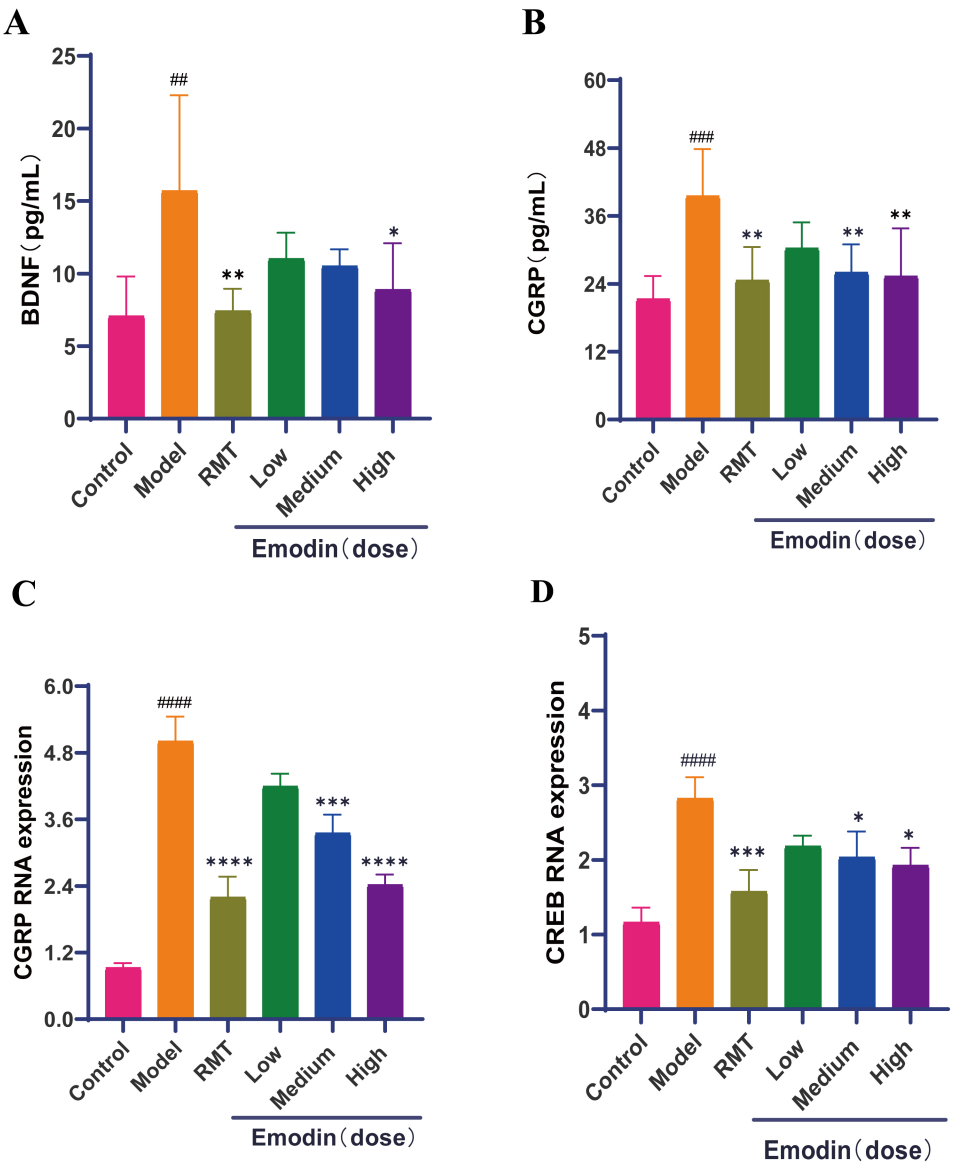

Fig. 3. Enzyme-linked immunosorbent assay (ELISA) and Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) assay. (A,B) ELISA assay was used to determine serum levels of Brain-derived neurotrophic factor (BDNF) and calcitonin gene-related peptide (CGRP). (C,D) RT-qPCR measurement of CREB and CGRP gene expression in brainstem tissue. Compared with the Control group $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$. Compared with the Model group $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

and the difference was statistically significant ($p < 0.01$). The COX-2 protein expression in the brainstem of RMT and emodin-H group rats was significantly lower than that in the Model group, and the difference was statistically significant ($p < 0.05$, $p < 0.01$). Again, there was no significant difference in the COX-2 protein expression in the brainstem of emodin-L and M group rats compared to the Model group ($p > 0.05$).

Discussion

Migraine is a prevalent and disabling neurological disorder characterized by moderate to severe headache, nausea, vomiting, and heightened sensitivity to sensory stimuli [33]. Despite its prevalence, the initial activation site of the migraine process remains unclear. Western medicine primarily employs anti-inflammatory and analgesic drugs for migraine treatment, with sumatriptan tablets being commonly used in clinical practice. Sumatriptan functions by inhibiting CGRP release through action on the 5-HT (1D) receptor in the perivascular trigeminal nerve, thereby blocking neurogenic vasodilation and alleviating headaches [34]. Although clinically effective, triptan drugs are associated with numerous adverse reactions, contraindications, and a certain risk of increasing cardiovascular and cerebrovascular events.

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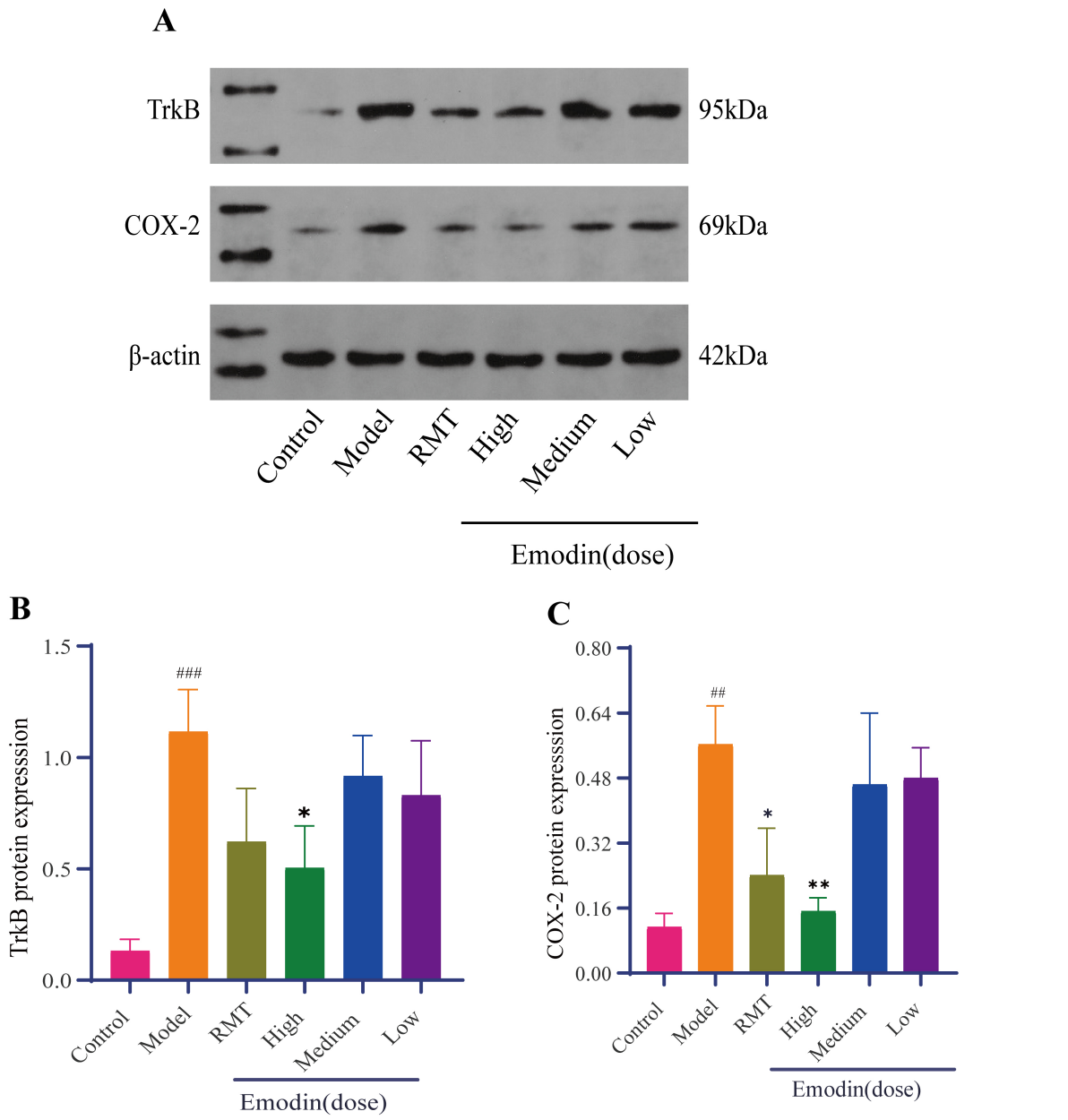

Fig. 4. Western blot for determination of TrkB and COX-2 protein levels in rat brainstem tissues. (A) Western blot analysis of TrkB and COX-2 protein levels shown in the banding pattern. (B) TrkB protein levels in each group. (C) COX-2 protein levels in each group. Compared with the Control group $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. Compared with the Model group $^{*}p < 0.05$, $^{**}p < 0.01$.

In China, traditional Chinese herbal medicine is frequently prescribed for migraine treatment. Numerous pharmacological studies have highlighted the potential of the traditional Chinese medicine monomer emodin in treating various neurological disorders. Emodin has been shown to inhibit the aggregation of amyloid-β peptide 1–42 and improve cognitive deficits in Alzheimer's disease transgenic mice [35,36]. The behavioral observations in this study revealed that the emodin-H group significantly ameliorated abnormal behavioral activities such as head scratching, cage climbing, and back-and-forth movement in migraine model rats. Additionally, all doses of emodin significantly increased the thermal pain threshold in the migraine model rats. These findings suggest that treatment with the Chinese herbal monomer emodin can enhance abnormal behavioral activities in migraine model rats.

Moreover, research indicates that emodin serves as a neuroprotective agent in an ischemic stroke rat model by activating the extracellular signal-regulated kinase-1/2 (ERK1/2) signaling pathway [37]. Studies have also demonstrated emodin's impact on neuropathic pain in rodent models. For instance, Wang *et al.* [38] observed that emodin alleviates neuropathic pain induced by chronic constriction injury (CCI) in rats. Early pharmacological inves-

tigations have suggested that emodin can inhibit pain signal transmission mediated by the P2X (2/3) receptor [39]. Additionally, emodin inhibits the release of calcitonin gene-related peptide in the trigeminal ganglia of rats with trigeminal neuralgia [16].

In the current experimental study, it was observed that the emodin-H group significantly downregulated the serum BDNF levels in migraine model rats and significantly decreased the expression of TrkB protein in the midbrain tissue of migraine model rats. Emodin-L, M, and H significantly downregulated the expression levels of mRNA CREB in the midbrain tissue of migraine model rats. Furthermore, emodin alleviated inflammation infiltration in midbrain tissue cells of migraine model rats and reduced the degree of cellular fibrosis. These findings suggest that emodin treatment exhibits effectiveness in mitigating migraine symptoms.

An additional study demonstrated that during migraine attacks, there was a significant increase in the number of COX-2 positive nerve cells in the PAG region [27]. COX-2 serves as a crucial peripheral mediator of inflammation and pain. Local COX activity in the dura mater can mediate peripheral sensitization [40]. The use of extracellular signal-regulated kinase (ERK) inhibitors has been shown to alleviate pathological pain and improve pain symptoms by reducing the expression of inflammatory factors such as COX-2 [36]. The aforementioned studies indicated a positive correlation between COX-2 expression and pain severity. The regulation of peripheral and central COX-2 expression can potentially alleviate migraine pain sensitization.

As per the results of this experimental study, compared with the Control group, the COX-2 protein expression in the brainstem of Model group rats was significantly increased. This suggests that COX-2 protein in the midbrain of migraine model rats is involved in the pathogenesis of migraine, and its increase is positively correlated with headache, aligning with previous research findings. Moreover, the COX-2 protein expression in the brainstem of RMT and emodin-H group rats was significantly lower than that of the Model group. This suggests that emodin may reduce central pain sensitization in the migraine rat model by decreasing the expression of COX-2 protein in midbrain tissue, thereby improving abnormal behavioral activities and increasing the thermal pain threshold of the migraine model rats.

CGRP is a crucial pain signaling factor in the pathogenesis of migraine, and targeting CGRP represents a novel therapeutic approach. CGRP, a 37-amino acid neuropeptide derived from the gene encoding calcitonin, was identified approximately 30 years ago [41]. CGRP is expressed in central and peripheral sites related to migraine [25,26]. In this experimental study, the expression levels of CGRP in the serum and midbrain mRNA of Model group rats were significantly higher than those in the Control group, with the highest content observed among all groups. This indicates that CGRP is involved in the pathogenesis of the migraine rat model and is positively correlated with the degree of headache.

The levels of CGRP expression in the serum and midbrain mRNA of the RMT, Hesperetin-L, M, and H group rats were significantly lower than those in the Model group. This suggests that Hesperetin may improve abnormal behavioral activities in the migraine rat model by downregulating the expression levels of CGRP in the serum and midbrain mRNA CGRP levels and increasing the thermal pain threshold.

However, it's important to note that a potential limitation of this study is that it was conducted in animal models and may not necessarily reflect the same effects in humans. Furthermore, although the study explored the pharmacological effects and analgesic mechanism of emodin in treating nitroglycerin-induced migraine, it did not investigate the potential side effects or long-term outcomes associated with the use of emodin as a treatment.

Conclusion

This study successfully demonstrated that emodin can significantly ameliorate abnormal behavioral activities, such as head scratching, cage climbing, and back-and-forth movement, in migraine model rats. Moreover, emodin was found to increase the thermal pain threshold of the migraine model rats. The mechanism of action appears to be associated with the downregulation of upstream COX-2 and CGRP expression, inhibition of the CREB/BDNF/TrkB pain signaling pathway, and relief of neurogenic inflammation. These findings provide support for the efficacy of emodin in the treatment of migraines.

Availability of Data and Materials

The data used to support the findings of this study are included in the article.

Author Contributions

SSW and YH conceived, designed, performed the experiments, and wrote the original draft. YLL, JC, QSH, and RH analyzed and interpreted the data, contributed reagents, materials, analysis tools or data. YHW and PC designed research, formal analysis, writing review & editing, and supervision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted strictly in accordance with the guidelines established by the Ethics Committee for An-

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imal Experiments. All animal experimental procedures were performed in strict adherence to the guiding principles of the Ethics Committee for Animal Experiments and approved by the Ethics Committee for Animal Experiments Welfare of Guizhou University of Traditional Chinese Medicine (No.20210137).

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Conflict of Interest

The authors declare no conflict of interest.

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