

The Mechanism of Emodin Negatively Regulating HMGB1 via the SIRT1 Pathway to Alleviate Pain Hypersensitivity in Rats With Neuropathic Pain

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Background: Neuropathic pain (NP) is chronic pain caused by damage to or disease of the somatosensory nervous system. Emodin is known to protect nerves from injury and inflammation, but its underlying mechanisms remain unclear. This study aims to investigate whether emodin negatively regulates the effect of high mobility group box 1 (HMGB1) on hyperalgesia in NP via the sirtuin 1 (SIRT1) pathway.

Methods: We established *in vivo* and *in vitro* models of NP to investigate emodin's specific mechanisms of action. A chronic constriction injury (CCI) model was created in adult rats. Paw withdrawal mechanical threshold and paw withdrawal thermal latency were used to assess hyperalgesia in the rats. Next, hematoxylin and eosin (H&E) staining was performed on the sciatic nerve and immunohistochemistry was used to assess changes in glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba-1) levels in spinal cord tissues. Then, enzyme-linked immunosorbent assay (ELISA) kits were used to measure the concentrations of tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6) in the serum of CCI rats. Western blotting and co-immunoprecipitation were used to examine the interaction between SIRT1 and HMGB1, as well as the expression of HMGB1-related pathways. Cell Counting Kit-8 (CCK-8) assay was used to evaluate the cytotoxicity of emodin at different concentrations on mouse microglial cell line (BV2) cells. RT-qPCR was used to analyze the transfection efficiency of si-SIRT1. A neuroinflammatory cell model induced by lipopolysaccharide (LPS) was established for the BV2. Transwell assay was used to evaluate cell migration. Western blotting and immunofluorescence were used to detect the expression of microglial markers and of pathways related to SIRT1 and HMGB1.

Results: Emodin reduced CCI-induced hyperalgesia and inflammation, upregulated SIRT1 expression, and inhibited the expression of HMGB1/Toll like receptor 4 (TLR4)/nuclear factor kappa-B (NF- κ B). SIRT1 inhibitor abolished the ameliorative effect of emodin on CCI-induced spinal cord inflammation. In the LPS-induced cell model, emodin reversed LPS-induced migration, activation, and inflammation of BV2 cells. Furthermore, in LPS-treated BV2 microglial cells, emodin inactivated the HMGB1/TLR4/NF- κ B pathway. Emodin also significantly inhibited the translocation of HMGB1 from the nucleus to the cytoplasm by upregulating SIRT1. Knockdown of SIRT1 abolished the protective effects of emodin against LPS-induced BV2 microglial cell migration, activation, and inflammation.

Conclusion: Emodin negatively regulates HMGB1 via the SIRT1 pathway to alleviate hyperalgesia in neuropathic pain both *in vivo* and *in vitro*. This research provides a new direction for clinical pharmacological treatment of NP and offers new insights into the mechanisms underlying NP.

Keywords: neuropathic pain; emodin; SIRT1; HMGB1

Introduction

Neuropathic pain (NP) refers to a pain syndrome caused by lesions of the central or peripheral nervous system, with common causes including physical/mechanical injury, metabolic or nutritional disorders, viral infection, and neurotransmitter dysfunction [1,2]. It is mainly characterized by spontaneous pain, hyperalgesia, and allody-

nia, and is a common type of chronic pain [3] that affects 7–10% of the general population [4]. NP is characterized by chronicity, poor treatment response, high medical costs, and a tendency to cause psychiatric symptoms such as depression and anxiety; it severely impairs patients' quality of life and imposes a substantial economic burden on individuals and society. Serotonin noradrenaline reuptake inhibitors, gabapentinoids and tricyclic antidepressants are

recommended as the preferred treatment options for NP [5]. However, existing drugs not only have serious side effects but also provide poor analgesic efficacy [6,7]. The limitations of these medications make treating NP a major clinical challenge. Therefore, there is an urgent need to develop more effective analgesics.

Sirtuin 1 (SIRT1), a protein deacetylase that depends on NAD^+ in humans, facilitates the extraction of acetyl groups from the lysine residues present in its substrate proteins. SIRT1 is highly conserved and predominantly nuclear; it can regulate target gene expression by altering histone acetylation levels and can deacetylate non-histone targets, thereby broadening the range of its target proteins [8]. Because of these functions, SIRT1 can participate in regulating various processes, including DNA damage repair, apoptosis, cell proliferation, metabolism, and inflammatory responses [9–11]. Moreover, in the spinal cord tissues of various pathological pain models, it has been observed that the expression level of SIRT1 was decreased [12,13]. A previous study has also shown that during exacerbations of multiple sclerosis (MS), SIRT1 levels in peripheral blood are reduced, and overexpression of SIRT1 has neuroprotective effects [14]. Therefore, SIRT1 may be a key target in neurological diseases and play an important role in their treatment. Although studies have elucidated the molecular mechanisms of SIRT1 and emodin in neuroprotection, it is still unclear whether emodin regulates neuropathic pain through SIRT1. High-mobility group box 1 (HMGB1), as an endogenous non-histone DNA-binding protein composed of 215 amino acid residues [15], maintains various cellular physiological processes by regulating chromatin structure and function [16]. HMGB1 is widely expressed in eukaryotic cells and mainly influences processes such as cell differentiation, migration, neurite outgrowth, wound healing, inflammation, bacterial killing, and tumor progression [17]. Toll like receptor 4 (TLR4), an important cell-surface receptor for HMGB1, is widely expressed on the membranes of microglia and astrocytes, and its activation can further trigger the nuclear factor kappa-B ($\text{NF-}\kappa\text{B}$) signaling pathway [18]. In research related to neuroinflammation, some scholars have discovered that upregulating SIRT1 or blocking HMGB1 can inhibit the TLR4/ $\text{NF-}\kappa\text{B}$ signaling pathway and exert neuroprotective effects [19]. Other reports have shown that SIRT1 can suppress HMGB1 expression in bone cancer [20]. However, in NP, whether SIRT1 regulates HMGB1-related signaling pathways still needs further investigation.

Emodin, a natural derivative of anthraquinone known as 1,3,8-trihydroxy-6-methylanthraquinone, can be found in *Rheum palmatum* as well as in various other plant species [21]. Emodin has been shown to possess multiple pharmacological properties, including anti-inflammatory, antitumor, neuroprotective, and immunosuppressive effects [21,22]. Furthermore, it has been found that in a rat chronic constriction injury (CCI) model, administration of emodin

significantly increased mechanical and thermal withdrawal thresholds [23], indicating that emodin has analgesic effects. Emodin also plays a significant role in modulating pain behaviors in different inflammatory models [24]. Emerging research indicates that emodin also suppresses microglial pyroptosis, activation, and inflammation, suggesting its neuroprotective effects [25–27]. Emodin alleviates NP by inhibiting the P2X4 purinergic receptor signaling pathway in CCI rats [28]. However, it remains unclear whether emodin alleviates hyperalgesia in NP by regulating HMGB1-related signaling pathways via SIRT1.

In the current research, we examined how emodin affects NP using both *in vivo* and *in vitro* methods, while also investigating its possible mechanisms. We tested the hypothesis that emodin alleviates hyperalgesia in NP by negatively regulating HMGB1 and related pathways via the SIRT1 pathway. This study may provide a new direction for clinical drug treatment of NP and offer new insights into the mechanisms of NP.

Materials and Methods

Animals

Fifty specific-pathogen-free (SPF) male Sprague–Dawley rats (6–7 weeks old, 150–170 g) were provided by the Sino-British SIPPR/BK Lab (Shanghai, China). Environmental conditions were controlled at $25 \pm 0.5^\circ\text{C}$, $55 \pm 5\%$ humidity, and a 12/12 h light/dark cycle, with *ad libitum* access to food and water. All experiments related to animals were conducted strictly in accordance with the ethical standards set by the National Institutes of Health (NIH) of the United States. All experimental protocols of this study were approved by the Ethics Committee of Zhejiang Chinese Medical University (Approval Number: SYXK 2021-0012 (Zhejiang)) ethics committee.

Chronic Constriction Injury (CCI)

After 7 days of acclimation, rats were anesthetized with an intraperitoneal injection of 1% pentobarbital sodium at 40 mg/kg body weight, and the CCI model was then established [29]. The left hind limb was shaved and disinfected with alcohol. The skin was lifted with forceps, and a vertical incision was made with scissors along the course of the sciatic nerve. After cutting the skin, the fascia was separated with forceps, and the muscle layer was bluntly dissected with cotton swabs to expose the sciatic nerve. A glass probe was used to isolate the sciatic nerve, and four ligatures (4-0 silk) were tied around the nerve at 1–2 mm intervals. The muscle, fascia, and skin were then sutured in sequence, and the wound was sprayed with povidone-iodine, completing the CCI procedure. In the sham-operated group, the sciatic nerve was exposed but not ligated.

Emodin Treatment and Groups

Rats were randomly assigned to the sham, CCI, CCI + emodin low-dose (L), CCI + emodin high-dose (H), and CCI + emodin high-dose + SIRT1 inhibitor EX-527 groups, with 10 rats in each group. Sham group: animals underwent the full surgical procedure except for sciatic nerve ligation; CCI group: animals underwent the CCI surgery; CCI + emodin L group: animals received emodin (30269, Sigma-Aldrich, St Louis, MO, USA) via intraperitoneal injection at 10 mg/kg after CCI surgery; CCI + emodin H group: animals received emodin via intraperitoneal injection at 50 mg/kg after CCI surgery; CCI + emodin H + SIRT1 inhibitor EX-527 group: animals received the inhibitor EX-527 (10 µg/day, E7034, Sigma Aldrich, St Louis, MO, USA) 30 minutes after emodin treatment. All administrations were performed once daily, starting 24 h after the CCI surgery for 15 consecutive days. Behavioral assessments were performed on postoperative days 3, 7, 11, and 15.

Behavioral Tests

Paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTl) were measured in each group to assess hyperalgesia at baseline and on postoperative days 3, 7, 11, and 15 after CCI.

PWMT: At 2:00 PM on postoperative days 3, 7, 11, and 15 after CCI, mechanical pain thresholds were measured in each group using Von Frey filaments. Rats were placed on a perforated metal platform and confined with a transparent plastic box. After 20–30 minutes, once the rats had acclimated and were not exhibiting exploratory behavior, the mechanical threshold test was performed. A series of Von Frey filaments (2.0 g, 4.0 g, 6.0 g, 8.0 g, 10.0 g, 15.0 g) were used, starting with the 2.0 g filament to test the left hind paw (the side of the model). The test site was the central plantar pad of the paw.

PWTl: Thermal sensitivity was measured 2 h after the mechanical threshold test. The hot-plate apparatus was preheated to 50 °C; once the target temperature was reached, each rat was placed on the plate and the time to the first lifting of the model side (left hind) paw was recorded as the paw withdrawal latency (PWL). Each rat was tested three times with 20-min intervals between trials, and the average value was taken. After each test, excreta were removed and the hot plate wiped with alcohol; testing of the next rat proceeded after the odor dissipated.

Sample Preparation

After behavioral testing on postoperative day 15, rats were anesthetized with an intraperitoneal injection of 1% pentobarbital sodium (40 mg/kg). Rats from each group were then dissected for sample collection. The abdominal aorta was fully exposed and transected with vascular scissors, and 2 mL of blood was collected into a 5-mL centrifuge tube for subsequent ELISA. After blood collection, the rats were euthanized by cervical dislocation and decap-

itation; the L4–L6 lumbar spinal cord segments were dissected and divided into three parts for histopathology, Western blotting, RT-PCR, and immunohistochemistry, respectively.

H&E Staining

The collected spinal cord tissues from each group were fixed in 10% paraformaldehyde for 12 h and embedded in paraffin. Tissue sections (4 µm) were cut with a microtome and stained using standard hematoxylin and eosin (H&E, G1120, Solarbio, Beijing, China) staining procedures. Finally, sections were dehydrated, mounted, and images were captured with a microscope (Olympus IX73, Hachinohe City, Tokyo, Japan).

Immunohistochemical Staining

Expression of Iba-1 and GFAP in spinal cord tissue was assessed by immunohistochemistry. The main steps were as follows: (1) Antigen retrieval: sections were subjected to antigen retrieval in Tris-EDTA (50X) antigen retrieval buffer (pH 9.0). (2) Endogenous peroxidase inactivation: Incubate with 3% hydrogen peroxide aqueous solution at room temperature for 10 minutes. After the inactivation treatment is completed, wash with PBS. (3) Serum blocking: a 5% normal goat serum working solution (stock: PBS 1:19 dilution) was added within the hydrophobic barrier to evenly cover the tissue, followed by blocking at room temperature for 30 min. (4) Primary antibody incubation: the blocking solution was gently removed, and Iba-1 antibody (AB178846, 1:2000, Abcam, Cambridge, UK) or GFAP antibody (AB7260, 1:2000, Abcam, Cambridge, UK) diluted in PBS was added. Slides were placed in a humidified chamber and incubated overnight at 4 °C. These slides were then washed in PBS on a shaker three times for 5 min each. (5) Incubate at room temperature with the secondary antibody (1:2000, ab6789, Abcam, Cambridge, UK) for 1 h. 3,3'-Diaminobenzidine (DM828, Dako, Glostrup, Denmark) was used to complete the color development. (6) Stained with hematoxylin, dehydrated and transparent, sealed with neutral gum. Histological analysis was performed using the Leica Application System module (Leica Microsystems GmbH, Wetzlar, Germany). For quantitative immunohistochemical analysis, three randomly selected regions were scanned and analyzed to determine the average numbers of GFAP and Iba-1-positive cells. Positive cells in spinal cord tissue were quantified using ImageJ software (NIH, Bethesda, MD, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

After centrifugation at 3000 ×g for 10 minutes, the blood and cell supernatants were obtained. The levels of tumor necrosis factor α (TNF-α) (PT516), interleukin-1β (IL-1β) (PI303), and interleukin-6 (IL-6) (PI328) were detected according to the manufacturer's instructions of the corresponding ELISA kits (Beyotime, Shanghai, China).

The absorbance of the samples was measured at 450 nm using an enzyme analyzer.

Co-Immunoprecipitation Assay

Spinal cord tissue samples were homogenized in a non-denaturing lysis buffer containing Protease and Phosphatase Inhibitor Cocktails. The samples were incubated with anti-SIRT1 (60303-1-Ig, 3 µg antibody per 3 mg protein, Proteintech Group, Wuhan, China) or anti-HMGB1 (10829-1-AP, 3 µg antibody per 3 mg protein, Proteintech Group, Wuhan, China) antibodies at 4 °C overnight. IGG (ab37355, Abcam, Cambridge, UK) antibody was used as the control group. After overnight incubation, protein A/G magnetic beads were added to the sample/antibody complexes and incubated at 4 °C for 2 h and then boiled. Subsequently, proteins were separated from the supernatant by SDS-PAGE and incubated with the anti-rabbit secondary antibody (1:2000, ab7090, Abcam, Cambridge, UK) or anti-mouse secondary antibody (1:2000, ab6789, Abcam, Cambridge, UK) for Western blot analysis.

Cell Culture and Treatment

Murine BV2 microglial cells were purchased from the National Collection of Authenticated Cell Cultures (GNM45, Shanghai, China). BV2 murine microglial cells were authenticated by short tandem repeat (STR) analysis and were confirmed to be free of mycoplasma contamination. Murine BV2 microglial cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified 5% CO₂ atmosphere. Cells were first pretreated with different concentrations of emodin (1, 10, 20, 30, 40, 50 µM), and then exposed to lipopolysaccharide (1 µg/mL, L2630, Sigma-Aldrich, St. Louis, MO, USA). The treatment duration was 24 h.

Cell Counting kit-8 Assay

BV2 microglial cells (5×10^3 cells/well) were treated with different concentrations of emodin (1, 10, 20, 30, 40, 50 µM), and then 10 µL of CCK-8 (C0037, Beyotime, Shanghai, China) solution was added. After incubating for 2 h, the absorbance of the samples was measured at 450 nm using a microplate reader (VersaMax, San Francisco, CA, USA).

Transfection

The SIRT1-specific siRNA and its scrambled control were commercially generated by HUA-GENE (Shanghai, China). Transfection was performed using Lipofectamine 3000, with siNC serving as the negative control. The primer for si-NC was forward 5'-UUCUCCGAACGUGUCACGUTT-3'. The primers for si-SIRT1-1 were forward 5'-AGUUGUUCUAGAAACAAUAGU-3'. The primers for si-SIRT1-2 were for-

ward 5'-CAACAAUGUAAUGAUUUAGA-3'. The primers for si-SIRT1-3 were forward 5'-GGUUGUAAUGAAGCUAUAGC-3'. Subsequently, the knockdown efficiencies of si-SIRT1-1, si-SIRT1-2 and si-SIRT1-3 were evaluated by RT-qPCR.

Transwell Assay

BV2 microglial cells (5×10^4 cells/well) were added to the upper chamber of Transwell inserts (8 µm pore size) in serum-free medium. The lower chamber was filled with 500 µL of DMEM containing 10% fetal bovine serum as a chemoattractant. Following a 24-hour incubation period, the cells remaining on the upper surface were eliminated using a cotton swab. The cells that migrated to the lower surface were then fixed in methanol for 20 minutes and subsequently stained with crystal violet for 15 minutes. Imaging of the migrated cells was conducted using a light microscope (Olympus, Hachinohe City, Tokyo, Japan).

Immunofluorescence Staining

Each group of BV2 microglial cells was fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. 5% BSA was used as the blocking reagent. Following primary incubation with Iba-1 (AB178846, Abcam, Cambridge, UK) and GFAP (AB7260, Abcam, Cambridge, UK) antibodies at 4 °C overnight, the cells were exposed to an Alexa-488-conjugated secondary antibody (ab150077, Abcam, Cambridge, UK) for 1 h at room temperature. Nuclei were then stained with 5 µg/mL DAPI for 5 minutes. Images were captured using a fluorescence microscope (BX63, Olympus, Tokyo, Japan).

Fluorescent Cell Co-Localization

Immunofluorescence staining was performed by removing the culture medium from each group, washing the cells with PBS, fixing them in 4% paraformaldehyde for 30 min, and rinsing them with PBS. Cells were then permeabilized with 0.3% Triton X-100 at 37 °C for 30 min, rinsed with PBS, and blocked with 5% BSA at 37 °C for 60 min. Primary antibodies [HMGB1 (1:50, AB79823, Abcam, Cambridge, UK), and SIRT1 (1:50, AB110304, Abcam, Cambridge, UK)] were diluted accordingly and incubated overnight at 4 °C. Then 50 µL of goat anti-rabbit IgG fluorescent antibodies (AB150077, Abcam, Cambridge, UK) and goat anti-mouse IgG fluorescent antibodies (AB150115, Abcam, Cambridge, UK) were added and incubated at 37 °C for 90 min. After PBS washes, nuclei were stained with DAPI working solution at 37 °C for 10 min, washed with PBS, mounted with glycerol-buffered mounting medium, and observed under a fluorescence microscope.

RT-qPCR

Total RNA was extracted from BV2 cells using the TRIzol method according to the manufacturer's

instructions. Reverse transcriptase was used to synthesize cDNA from the total RNA (D7180L, Beyotime, Shanghai, China), and real-time PCR was used to amplify the cDNA using a thermal cycler. The primers for SIRT1 were forward 5'-TCCACGCCCCACAAAAGGAAT-3' and reverse 5'-CATTCGGGCCTCTCCGTATC-3'. The primers for GAPDH were forward 5'-GGGTCCCAGCTTAGGTTTCATC-3' and reverse 5'-TACGGCCAAATCCGTTTACACA-3'. All measurements were performed in triplicate to eliminate operational errors. The relative expression levels of the genes were calculated using the $2^{-\Delta\Delta C_t}$ method.

Western Blotting

Spinal cord tissues and BV2 cells were lysed in protein lysis buffer and centrifuged at 4 °C at 12,000 rpm for 15 min. The supernatants were collected, and the total protein concentration was measured. Proteins were separated by 10% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 50.0 g/L skim milk at room temperature for 1 h, then incubated with primary antibodies against SIRT1 (1:2000, AB189494, Abcam, Cambridge, UK), HMGB1 (10829-1-AP, 1:5000, Proteintech Group, Wuhan, China), Ac-HMGB1 (1:2000, A16002, ABclonal, Hubei, China), TLR4 (1:1000, 19811-1-AP, Proteintech Group, Wuhan, China), NF- κ B p65 (1:1000, AF5006, Affinity, MI, USA), GAPDH (1:3000, AF7021, Affinity, MI, USA), p-NF- κ B p65 (1:1000, AF3387, Affinity, MI, USA), CD11b (1500, AB133357, Abcam, Cambridge, UK), Iba-1 (1000, AB178846, Abcam, Cambridge, UK), COX2 (1000, AB179800, Abcam, Cambridge, UK), and iNOS (2000, AB178945, Abcam, Cambridge, UK) overnight at 4 °C. Horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000, ab7090, Abcam, Cambridge, UK) was incubated at room temperature for 2 h. Bands were visualized by electrochemiluminescence (ECL) and semi-quantified using ImageJ software.

Statistical Analysis

In this study, results are presented as the mean $\pm$ standard deviation. Statistical evaluations were conducted utilizing GraphPad Prism 7 software (La Jolla, CA, USA). To compare multiple groups, a one-way analysis of variance (ANOVA) was employed, followed by a Tukey's post hoc analysis. PWMT and PWTL results were analyzed using two-way repeated measures ANOVA. Between-group differences were assessed using *t*-tests. Statistical significance was defined as a *p*-value less than 0.05.

Results

Effect of Emodin on Hyperalgesia in Rats With Neuropathic Pain

To investigate the effect of emodin on pain hypersensitivity in CCI rats, we assessed paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTL) at multiple time points. Compared with the sham-operated group, CCI rats showed significantly lower PWMT and PWTL starting on day 3 after surgery (Fig. 1A,B) ($p < 0.001$). Compared with the CCI group, treatment with low- and high-dose emodin (emodin-L and emodin-H) increased PWMT and PWTL, with the high-dose group producing a more pronounced effect (Fig. 1A,B) ($p < 0.05$). This indicates that emodin can reverse CCI-induced pain hypersensitivity.

Emodin Inhibits Spinal Cord Nerve Injury and Inflammation in CCI Rats

Pathological changes and inflammatory cell infiltration in the spinal cord were examined by H&E staining. The sciatic nerve structure in the sham-operated group was normal. In the CCI model group, the spinal cord structure was markedly disrupted, with the most severe axonal damage, the most pronounced inflammatory response with extensive lymphocyte infiltration, and obvious glial cell proliferation. In the emodin low-dose group, axonal rupture was still evident, but the inflammatory response and glial cell proliferation were slightly attenuated compared with the model group. Compared with the low-dose group, the emodin high-dose group showed reduced inflammation, improved lymphocyte infiltration, and decreased glial cell proliferation (Fig. 2A). Additionally, we used immunohistochemistry to assess changes in GFAP and Iba-1 levels in spinal cord tissues. Compared with the sham-operated group, CCI model rats showed markedly increased GFAP and Iba-1 expression in the spinal cord. Compared with the CCI group, the Emodin-L group showed decreased Iba-1 and GFAP levels in the spinal cord, with a more pronounced decrease in the Emodin-H group (Fig. 2B) ($p < 0.05$). To assess the release of inflammatory mediators, ELISA was used to quantify the concentrations of TNF- α , IL-6, and IL-1 β . Compared with the sham-operated group, CCI surgery significantly increased the concentrations of these inflammatory mediators ($p < 0.001$). Emodin treatment effectively reduced the CCI-induced release of inflammatory mediators (Fig. 2C) ($p < 0.01$). In summary, these findings indicate that emodin suppresses nerve injury and neuroinflammation. The emodin-H group (50 mg/kg) showed the strongest effect; therefore, this dose was selected for subsequent experiments.

SIRT1 and HMGB1 Interaction

To investigate the relationship between SIRT1 and HMGB1, Western blot analysis was performed. Com-

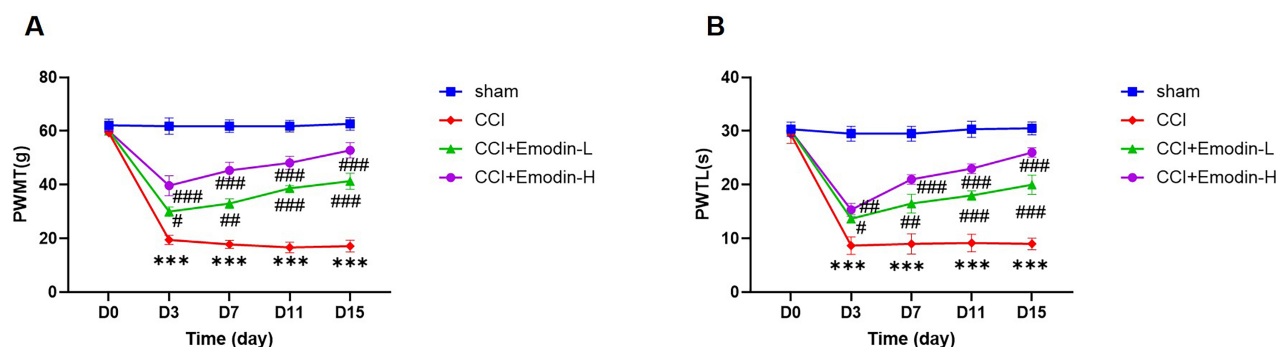


Fig. 1. Values of PWMT and PWTL in each group. (A) PWMT values of rats in each group at different time points. (B) PWTL values of rats in each group at different time points. $n = 6$. *** $p < 0.001$ versus the sham group, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus the CCI group. PWMT, paw withdrawal mechanical threshold; PWTL, paw withdrawal thermal latency; CCI, chronic constriction injury.

pared with the sham-operated group, CCI induction significantly decreased SIRT1 expression and significantly increased HMGB1 expression ($p < 0.001$); however, emodin-H treatment reversed this effect (Fig. 3A,B) ($p < 0.01$). Additionally, we performed co-immunoprecipitation to determine whether SIRT1 and HMGB1 bind to each other. As shown in Fig. 3C, HMGB1 was detected in SIRT1 immunoprecipitation, and SIRT1 was detected in HMGB1 immunoprecipitation, whereas neither was detected in the control IgG precipitates, indicating an interaction between SIRT1 and HMGB1.

Emodin Alleviates Inflammation in Rat Neuropathic Pain by Negatively Regulating HMGB1 Via the SIRT1 Pathway in Vivo

To determine whether the inhibition of SIRT1 blocks the protective effect of emodin by preventing the downregulation of HMGB1 in CCI, we treated spinal cord tissue from CCI rats with a SIRT1 inhibitor and performed subsequent experiments. Immunofluorescence showed that, compared with the CCI + Emodin-H group, the CCI + Emodin-H + SIRT1 inhibitor EX-527 group exhibited increased Iba-1 and GFAP levels (Fig. 4A,B) ($p < 0.001$). Subsequently, we conducted ELISA on the spinal cord tissues induced by CCI after treatment with the SIRT1 inhibitor EX-527. The results showed that compared with the CCI + Emodin-H group, the concentrations of TNF- α , IL-6, and IL-1 β in the CCI + Emodin-H + SIRT1 inhibitor EX-527 group increased (Fig. 4C) ($p < 0.001$). Based on the above results, we conducted further Western blot analysis and found that compared with the sham group, the expression of SIRT1 in the CCI group was significantly decreased, while the expression levels of TLR4, acHMGB1, and p-p65 were significantly increased. Emodin significantly increased the expression of SIRT1, while the expression levels of TLR4, acHMGB1 and p-p65 were significantly decreased. The SIRT1 inhibitor EX-527 reversed the upregulation of SIRT1 expression and the downregulation of TLR4, acHMGB1, and p-p65 expression caused by emodin (Fig. 4D) ($p <$

0.05). The SIRT1 inhibitor EX-527 also blocked the inhibitory effect of emodin on the acetylation of HMGB1 (Fig. 4D). These results indicate that emodin reduces the acetylation level of HMGB1 by promoting SIRT1, thereby inhibiting the HMGB1 signaling pathway and alleviating pain hypersensitivity in rat neuropathic pain.

Emodin Reduces LPS-Induced Migration, Activation and Inflammatory Response of BV2 Microglial Cells by Targeting SIRT1 in Vitro

Based on the above findings that emodin alleviated neuropathic pain in rats *in vivo* by regulating HMGB1-related pathways via SIRT1, we next investigated whether emodin attenuated LPS-induced migration, activation, and inflammatory responses in BV2 microglial cells *in vitro* by targeting SIRT1. The effect of emodin on BV2 microglial cell viability was assessed using cytotoxicity assays. CCK-8 assay results showed that, with increasing concentrations of emodin, BV2 cell viability was significantly decreased at 20 μ M (Fig. 5A) ($p < 0.001$). Therefore, 20 μ M was selected for subsequent cell-based experiments. To ensure the validity of the results, we constructed three SIRT1 knock-down sequences and verified their knockdown efficiency. The results showed that si-SIRT1-3 was the most effective, so si-SIRT1-3 was selected for the subsequent experiments (Fig. 5B) ($p < 0.001$). To determine whether emodin acted through targeting SIRT1 in LPS-induced responses, biological events in emodin-treated, LPS-stimulated BV2 cells were evaluated after si-SIRT1 transfection. The Transwell assay showed that LPS significantly increased the migratory capacity of BV2 cells, while emodin reduced migration (Fig. 5C) ($p < 0.001$). Treatment with si-SIRT1 reversed the emodin-induced reduction in the number of migrating cells (Fig. 5C). Furthermore, immunofluorescence staining and Western blot analysis showed that LPS significantly increased CD11b and Iba-1 expression in BV2 cells, while emodin reduced the expression of these markers (Fig. 5D,E) ($p < 0.001$). si-SIRT1 treatment reversed emodin's inhibitory effect on CD11b and Iba-1 expression in the cells

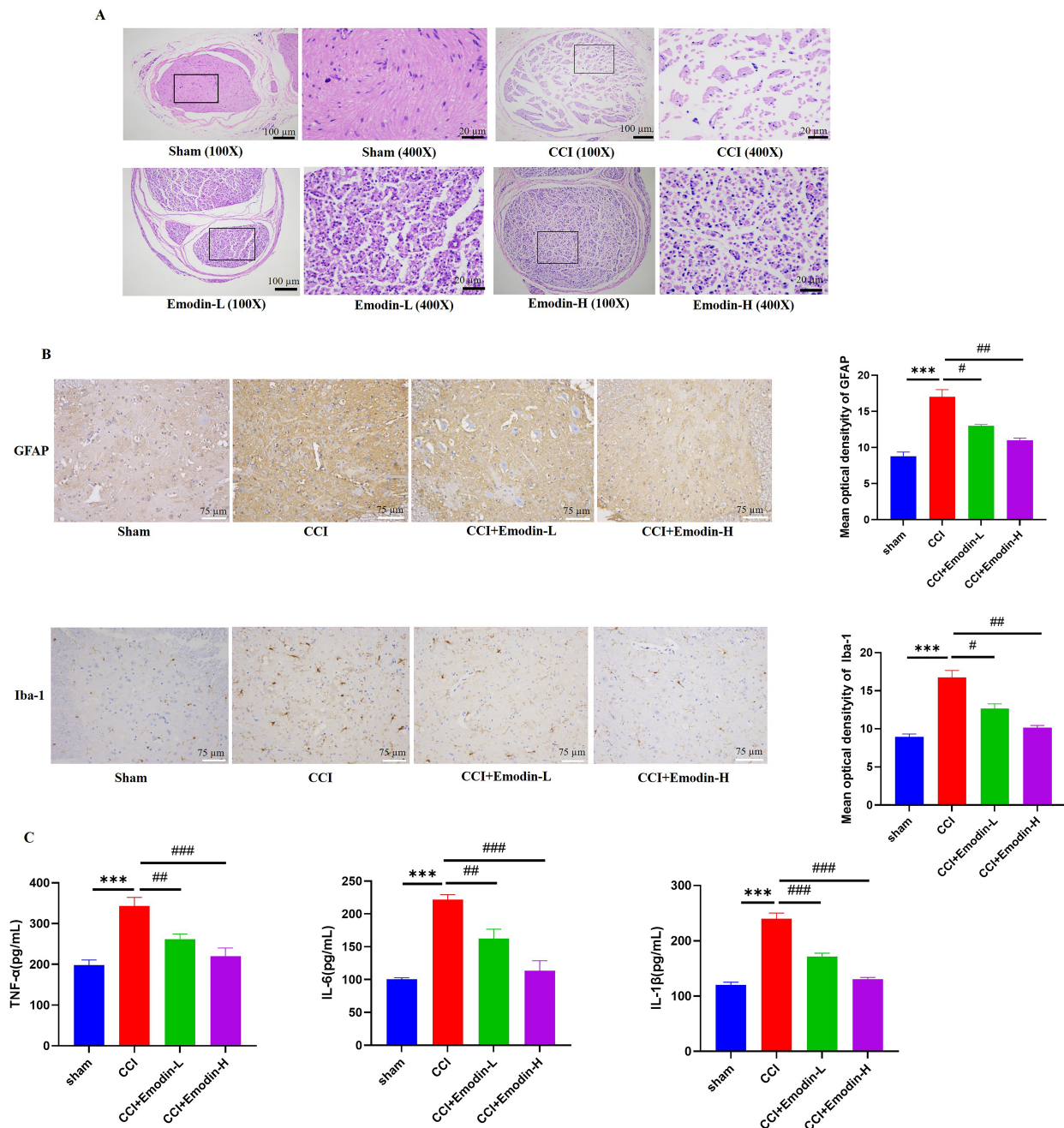


Fig. 2. Effects of emodin on nerve injury and inflammation in CCI rats. (A) H&E staining was used to assess the pathological changes in spinal cord tissues from rats in each group. (B) IHC analysis was conducted to examine the expression of GFAP and Iba-1 in spinal cord tissues from rats in each group. (C) ELISA was employed to measure inflammatory factor levels in each group. $n = 3$. *** $p < 0.001$, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium-binding adapter molecule 1; ELISA, enzyme-linked immunosorbent assay.

(Fig. 5D,E) ($p < 0.05$). The expression of proinflammatory cytokines (TNF- α , IL-1 β , IL-6) was significantly increased in LPS-induced BV2 microglial cells, and was markedly reduced by emodin (Fig. 5F) ($p < 0.01$). Compared with emodin, si-SIRT1 increased the expression of proinflammatory cytokines (TNF- α , IL-1 β , IL-6) (Fig. 5F) ($p < 0.01$). Moreover, emodin decreased the expression of iNOS

and COX2 that were upregulated in LPS-induced BV2 microglial cells (Fig. 5G) ($p < 0.01$). Treatment with si-SIRT1 reversed the emodin-induced downregulation of iNOS and COX2 in the cells ($p < 0.05$).

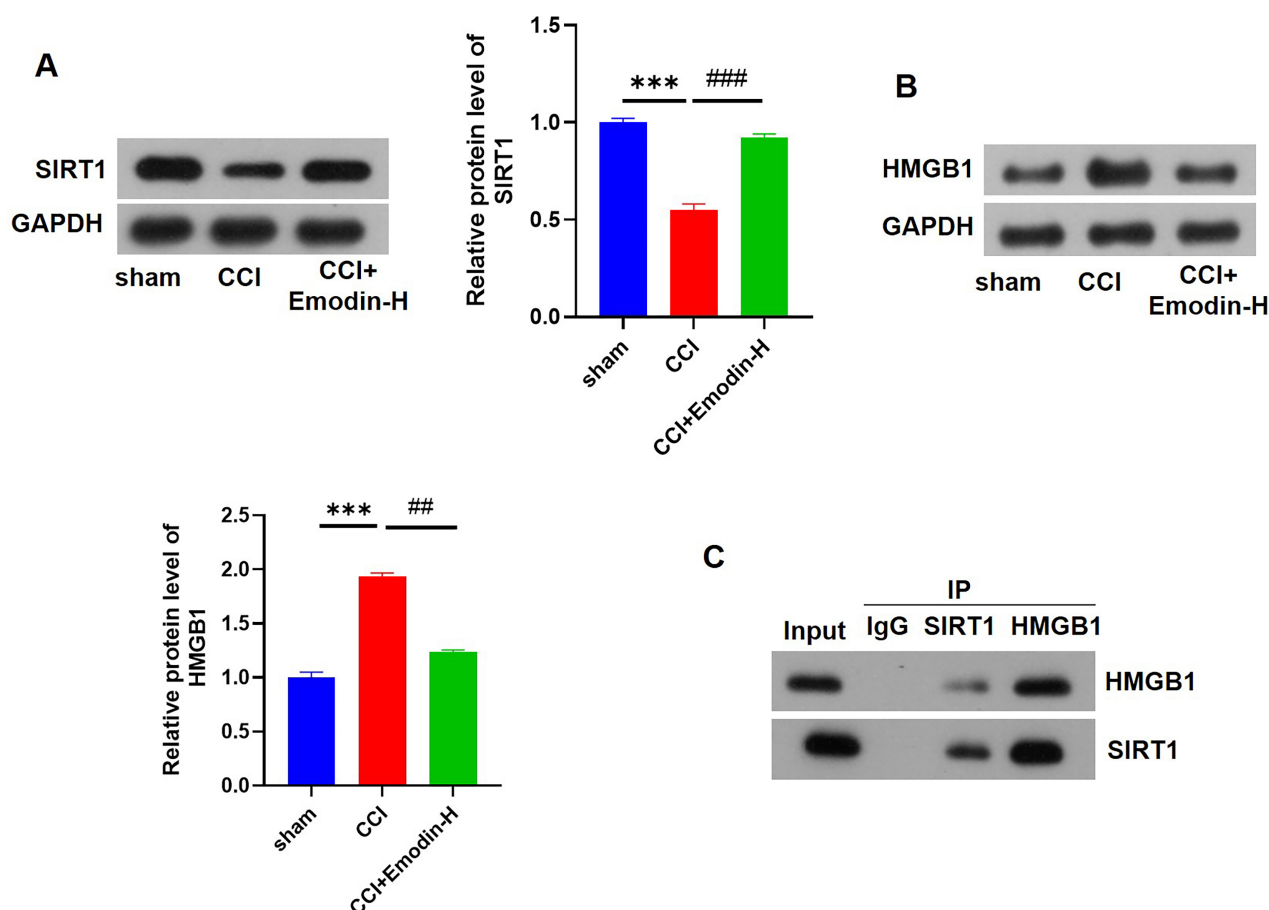


Fig. 3. The relationship between SIRT1 and HMGB1. (A,B) Western blotting was used to analyze the expression of SIRT1 and HMGB1 in each group. (C) Co-Immunoprecipitation assay was used to verify the relationship between SIRT1 and HMGB1. $n = 3$. *** $p < 0.001$, ## $p < 0.01$, ### $p < 0.001$. SIRT1, sirtuin 1; HMGB1, high mobility group box 1; IP, immunoprecipitation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Emodin Alleviates LPS-Induced Inflammation in BV2 Microglial Cells by Regulating HMGB1 Through SIRT1 in Vitro

To determine whether emodin alleviates LPS-induced migration, activation, and inflammatory responses of BV2 microglial cells by targeting SIRT1 to regulate HMGB-related pathways, we performed fluorescence colocalization analysis. Immunofluorescence results showed that, compared with the control group, HMGB1 levels were increased in the LPS-treated BV2 group and HMGB1 was localized to both the nucleus and the cytoplasm, indicating that LPS-activated BV2 microglia induce HMGB1 acetylation and its translocation from the nucleus to the cytoplasm (Fig. 6A). Compared with the LPS + BV2 group, the LPS + BV2 + Emodin group showed increased SIRT1 levels and decreased HMGB1 levels with HMGB1 primarily localized to the nucleus, indicating that emodin increases SIRT1, reduces HMGB1 levels and HMGB1 acetylation, and inhibits BV2 microglia-induced translocation of HMGB1 from the

nucleus to the cytoplasm. Compared with the LPS + BV2 + Emodin and LPS + BV2 + Emodin + siRNA-NC groups, the si-SIRT1 group showed markedly reduced SIRT1 levels and increased HMGB1 levels with HMGB1 present in both the nucleus and the cytoplasm, indicating that SIRT1 reduction can induce HMGB1 acetylation and its translocation from the nucleus to the cytoplasm (Fig. 6A).

Based on the above results, we further performed Western blot analysis and found that, compared with the control group, SIRT1 expression was significantly decreased in the LPS + BV2 group, while TLR4, acetylated HMGB1 (ac-HMGB1), and phosphorylated p65 (p-p65) expression were significantly increased ($p < 0.001$). Emodin significantly increased SIRT1 expression, while the expression of TLR4, ac-HMGB1, and p-p65 was significantly decreased (Fig. 6B) ($p < 0.01$). Treatment with si-SIRT1 reversed the emodin-mediated upregulation of SIRT1 and downregulation of TLR4, ac-HMGB1, and p-p65. Immunofluorescence staining of p65 further con-

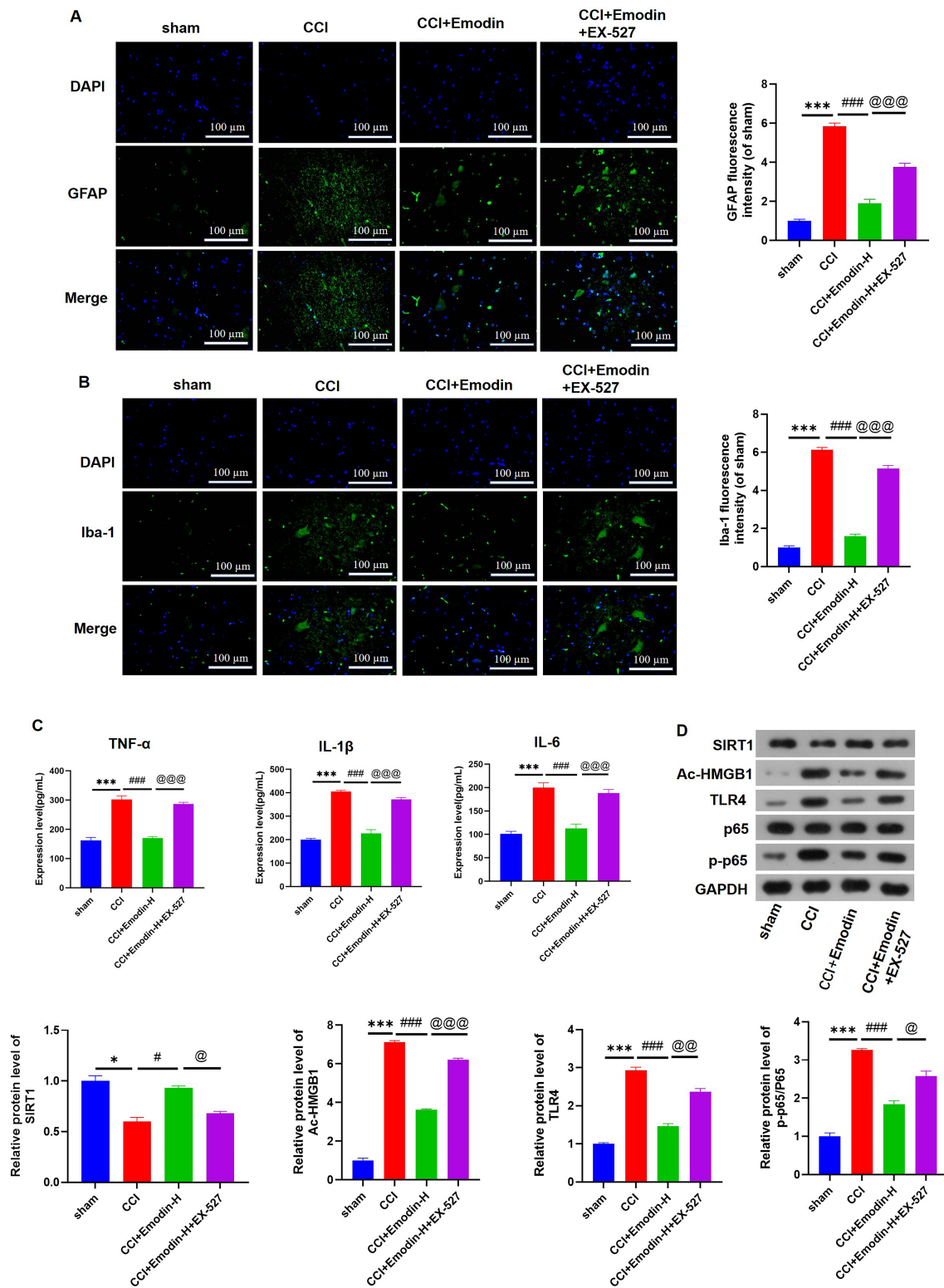


Fig. 4. Emodin negatively regulates HMGB1 through the SIRT1 pathway to alleviate neurogenic pathological pain. (A,B) Immunofluorescence analysis of the levels of Iba-1 and GFAP in each group. (C) ELISA for detecting inflammatory factor levels in each group. (D) Western blot analysis of the protein expression of SIRT1 and HMGB1-related pathways in each group. $n = 3$. * $p < 0.05$, *** $p < 0.001$; # $p < 0.05$, ### $p < 0.001$; @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$.

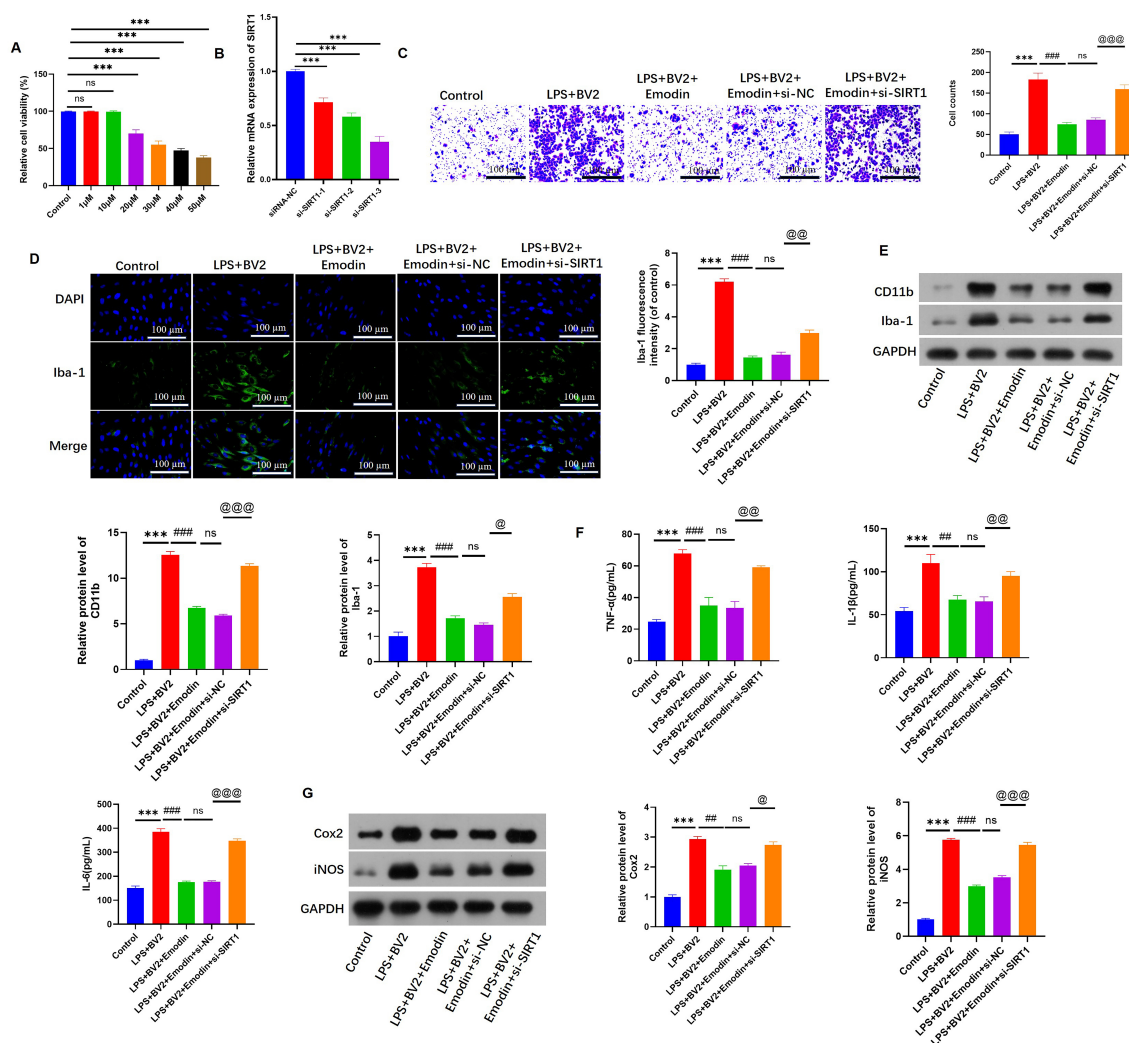


Fig. 5. Effects of emodin-mediated SIRT1 regulation on LPS-induced migration, activation, and inflammatory responses in BV2 microglial cells. (A) CCK-8 assay to determine cell viability. (B) RT-qPCR showing the efficiency of SIRT1 knockdown. (C) Transwell assay showing the migratory ability of cells. (D) Immunofluorescence to evaluate the expression of Iba-1 in cells of each group. (E) WB to detect the expression of CD11b and Iba-1 in cells of each group. (F) ELISA analysis of inflammatory factor levels in each group. (G) WB to detect the expression of iNOS and COX2 in cells of each group. $n = 3$. *** $p < 0.001$; ## $p < 0.01$; ### $p < 0.001$; @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$; ns, no significance. LPS, lipopolysaccharide; BV2, mouse microglial cell line; CCK-8, Cell Counting Kit-8.

firmed these findings (Fig. 6C) ($p < 0.001$). Therefore, these findings suggest that emodin promotes SIRT1 expression, reduces HMGB1 acetylation, inhibits HMGB1 translocation from the nucleus to the cytoplasm, decreases extracellular HMGB1 release, and suppresses HMGB1 signaling.

Discussion

NP is a chronic neurological disorder characterized by hyperalgesia, abnormal pain (dysesthesia), and spontaneous pain. Due to its high prevalence [30] and heterogeneity, the underlying mechanisms remain unclear, and current analgesics show limited efficacy. These characteristics make NP difficult to manage and underscore the

need to develop new therapeutic strategies. Recent studies have revealed emodin's analgesic properties in various neuropathic pain models induced by nerve injury and CCI [31,32]. The spinal cord is a key center for signal transmission, and emodin has attracted increasing attention for its therapeutic potential in treating spinal nociception. Chen *et al.* [28] and Badshah *et al.* [33] found that emodin relieved the pain and spinal inflammation of NP by regulating the P2X4 purinoceptor signaling and PPAR-gamma pathway. Emodin has also been reported to restore the viability of LPS-treated BV2 cells [26]. In the present study, we investigated the anti-inflammatory mechanisms by which emodin regulates HMGB1-related pathways via SIRT1 in a rat CCI model and a BV2 neuroinflammation cell model.

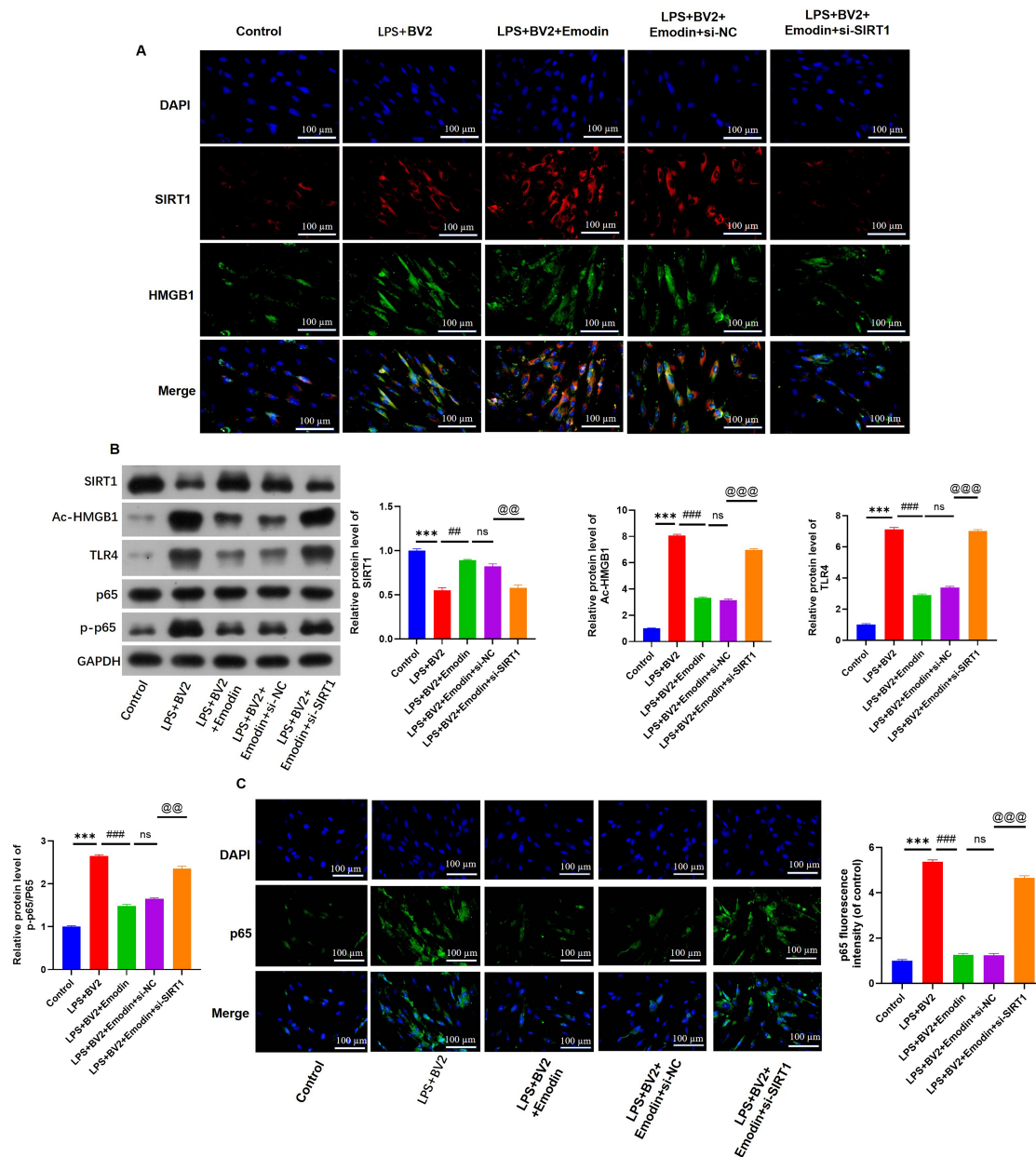


Fig. 6. Emodin regulates HMGB1 via SIRT1 to modulate LPS-induced inflammation in BV2 microglial cells. (A) Fluorescence localization of HMGB1 and SIRT1 immunofluorescence staining in each group of cells. (B) Western blot analysis of protein expression of SIRT1 and HMGB1-related pathways in each group. (C) Immunofluorescence detection of protein expression of p65 in each group. $n = 3$. *** $p < 0.001$; ## $p < 0.01$, ### $p < 0.001$; @@ $p < 0.01$, @@@ $p < 0.001$; ns, no significance.

This study may provide new insights into the mechanisms underlying NP and support the development of novel therapeutic strategies.

Plant-derived natural compounds have attracted widespread attention because of their low toxicity, minimal side effects, and strong therapeutic potential. Emodin is an active constituent of rhubarb with anticancer, antiapoptotic, and anti-inflammatory properties [34], and has been reported to improve NP [23]. However, studies on the mechanisms by which emodin alleviates NP *in vivo* and *in vitro* remain limited. In this study, behavioral tests

showed that emodin reversed CCI-induced hyperalgesia. H&E staining results also indicated that emodin treatment partially alleviated axonal injury and inflammatory responses in the sciatic nerve. A previous study reported that sciatic nerve ligation caused peripheral nerve injury, and the activation of spinal microglia cells led to an early response from the central nervous system, followed by the activation and proliferation of astrocytes [35]. Activation of microglia and astrocytes is critical for sustaining the central sensitization and hyperalgesia associated with NP. Iba-1 and GFAP are marker proteins for activated

microglia and astrocytes, respectively; numerous studies have shown increased GFAP immunoreactivity in the spinal cord in CCI animal models, and specifically, GFAP levels were significantly elevated in CCI rats, whereas emodin treatment significantly reversed this increase [36,37]. Furthermore, activated microglia can secrete large amounts of inflammatory factors such as IL-6 and TNF- α , triggering inflammatory responses and exacerbating the progression of NP [38]. Emodin attenuated the CCI-induced upregulation of classical inflammatory mediators in previous studies [11,12]. The results of this present study showed that TNF- α , IL-1 β , and IL-6 levels were markedly increased in rats in the CCI group, and emodin intervention reduced these levels. In addition, we examined the effects of emodin on LPS-induced inflammation in BV2 microglial cells *in vitro*, since it has been previously shown that LPS accelerates the migration of BV2 cells [39]. Consistently, our study showed that LPS increased the number of migrating BV2 microglial cells, whereas emodin reduced this increase, in line with a recent study that reported that emodin exerts protective effects against microglial activation [27]. Experimental results showed that emodin reduced the expression of microglial activation markers CD11b and Iba-1 in LPS-stimulated BV2 cells. Also, microglia play an important role in CNS neuroinflammation [40]. Activation of microglia may trigger the secretion of pro-inflammatory cytokines, thereby causing severe neuroinflammatory responses and leading to central sensitization [41,42]. Notably, considerable evidence indicates that emodin exerts anti-inflammatory effects on microglia in autoimmune encephalomyelitis [27,43], and COX-2 and iNOS are pro-inflammatory enzymes involved in microglial inflammation [44]. This study also found that emodin reduced the expression of COX-2, iNOS, TNF- α , IL-6, and IL-1 β in LPS-exposed BV2 microglial cells, reflecting the anti-inflammatory effect of emodin in BV2 cells during the LPS response. Together, these findings indicate that emodin alleviates hyperalgesia and spinal central sensitization in NP by suppressing neuroinflammation.

SIRT1, an important NAD⁺-dependent deacetylase in the body, has diverse biological functions across various cell types, such as regulating cell differentiation and participating in inflammation [45]. SIRT1 has been shown to be a therapeutic target for chronic neuropathic pain [46]. In this study, following spinal cord injury in the CCI model, SIRT1 expression was reduced, and emodin upregulated its expression. A similar result was reported by Liu *et al.* [47]. Meanwhile, emodin decreased HMGB1 expression, suggesting that SIRT1 and HMGB1 may be involved in the pathological processes of NP. Le *et al.* [48] confirmed that SIRT1 is involved in the acetylation of HMGB1 and may negatively regulate HMGB1. This finding is consistent with our results, as co-immunoprecipitation showed an interaction between SIRT1 and HMGB1 in spinal cord tissues, suggesting

that SIRT1 regulates HMGB1. These results indicate that emodin may suppress HMGB1 expression and its related pathways by promoting SIRT1 expression, thereby influencing the progression of NP.

HMGB1 is a nuclear protein that is highly conserved across all cell types and plays a role in regulating innate immune responses in both intracellular and extracellular settings [49]. Extracellular HMGB1 can recognize and bind specific receptors to participate in the regulation of various physiological processes, including cell survival, proliferation, and inflammation. Wang *et al.* [50] found that inhibition of HMGB1 promotes apoptosis and exerts antitumor effects in glioma. As a key regulator of neuroinflammation, HMGB1 is released through two main routes: passive release from injured neurons and active secretion by activated immune cells, such as microglia. Thus, it acts as a critical mediator linking acute brain injury to secondary inflammatory responses [51]. It has been reported that acetylation of HMGB1 promotes its translocation from the nucleus to the cytoplasm, whereas upregulation of the deacetylase SIRT1 inhibits this translocation [52], suggesting that the inhibition may be related to HMGB1 acetylation levels. In a previous study, emodin alleviated sepsis-induced lung injury *in vivo* and *in vitro* by promoting SIRT1-mediated deacetylation and inhibiting the HMGB1/NF- κ B pathway [47]. However, the mechanism by which emodin may alleviate hyperalgesia in neuropathic pain by negatively regulating HMGB1-related signaling pathways via SIRT1 remains unclear. To further validate this mechanism, we first treated CCI model rats *in vivo* with the SIRT1-specific inhibitor EX-527 to determine whether SIRT1 regulates the HMGB1 signaling pathway in CCI. The results showed that expression levels of Iba-1, GFAP, and inflammatory cytokines were higher in the EX-527 (SIRT1 inhibitor) group than in the emodin-treated group. Western blot analysis indicated that acetylated HMGB1 was also significantly upregulated after EX-527 treatment. Meanwhile, the expression levels of HMGB1 pathway-related proteins TLR4 and phosphorylated p65 (p-p65) were markedly increased.

A study found that HMGB1 expression is significantly upregulated in LPS-stimulated BV2 cells [53]. After LPS induction, HMGB1 can translocate from the nucleus, traverse the cytoplasm, enter vesicles, and be released via a nonclassical secretion mechanism [54]. Interferon (IFN) induces HMGB1 translocation from the nucleus to the cytoplasm by activating the JAK/STAT1 pathway [55], suggesting that inflammatory stimuli promote HMGB1 nuclear translocation in cells. Based on the *in vivo* results, we validated this mechanism *in vitro* in LPS-stimulated BV2 neuroinflammatory cells. We further generated SIRT1-knockdown BV2 cells via lentiviral infection. Immunofluorescence co-localization and Western blot analysis showed that emodin increased SIRT1 expression and reduced LPS-induced HMGB1 acetylation and nuclear translocation, whereas SIRT1 knockdown markedly pro-

moted HMGB1 acetylation and nuclear translocation and increased the expression of inflammatory cytokines. These results indicate that emodin suppresses HMGB1 expression and translocation via a SIRT1-mediated mechanism involving HMGB1 acetylation, thereby influencing the development of NP. Identification of this mechanism may provide a novel theoretical framework for understanding the development and treatment of NP.

Conclusion

Taken together, this study shows that emodin alleviates hyperalgesia and inhibits inflammation, supporting its potential therapeutic application in NP. Moreover, NP-induced hyperalgesia is alleviated by emodin-mediated negative regulation of HMGB1 via the SIRT1 pathway. This research has the potential to support a novel approach to NP management and provide novel perspectives on its underlying mechanisms.

Availability of Data and Materials

All data included in this study are available from the corresponding author upon reasonable request.

Author Contributions

CFL had the original conception of the work. GHL and HYZ collected the data. CFL, YF and JQM performed the research. XYL analyzed the data. CFL drafted the manuscript. All authors contributed to the important editorial changes in the manuscript. 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

This study was approved by the Experimental Animal Management and Ethics Committee of Zhejiang Chinese Medical University (Approval Number: SYXK 2021-0012 (Zhejiang)). All procedures complied with the ARRIVE Guidelines 2.0.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Widerström-Noga E. Neuropathic Pain and Spinal Cord Injury: Phenotypes and Pharmacological Management. *Drugs*. 2017; 77: 967–984. <https://doi.org/10.1007/s40265-017-0747-8>.
- [2] St John Smith E. Advances in understanding nociception and neuropathic pain. *Journal of Neurology*. 2018; 265: 231–238. <https://doi.org/10.1007/s00415-017-8641-6>.
- [3] Tsuda M, Masuda T, Kohno K. Microglial diversity in neuropathic pain. *Trends in Neurosciences*. 2023; 46: 597–610. <https://doi.org/10.1016/j.tins.2023.05.001>.
- [4] Ghazisaeidi S, Muley MM, Salter MW. Neuropathic Pain: Mechanisms, Sex Differences, and Potential Therapies for a Global Problem. *Annual Review of Pharmacology and Toxicology*. 2023; 63: 565–583. <https://doi.org/10.1146/annurev-pharmtox-051421-112259>.
- [5] Attal N, Bouhassira D, Colvin L. Advances and challenges in neuropathic pain: a narrative review and future directions. *British Journal of Anaesthesia*. 2023; 131: 79–92. <https://doi.org/10.1016/j.bja.2023.04.021>.
- [6] Finnerup NB, Sindrup SH, Jensen TS. The evidence for pharmacological treatment of neuropathic pain. *Pain*. 2010; 150: 573–581. <https://doi.org/10.1016/j.pain.2010.06.019>.
- [7] Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, *et al*. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *The Lancet. Neurology*. 2015; 14: 162–173. [https://doi.org/10.1016/S1474-4422\(14\)70251-0](https://doi.org/10.1016/S1474-4422(14)70251-0).
- [8] Liang SP, Wang XZ, Piao MH, Chen X, Wang ZC, Li C, *et al*. Activated SIRT1 contributes to DPT-induced glioma cell parthanatos by upregulation of NOX2 and NAT10. *Acta Pharmacologica Sinica*. 2023; 44: 2125–2138. <https://doi.org/10.1038/s41401-023-01109-3>.
- [9] Hao C, Zhu PX, Yang X, Han ZP, Jiang JH, Zong C, *et al*. Overexpression of SIRT1 promotes metastasis through epithelial-mesenchymal transition in hepatocellular carcinoma. *BMC Cancer*. 2014; 14: 978. <https://doi.org/10.1186/1471-2407-14-978>.
- [10] Yang H, Bi Y, Xue L, Wang J, Lu Y, Zhang Z, *et al*. Multifaceted Modulation of SIRT1 in Cancer and Inflammation. *Critical Reviews in Oncogenesis*. 2015; 20: 49–64. <https://doi.org/10.1615/critrevoncog.2014012374>.
- [11] Chen C, Zhou M, Ge Y, Wang X. SIRT1 and aging related signaling pathways. *Mechanisms of Ageing and Development*. 2020; 187: 111215. <https://doi.org/10.1016/j.mad.2020.111215>.
- [12] Zhong X, Wang W, Mao Z, Gao F, Guo R, Wei X, *et al*. Activation of liver x receptors prevents the spinal LTP induced by skin/muscle retraction in the thigh via SIRT1/NF-Kb pathway. *Neurochemistry International*. 2019; 128: 106–114. <https://doi.org/10.1016/j.neuint.2019.04.002>.
- [13] Yu X, Zhang S, Zhao D, Zhang X, Xia C, Wang T, *et al*. SIRT1 inhibits apoptosis in *in vivo* and *in vitro* models of spinal cord injury via microRNA-494. *International Journal of Molecular Medicine*. 2019; 43: 1758–1768. <https://doi.org/10.3892/ijmm.2019.4106>.
- [14] Nimmagadda VKC, Makar TK, Chandrasekaran K, Sagi AR, Ray J, Russell JW, *et al*. SIRT1 and NAD⁺ precursors: Therapeutic targets in multiple sclerosis a review. *Journal of Neuroimmunology*. 2017; 304: 29–34. <https://doi.org/10.1016/j.jneuroim.2016.07.007>.
- [15] Thomas JO. HMG1 and 2: architectural DNA-binding proteins. *Biochemical Society Transactions*. 2001; 29: 395–401. <https://doi.org/10.1042/bst0290395>.

- [//doi.org/10.1042/bst0290395](https://doi.org/10.1042/bst0290395).
- [16] Chen R, Kang R, Tang D. The mechanism of HMGB1 secretion and release. *Experimental & Molecular Medicine*. 2022; 54: 91–102. <https://doi.org/10.1038/s12276-022-00736-w>.
 - [17] Chen R, Zou J, Zhong X, Li J, Kang R, Tang D. HMGB1 in the interplay between autophagy and apoptosis in cancer. *Cancer Letters*. 2024; 581: 216494. <https://doi.org/10.1016/j.canlet.2023.216494>.
 - [18] Jin X, Liu MY, Zhang DF, Zhong X, Du K, Qian P, *et al*. Natural products as a potential modulator of microglial polarization in neurodegenerative diseases. *Pharmacological Research*. 2019; 145: 104253. <https://doi.org/10.1016/j.phrs.2019.104253>.
 - [19] Xu X, Piao HN, Aosai F, Zeng XY, Cheng JH, Cui YX, *et al*. Arctigenin protects against depression by inhibiting microglial activation and neuroinflammation via HMGB1/TLR4/NF- κ B and TNF- α /TNFR1/NF- κ B pathways. *British Journal of Pharmacology*. 2020; 177: 5224–5245. <https://doi.org/10.1111/bph.15261>.
 - [20] Yang C, Kang F, Huang X, Zhang W, Wang S, Han M, *et al*. Melatonin attenuates bone cancer pain via the SIRT1/HMGB1 pathway. *Neuropharmacology*. 2022; 220: 109254. <https://doi.org/10.1016/j.neuropharm.2022.109254>.
 - [21] Dong X, Fu J, Yin X, Cao S, Li X, Lin L, *et al*. Emodin: A Review of its Pharmacology, Toxicity and Pharmacokinetics. *Phytotherapy Research: PTR*. 2016; 30: 1207–1218. <https://doi.org/10.1002/ptr.5631>.
 - [22] Cui Y, Chen LJ, Huang T, Ying JQ, Li J. The pharmacology, toxicology and therapeutic potential of anthraquinone derivative emodin. *Chinese Journal of Natural Medicines*. 2020; 18: 425–435. [https://doi.org/10.1016/S1875-5364\(20\)30050-9](https://doi.org/10.1016/S1875-5364(20)30050-9).
 - [23] Chen P, Lin D, Wang C, Song C, Wang W, Qu J, *et al*. Proteomic Analysis of Emodin Treatment in Neuropathic Pain Reveals Dysfunction of the Calcium Signaling Pathway. *Journal of Pain Research*. 2021; 14: 613–622. <https://doi.org/10.2147/JPR.S290681>.
 - [24] Zhang X, Li J, Guan L. Emodin Reduces Inflammatory and Nociceptive Responses in Different Pain-and Inflammation-Induced Mouse Models. *Combinatorial Chemistry & High Throughput Screening*. 2023; 26: 989–1000. <https://doi.org/10.2174/1386207325666220514145252>.
 - [25] Li S, Tang S, Dai L, Jian Z, Li X. Emodin relieves morphine-stimulated BV2 microglial activation and inflammation through the TLR4/NF- κ B/NLRP3 pathway. *Neuroreport*. 2024; 35: 518–528. <https://doi.org/10.1097/WNR.0000000000002034>.
 - [26] Jiang W, Liu Z, Wu S, Meng T, Xu LL, Liu JF, *et al*. Neuroprotection of Emodin by Inhibition of Microglial NLRP3 Inflammasome-Mediated Pyroptosis. *Journal of Integrative Neuroscience*. 2023; 22: 48. <https://doi.org/10.31083/j.jin2202048>.
 - [27] Cui YR, Bu ZQ, Yu HY, Yan LL, Feng J. Emodin attenuates inflammation and demyelination in experimental autoimmune encephalomyelitis. *Neural Regeneration Research*. 2023; 18: 1535–1541. <https://doi.org/10.4103/1673-5374.358612>.
 - [28] Chen P, Gong Q, Wang H, Wang C, Wang W, Wu J, *et al*. Analgesic Mechanism of Emodin in Neuropathic Pain Through Inhibiting P2X4 Purinoceptor Signaling. *Molecular Neurobiology*. 2025; 62: 10210–10227. <https://doi.org/10.1007/s12035-025-04906-5>.
 - [29] Chen P, Wang C, Gong Q, Chai Y, Chen Y, Song C, *et al*. Alterations of endogenous pain-modulatory system of the cerebral cortex in the neuropathic pain. *iScience*. 2023; 26: 106668. <https://doi.org/10.1016/j.isci.2023.106668>.
 - [30] Baskozos G, Hébert HL, Pascal MM, Themistocleous AC, Macfarlane GJ, Wynick D, *et al*. Epidemiology of neuropathic pain: an analysis of prevalence and associated factors in UK Biobank. *Pain Reports*. 2023; 8: e1066. <https://doi.org/10.1097/PR9.0000000000001066>.
 - [31] Yang HY, Wu J, Lu H, Cheng ML, Wang BH, Zhu HL, *et al*. Emodin suppresses oxaliplatin-induced neuropathic pain by inhibiting COX2/NF- κ B mediated spinal inflammation. *Journal of Biochemical and Molecular Toxicology*. 2023; 37: e23229. <https://doi.org/10.1002/jbt.23229>.
 - [32] Li L, Sheng X, Zhao S, Zou L, Han X, Gong Y, *et al*. Nanoparticle-encapsulated emodin decreases diabetic neuropathic pain probably via a mechanism involving P2X3 receptor in the dorsal root ganglia. *Purinergic Signalling*. 2017; 13: 559–568. <https://doi.org/10.1007/s11302-017-9583-2>.
 - [33] Badshah I, Qazi NG, Ali F, Minhas AM, Alvi AM, Kandeel M, *et al*. Emodin alleviates chronic constriction injury-induced neuropathic pain and inflammation via modulating PPAR- γ pathway. *PloS One*. 2023; 18: e287517. <https://doi.org/10.1371/journal.pone.0287517>.
 - [34] Shrimali D, Shanmugam MK, Kumar AP, Zhang J, Tan BKH, Ahn KS, *et al*. Targeted abrogation of diverse signal transduction cascades by emodin for the treatment of inflammatory disorders and cancer. *Cancer Letters*. 2013; 341: 139–149. <https://doi.org/10.1016/j.canlet.2013.08.023>.
 - [35] Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nature Neuroscience*. 2007; 10: 1361–1368. <https://doi.org/10.1038/nn1992>.
 - [36] Zhao B, Pan Y, Xu H, Song X. Kindlin-1 Regulates Astrocyte Activation and Pain Sensitivity in Rats With Neuropathic Pain. *Regional Anesthesia and Pain Medicine*. 2018; 43: 547–553. <https://doi.org/10.1097/AAP.0000000000000780>.
 - [37] Dieb W, Hafidi A. Astrocytes are involved in trigeminal dynamic mechanical allodynia: potential role of D-serine. *Journal of Dental Research*. 2013; 92: 808–813. <https://doi.org/10.1177/0022034513498898>.
 - [38] Zhou LJ, Peng J, Xu YN, Zeng WJ, Zhang J, Wei X, *et al*. Microglia Are Indispensable for Synaptic Plasticity in the Spinal Dorsal Horn and Chronic Pain. *Cell Reports*. 2019; 27: 3844–3859.e6. <https://doi.org/10.1016/j.celrep.2019.05.087>.
 - [39] You Z, Yang Z, Cao S, Deng S, Chen Y. The Novel KLF4/BIG1 Regulates LPS-mediated Neuro-inflammation and Migration in BV2 Cells via PI3K/Akt/NF- κ B Signaling Pathway. *Neuroscience*. 2022; 488: 102–111. <https://doi.org/10.1016/j.neuroscience.2022.01.014>.
 - [40] Ayata P, Amit I, Cuda CM. Editorial: Microglia in neuroinflammation. *Frontiers in Immunology*. 2023; 14: 1227095. <https://doi.org/10.3389/fimmu.2023.1227095>.
 - [41] Atta AA, Ibrahim WW, Mohamed AF, Abdelkader NF. Microglia polarization in nociplastic pain: mechanisms and perspectives. *Inflammopharmacology*. 2023; 31: 1053–1067. <https://doi.org/10.1007/s10787-023-01216-x>.
 - [42] Wang H, Wen H, Yang Y, Zhong J, Zhang W, Qing G, *et al*. CCL2 activates NLRP3 inflammasomes to regulate microglial phenotype transformation to promote central sensitisation in chronic migraine. *International Immunopharmacology*. 2025; 162: 115133. <https://doi.org/10.1016/j.intimp.2025.115133>.
 - [43] Zheng K, Lv B, Wu L, Wang C, Xu H, Li X, *et al*. Protecting effect of emodin in experimental autoimmune encephalomyelitis mice by inhibiting microglia activation and inflammation via Myd88/PI3K/Akt/NF- κ B signalling pathway. *Bioengineered*. 2022; 13: 9322–9344. <https://doi.org/10.1080/21655979.2022.2052671>.
 - [44] Kim SR, Seong KJ, Kim WJ, Jung JY. Epigallocatechin Gallate Protects against Hypoxia-Induced Inflammation in Microglia via NF- κ B Suppression and Nrf-2/HO-1 Activation. *International Journal of Molecular Sciences*. 2022; 23: 4004. <https://doi.org/10.3390/ijms23074004>.
 - [45] Wang R, Xie Y, Qiu J, Chen J. The Effects of Dexmedetomidine in a Rat Model of Sepsis-Induced Lung Injury are Me-

- diated Through the Adenosine Monophosphate-Activated Protein Kinase (AMPK)/Silent Information Regulator 1 (SIRT1) Pathway. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. 2020; 26: e919213. <https://doi.org/10.12659/MSM.919213>.
- [46] Song FH, Liu DQ, Zhou YQ, Mei W. SIRT1: A promising therapeutic target for chronic pain. *CNS Neuroscience & Therapeutics*. 2022; 28: 818–828. <https://doi.org/10.1111/cns.13838>.
- [47] Liu FJ, Gu TJ, Wei DY. Emodin alleviates sepsis-mediated lung injury via inhibition and reduction of NF- κ B and HMGB1 pathways mediated by SIRT1. *The Kaohsiung Journal of Medical Sciences*. 2022; 38: 253–260. <https://doi.org/10.1002/kjm2.12476>.
- [48] Le K, Chibaatar Daliv E, Wu S, Qian F, Ali AI, Yu D, *et al*. SIRT1-regulated HMGB1 release is partially involved in TLR4 signal transduction: A possible anti-neuroinflammatory mechanism of resveratrol in neonatal hypoxic-ischemic brain injury. *International Immunopharmacology*. 2019; 75: 105779. <https://doi.org/10.1016/j.intimp.2019.105779>.
- [49] Yang H, Wang H, Andersson U. Targeting Inflammation Driven by HMGB1. *Frontiers in Immunology*. 2020; 11: 484. <https://doi.org/10.3389/fimmu.2020.00484>.
- [50] Wang Y, Wang S, Wang Y, Wang C, Tang Y, Zhang C, *et al*. Glucose regulates the HMGB1 signaling pathway through SIRT1 in glioma. *Cellular Signalling*. 2024; 118: 111137. <https://doi.org/10.1016/j.cellsig.2024.111137>.
- [51] Kim H, Kim BJ, Koh S, Cho HJ, Jin X, Kim BG, *et al*. High mobility group box 1 in the central nervous system: regeneration hidden beneath inflammation. *Neural Regeneration Research*. 2025; 20: 107–115. <https://doi.org/10.4103/NRR.NR-R-D-23-01964>.
- [52] Shen P, Lin W, Huang Y, Ba X, Han L, Li T, *et al*. Wutou decoction attenuates rheumatoid arthritis in rats through SIRT1-mediated deacetylation of the HMGB1/NF- κ B pathway. *Journal of Ethnopharmacology*. 2025; 337: 118921. <https://doi.org/10.1016/j.jep.2024.118921>.
- [53] Chibaatar E, Le K, Abdoulaye IA, Wu S, Guo Y. Melatonin Ameliorates Lipopolysaccharide-Induced Microglial Inflammation via Triggering SIRT1/HMGB1 Signaling Axis. *Journal of Molecular Neuroscience: MN*. 2021; 71: 691–701. <https://doi.org/10.1007/s12031-020-01699-1>.
- [54] Pisetsky DS. The translocation of nuclear molecules during inflammation and cell death. *Antioxidants & Redox Signaling*. 2014; 20: 1117–1125. <https://doi.org/10.1089/ars.2012.5143>.
- [55] Lu B, Antoine DJ, Kwan K, Lundbäck P, Wähämaa H, Schierbeck H, *et al*. JAK/STAT1 signaling promotes HMGB1 hyperacetylation and nuclear translocation. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111: 3068–3073. <https://doi.org/10.1073/pnas.1316925111>.