

# Dexmedetomidine alleviates inflammation-induced neuropathic pain by suppressing NLRP3 via activation of Nrf2

**Wenyan Shan**

Hunan Provincial People's Hospital

**Xiaoyun Liao**

Hunan Provincial People's Hospital

**Yixun Tang**

Hunan Provincial People's Hospital

**Jitong Liu** (✉ [liujitong1971@126.com](mailto:liujitong1971@126.com))

Hunan Provincial People's Hospital

---

## Research

**Keywords:** Neuropathic pain, Dexmedetomidine, Chronic constriction injury, Nrf2, NLRP3

**Posted Date:** November 13th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-106982/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at Experimental and Therapeutic Medicine on July 22nd, 2021. See the published version at <https://doi.org/10.3892/etm.2021.10479>.

# Abstract

**Objective:** To investigate the mechanism of dexmedetomidine (DEX) involving Nrf2-dependent inhibition of NLRP3 in relieving neuropathic pain in chronic constriction injury (CCI) rat models.

**Methods:** The CCI rat models were constructed through sciatic nerve ligation. The CCI rats were treated with DEX, Nrf2 inhibitor (ML385), NLRP3 antagonist (MCC950) and NLRP3 activator (Nigericin). Mechanical withdrawal threshold (MWT) was measured to test the pain sensitivity of CCI rats. H&E staining detected spinal injury of the rats and TUNEL staining was applied to test apoptosis in the spinal cords. ELISA measured the expressions of inflammatory factors. The expressions of Nrf2 and NLRP3 were also detected.

**Results:** Decreased MWT, enhanced spinal cord injury, promoted apoptosis and increased inflammatory factors were detected in CCI rats. The expressions of the above indicators were retraced in DEX-treated CCI rats. Increased MWT, reduced spinal cord injury, inhibited apoptosis and decreased inflammatory factors were detected in rats treated with MCC950 or ML385 while opposite expression patterns were found in rats treated with Nigericin. The expressions of these indicators were retraced in both DEX+ML385 group and MCC950+ML385 group compared to ML385 group and MCC950 group respectively.

**Conclusion:** DEX reduces neuropathic pain of CCI rats by suppressing NLRP3 through activation of Nrf2.

## Introduction

Inflammatory responses play a pivotal role in various neurodegenerative diseases such as Alzheimer's disease, multiple sclerosis and Parkinson's disease [1]. Neuroinflammation is characterized by activation of pro-inflammatory cytokines and chemokines which promote chronic widespread pain in central nervous system [2]. Chronic neuropathic pain may complicate neuropathic symptoms and treatment decisions, leading to poor outcomes and impaired life quality of patients [3]. Diagnosis of neuropathic pain is complex for its diverse clinical features and pharmacological treatment is the most widely used approach in neuropathic pain [4]. Further research and clinical trials should be implemented to improve the diagnosis and treatment of neuropathic pain. The present study intends to validate the anti-inflammatory effect of dexmedetomidine (DEX) on inflammation-induced neuropathic pain in chronic constriction injury (CCI) rat models.

DEX is a potent and highly selective  $\alpha_2$ -adrenoceptor agonist with sedative, anxiolytic, sympatholytic, and analgesic abilities [5]. This drug is used to induce short- and longer-term sedation among patients in intensive care unit and has effective suppression on delirium [6]. DEX was reported to have the potential for prevention of acute pain in adults performed with abdominal surgery [7]. Moreover, a steadily growing number of studies have investigated the inhibitory effect of DEX on neuropathic pain. For instance, DEX suppressed neuropathic pain by inhibiting P2  $\times$  7R through regulation of ERK in a rat model of CCI [8].

NLRP3, belonging to the protein family of nucleotide-binding oligomerization domain-like receptor (NLR), is one of the pattern recognition receptors which contribute to the formation of inflammasomes [9]. Among those inflammasomes, NLRP3 inflammasome has been intensively investigated and verified to play a critical role in innate immunity and pathology of human diseases [10]. NLRP3 functions as an inflammation promoter through cleavage of pro-inflammatory cytokines IL-1 $\beta$  and IL-18 by stimulating caspase-1 [11]. Peter M. Grace et al. found that morphine promoted the intensity and duration of neuropathic pain by activating NLRP3 inflammasome [12]. However, little attention has been attached to whether NLRP3 was implicated in DEX-induced reduction of inflammatory responses and neuropathic pain.

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a multifunctional protein that modulates antioxidant as well as other cytoprotective genes and is involved in inflammatory processes [13, 14]. Up-regulation of Nrf2 by Plumbagin was found in the alleviation of CCI-induced neuropathic pain [15]. In addition, a new biphenyl diester derivative AB-38b attenuated diabetic nephropathy by suppressing NLRP3 via Nrf2 activation [16]. However, the regulation of NLRP3 through Nrf2 signaling is rarely discussed in neuropathic pain.

A recent study claimed that DEX exerted neuroprotective effect via Nrf2 signaling pathway [17]. The present research utilizes NLRP3 antagonist MCC950, NLRP3 activator Nigericin and Nrf2 inhibitor ML385 to determine the effects of NLRP3 and Nrf2 in neuropathic pain, and verifies that DEX exerted suppression on neuropathic pain by inhibiting NLRP3-mediated inflammation via activation of Nrf2.

## Materials And Methods

### Animal experiments

Male Sprague-Dawley rats (n = 108, 200 ~ 220 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd. The rats were subjected to a 12 h/12 h light-dark cycle (light: 8 am to 8 pm; dark: 8 pm to 8 am) at room temperature (23  $\pm$  1  $^{\circ}$ C) and fed with standard food and water for at least one week. All the animal experiments were approved by the Animal Protection and Use Committee of Hunan Provincial People's Hospital and strictly followed the "Guidelines for the Use and Management of Laboratory Animals" issued by the National Institutes of Health (NIH).

The rats were subjected to sciatic nerve ligation operation after being anesthetized using pentobarbital sodium (40 mg/kg). An incision was made at the biceps femoris in the left thigh to expose the sciatic nerve. Four sterile 4 - 0 chromic gut sutures (ethicon, Somerville, NJ), 1 mm apart, were loosely tied to the proximal end of the trifurcation of the sciatic nerve so long as each suture could detect small twitches of the hind limb. After ligation, the muscle and skin were immediately stitched. Rats that received sciatic nerve ligation were established as chronic constriction injury (CCI) models (Model group). Rats in sham group were sham-operated (no nerve ligation was performed). Rats in Control group were left totally untreated (no surgery or drug treatment).

After model establishment, rats were subjected to corresponding treatments and accordingly grouped into DEX group (intraperitoneally injected with 5 µg/kg DEX for post-operational 7 days, Nhwua Pharmaceutical, Jiangxi, China), MCC950 group (intraperitoneally injected with 50 µg/kg NLRP3 antagonist MCC950 for post-operational 7 days, MedChemExpress, Shanghai, China), Nigericin group (intraperitoneally injected with 1 mg/kg NLRP3 activator Nigericin for post-operational 7 days, MedChemExpress, Shanghai, China), DEX + ML385 group (intraperitoneally injected with 5 µg/kg DEX and 30 mg/kg Nrf2 inhibitor ML385 for post-operational 7 days, Selleck, Shanghai, China), DEX + Nigericin group (intraperitoneally injected with 5 µg/kg DEX and 1 mg/kg Nigericin for post-operational 7 days) and MCC950 + ML385 group (intraperitoneally injected with 50 µg/kg MCC950 and 30 mg/kg ML385 for post-operational 7 days). The animal groups and treatment are listed in Table 2. Rats subjected to behavioral tests received drug treatment for post-operational 14 days. Experiment timetable is shown in Fig. 1.

Table 2  
Corresponding treatment strategy for rats in each group

Groups	Treatment	Behavioral tests	H&E staining	TUNEL	ELISA	Gene expression
Control group (n = 6)	Untreated (no surgery or drug treatment)	√	√	√	√	√
Model group (n = 6)	CCI models	√	√	√	√	√
Sham group (n = 6)	Sham-operated, no nerve ligation was performed	√	√	√	√	√
DEX group (n = 6)	Intraperitoneally injected with 5 µg/kg DEX for post-operational 7 days	√	√	√	√	√
MCC950 group (n = 6)	Intraperitoneally injected with 50 µg/kg MCC950 for post-operational 7 days	√	√	√	√	√
Nigericin group (n = 6)	Intraperitoneally injected with 1 mg/kg Nigericin for post-operational 7 days	√	√	√	√	√
DEX + ML385 group (n = 6)	Intraperitoneally injected with 5 µg/kg DEX and 30 mg/kg ML385 for post-operational 7 days	√	√	√	√	√
DEX + Nigericin group (n = 6)	Intraperitoneally injected with 5 µg/kg DEX and 1 mg/kg Nigericin for post-operational 7 days	√	√	√	√	√
MCC950 + ML385 group (n = 6)	Intraperitoneally injected with 50 µg/kg MCC950 and 30 mg/kg ML385 for post-operational 7 days	√	√	√	√	√

Note: CCI, chronic constriction injury; DEX, dexmedetomidine

### Animal behaviors

Rats were subjected to behavioral tests 0, 1, 3, 7 and 14 after operation. These rats were accordingly assigned into Control, Sham, Model, DEX, MCC950, Nigericin, DEX + ML385, MCC950 + ML385 and DEX + Nigericin groups (n = 6 in each group). The behavioral tests were conducted from 9 am to 12 am in a noiseless environment. The rats were placed in a wire cage for 30 minutes to adjust to the environment before experiment. Mechanical withdrawal threshold (MWT) of the rats was measured every 5 min for three times (each time for 2 s) using automated dynamic plantar aesthesiometer (Ugo Basile, Varese,

Italy). Von Frey filaments were vertically placed on the inner plantar surface of rat's right hind paw and increasing pressure was put on to bend the filaments (from 0.6, 1, 2, 4, 6, 8, 10, 15, 25 to 60 g; break force = 60 g). The minimum retraction force (G) of rat's right hind paw was recorded and the average value of the power that induced a reliable retreat was recorded as threshold. Quick withdrawal or licking of paws in response to stimuli was considered a positive reaction.

### **H&E staining**

Spinal cords (L4 ~ L6) were extracted from rats of the Control, Sham, Model, DEX, MCC950, Nigericin, DEX + ML385, MCC950 + ML385 and DEX + Nigericin groups (n = 6 in each group) 7 days after operation. The spinal cords were fixed in 4% paraformaldehyde and then dehydrated in 30% sucrose at 4 °C overnight. The spinal cords were embedded in paraffin and cut into 5 µm sections (10 slices of each spinal cord) for hematoxylin and eosin staining. Finally, the spinal cord sections were observed using a microscope (Olympus ABX50, Tokyo, Japan). The scoring criteria of spinal cord injury are as follows: 0 = no lesion; 1 = gray matter contained 1 ~ 5 eosinophilic neurons; 2 = gray matter contained 5 ~ 10 eosinophilic neurons; 3 = gray matter contained more than 10 eosinophilic neurons; 4 = infarction of less than 1/3 of the gray matter area; 5 = infarction of 1/3 to 1/2 of the gray matter area; 6 = infarction of more than 1/2 of the gray matter area.

### **TUNEL staining**

Spinal cords (L4 ~ L6) were extracted from rats of the Control, Sham, Model, DEX, MCC950, Nigericin, DEX + ML385, MCC950 + ML385 and DEX + Nigericin groups (n = 6 in each group) 7 days after operation. Ten slices were obtained from each spinal cord, and sections with a thickness of about 5 µm were dewaxed with xylene and dehydrated using gradient alcohol. After that, the spinal sections were treated with proteinase K for 30 min and immersed in blocking solution at room temperature for 10 min. TUNEL detection solution (Beyotime Biotechnology, Shanghai, China) was then added for 60 min of incubation at 37 °C in the dark. Finally, the spinal sections were washed with PBS for three times and observed using a microscope. Apoptosis of 15 randomly selected areas of each spinal cord section was assessed. Apoptosis rate (%) = number of brown cells/total number of cells × 100%.

### **Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)**

Total RNA was extracted from L4-L6 segments of the spinal cords of the rats from the Control, Sham, Model, DEX and DEX + ML385 groups (n = 6 in each group) 7 days after operation using Trizol (Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions. Then the RNAs were reversely transcribed into cDNAs through Reverse Transcription System (Promega, WI, USA). The expressions of the genes were detected using LightCycler 480 (Roche, IN, USA) in accordance with the instructions of the fluorescence quantitative PCR kit (SYBR Green PCR kit, Takara Bio, Inc., Otsu, Japan). The reaction conditions were as follows: 5 min of pre-degeneration at 95°C, followed by 40 cycles of 10 s of degeneration at 95°C, 10 s of annealing at 60°C and 20 s of expansion at 72°C. The relative quantitation was performed using comparative  $2^{-\Delta\Delta Ct}$  method, with GAPDH applied as internal reference. The primer sequences are shown in Table 1.

Table 1  
Primer sequences

Name	Sequence
Nrf2	F: 5'- AGGTTGCCACATTCCCAAA-3'
	R: 5'- AGTGACTGAAACGTAGCCGA-3'
GAPDH	F: 5'-TTCTGGGATACACGGAGCAC-3'
	R: 5'-TACCAGCACCAGCGTCAAAG-3'
Note: F, forward; R, reverse	

## Western blot

The L4-L6 segments of the spinal cords extracted from rats of the Control, Sham, Model, DEX, MCC950, Nigericin, DEX + ML385, MCC950 + ML385 and DEX + Nigericin groups (n = 6 in each group) 7 days after operation were mixed with RIPA buffer containing protease inhibitor and phosphatase inhibitor. Then the segments were centrifuged at 13000 rpm for 30 min at 4°C for obtaining of total proteins or nucleoprotein using nuclear protein extraction kit (Solarbio Science and Technology Corporation, Beijing, China). The concentration of proteins was measured using a BCA kit. Then the proteins, separated by SDS-PAGE, were transferred onto a PVDF membrane for co-incubation with primary antibodies (abcam, Cambridge, UK) of GAPDH (1:10000, ab181602), Nrf2 (1:100, ab137550), NLRP3 (1:500, ab214185) and HO-1 (#82206, 1:1000, Cell signaling technology, Danvers, USA) at 4°C overnight. After being washed with PBST for three times, the membrane was incubated with the secondary antibodies of goat anti rabbit IgG (1:5000, Beijing ComWin Biotech Co.,Ltd., Beijing, China) or goat anti rat IgG (1:2000, ab205719) at room temperature for 30 min. Finally, the membrane was subjected to washing with PBST for four times and allowed for color development with ECL. The brands were then detected using chemiluminescence imaging system (GE Healthcare, Beijing, China).

## ELISA

The whole blood of rats from the Control, Sham, Model, DEX, MCC950, Nigericin, DEX + ML385, MCC950 + ML385 and DEX + Nigericin groups (n = 6 in each group) was mixed with EDTA and then centrifuged at 1000 g for 10 min at 4°C to remove the yellow supernatant. The expressions of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10 were detected using a commercial ELISA kit (R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions.

## Statistical analysis

The statistics were analyzed by GraphPad Prism 7.0. T was used to compare differences between two groups. One-way analysis of variance was applied for multi-group comparison.  $P < 0.05$  was considered statistically significant.

# Results

## **DEX reduces neuropathic pain of CCI rats**

Mechanical withdrawal threshold (MWT) was measured to assess rats' sensitivity to pain. MWT of rats was decreased in Model group compared with Control group while increased in DEX group compared with Model group (Fig. 2A,  $P < 0.05$ ). This result indicated that rats in Model group had the highest pain sensitivity whereas DEX reduced the pain of CCI rats.

H&E staining showed that the spinal injury area was expanded in Model group compared with Control group while lessened in DEX group compared with Model group (Fig. 2B,  $P < 0.05$ ). TUNEL positive cells were decreased in Model group compared with Control group while increased in DEX group compared with Model group (Fig. 2C,  $P < 0.05$ ), suggesting DEX could decrease cell apoptosis in spinal cord tissues of CCI rats. TNF- $\alpha$ , IL-1 $\beta$  and IL-6 were increased in Model group compared with Control group while decreased in DEX group compared with Model group (Fig. 2D,  $P < 0.05$ ). The expression pattern of IL-10 was different from those pro-inflammatory cytokines (Fig. 2D,  $P < 0.05$ ). According to the results of qRT-PCR and Western blot, Nrf2 was down-regulated in Model group compared with Control group while up-regulated in DEX group compared with Model group (Fig. 2E-F,  $P < 0.05$ ) Meanwhile, different expression pattern of NLRP3 was also detected (Fig. 2F,  $P < 0.05$ ). There was no significant difference between Sham group and Control group. The above results demonstrate that DEX suppresses inflammation in the spinal cords to reduce neuropathic pain of CCI rats.

## **NLRP3 increases neuropathic pain of CCI rats**

After CCI rats were injected with NLRP3 antagonist MCC950 or NLRP3 activator Nigericin, MWT was found to be increased in MCC950 group while decreased in Nigericin group (Fig. 3A,  $P < 0.05$ , vs Model group). Western blot detected down-regulated NLRP3 in MCC950 group and up-regulated NLRP3 in Nigericin group (Fig. 3B,  $P < 0.05$ , vs Model group). The result demonstrated the successful suppression or activation of NLRP3 in CCI rats. H&E staining showed that the spinal injury was reduced in MCC950 group while expanded in Nigericin group (Fig. 3C,  $P < 0.05$ , vs Model group). According to the results of TUNEL staining, apoptosis was suppressed in MCC950 group while promoted in Nigericin group (Fig. 3D,  $P < 0.05$ , vs Model group). Decreases of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and increase of IL-10 were found in MCC950 group; different expression patterns of these cytokines were detected in Nigericin group (Fig. 3E,  $P < 0.05$ , vs Model group). Given the above, NLRP3 can promote neuropathic pain of CCI rats.

## **DEX reduces neuropathic pain by activating Nrf2 in CCI rats**

After CCI rats were injected with Nrf2 inhibitor ML385 followed by DEX treatment, rats in DEX + ML385 group had lower MWT compared to DEX group (Fig. 4A,  $P < 0.05$ ). Western blot and qRT-PCR tested the expression of Nrf2 (Fig. 4B-C,  $P < 0.05$ ). Nrf2 was down-regulated in DEX + ML385 group compared with DEX group, indicating the effectiveness of ML385 injection. According to H&E staining and TUNEL staining respectively, spinal injury and apoptosis were aggravated in DEX + ML385 group (Fig. 4D-E,  $P < 0.05$ , vs DEX group). TNF- $\alpha$ , IL-1 $\beta$  and IL-6 were over-expressed while IL-10 was under-expressed in DEX + ML385 group (Fig. 4F,  $P < 0.05$ , vs DEX group). Moreover, NLRP3 was also up-regulated in DEX + ML385

group (Fig. 4G,  $P < 0.05$ , vs DEX group). To conclude, DEX promotes the expression of Nrf2 to relieve neuropathic pain of CCI rats.

### **DEX releases neuropathic pain of CCI rats by suppressing NLRP3 via activation of Nrf2**

To investigate the pain relief mechanism of DEX, CCI rats were injected with MCC950 + ML385 or Nigericin + DEX. First, MWT was decreased in MCC950 + ML385 group and increased in Nigericin + DEX group (Fig. 5A,  $P < 0.05$ , vs MCC950 group and Nigericin group respectively). Western blot detected up-regulated NLRP3 in MCC950 + ML385 group compared to MCC950 group and down-regulated NLRP3 in Nigericin + DEX group compared to Nigericin group (Fig. 5B,  $P < 0.05$ ). Spinal cords injury and apoptosis were enhanced in MCC950 + ML385 group while suppressed in Nigericin + DEX group (Fig. 5C-D,  $P < 0.05$ , vs MCC950 group and Nigericin group respectively). Moreover, increases of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and decrease of IL-10 were found in MCC950 + ML385 group; different expression patterns of these cytokines were detected in Nigericin + DEX group (Fig. 5E,  $P < 0.05$ , vs MCC950 group and Nigericin group respectively). Taken together, DEX inhibits NLRP3 by activating Nrf2 to suppress neuropathic pain of CCI rats.

## **Discussion**

Over the past decades, pharmacotherapy has been considerably recommended for management of neuropathic pain but still with clinical deficiency [18]. The present study is committed to discovery of the mechanism of DEX in relieving neuropathic pain. Collected evidence in this study supports that DEX inhibits NLRP3 by stimulating Nrf2 to suppress neuropathic pain in CCI rat models.

First of all, we demonstrated the neuroprotective role of DEX in a rat model of neuropathic pain. DEX reduced TNF- $\alpha$ , IL-1 $\beta$  and IL-6 while increased IL-10 in CCI rats; meanwhile, the down-regulation of NLRP3 and up-regulation of Nrf2 were also detected. DEX was found to attenuate neuropathic pain by progressing anti-inflammatory activity in chronic constriction injury [19]. Consistent with our results, H.S.M. Farghaly et al. demonstrated that DEX decreased TNF- $\alpha$  and IL-6 to relieve neuropathic pain [20].

Abnormal expression pattern of NLRP3 was also found in CCI rat model which encourage us to dig the possible relationship between NLRP3 and DEX or the mechanism for implication of NLRP3 in neuropathic pain. NLRP3 was found to promote neuropathic pain and inflammatory responses in CCI rats. Despite the absence of research on interaction between DEX and NLRP3, involvement of NLRP3 has been intensively investigated in research on neuropathic pain. For instance, microRNA-223 ameliorated morphine analgesic tolerance to neuropathic pain by down-regulating NLRP3 [21]. Paclitaxel activated neuropathic pain by stimulating NLRP3 inflammasome and pro-inflammatory factor IL-1 $\beta$  [22].

Afterwards, we confirmed that DEX relieved neuropathic pain via the activation of Nrf2. The DEX-treated CCI rats after receiving ML385 obtained an aggravation of neuropathic pain as well as up-regulation of NLRP3. In accordance with our study, the expression of Nrf2 was lowered in a rat model of chronic neuropathic pain [23]. Nrf2 may alleviate oxaliplatin-induced peripheral neuropathy by maintaining

mitochondrial homeostasis and suppressing oxidative stress [24]. However, there is lack of research on the regulation of Nrf2 in DEX-induced neuroprotection.

The NLRP3/Nrf2 pathway has been considerably discussed in many pharmacological and pathological conditions such as liver injury [25] and colitis [26]. In the present study, NLRP3 was up-regulated in MCC950-treated CCI rats in response to ML385 injection, indicating the suppressive effect of Nrf2 on NLRP3 expression. Furthermore, DEX in this study was proved to down-regulate NLRP3 in Nigericin-treated CCI rats with reduced MWT as well as attenuated spinal cord injury and inflammatory responses. As the protective effect of Nrf2 is demonstrated in the above paragraph, we finally drew a conclusion that DEX released neuropathic pain of CCI rats by suppressing NLRP3 via activation of Nrf2. Additionally, Nrf2 was found to modulate HO-1 in the suppression of murine lupus nephritis by dietary oleuropein and peracetylated oleuropein [27]. Further research could be done to explore if there is a downstream target of Nrf2 in the management of neuropathic pain.

## Conclusions

In summary, DEX ameliorates inflammation-induced neuropathic pain in CCI rat models. The NLRP3/Nrf2 pathway is verified to modulate neuropathic pain and inflammatory responses. We demonstrate that DEX inhibits neuropathic pain by activating Nrf2 via the suppression of NLRP3. The revealing of the mechanism of DEX may alleviate the burden of neuropathic pain in patients and improve the pharmacotherapeutic treatment of neuropathy.

## Abbreviations

DEX: Dexmedetomidine

NLR: nucleotide-binding oligomerization domain-like receptor

Nrf2: Nuclear factor-erythroid 2-related factor 2

NIH: National Institutes of Health

CCI: Chronic constriction injury

MWT: Mechanical withdrawal threshold

## Declarations

### Acknowledgements

Thanks for all the contributors and participants.

### Authors' contribution

SWY conceived the ideas. SWY designed the experiments. LXY performed the experiments. TYX analyzed the data. LJT and SWY provided critical materials. SWY and LXY wrote the manuscript. LJT supervised the study. All the authors have read and approved the final version for publication.

## **Funding**

This study was funded by the grant from Clinical Research Center for Anesthesiology of ERAS in Hunan Province (Grant No. 2018SK7001).

## **Availability of data and materials**

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

## **Ethical approval and consent to participate**

All the animal experiments were approved by the Animal Protection and Use Committee of Hunan Provincial People's Hospital and strictly followed the "Guidelines for the Use and Management of Laboratory Animals" issued by the National Institutes of Health (NIH).

## **Consent for publication**

Not applicable

## **Competing interest**

The authors declare there is no conflict of interest.

## **References**

1. Chen WW, Zhang X, Huang WJ. Role of neuroinflammation in neurodegenerative diseases (Review). *Mol Med Rep.* 2016;13:3391-3396.
2. Ji RR, Nackley A, Huh Y, Terrando N, Maixner W. Neuroinflammation and Central Sensitization in Chronic and Widespread Pain. *Anesthesiology.* 2018;129:343-366.
3. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, et al. Neuropathic pain. *Nat Rev Dis Primers.* 2017;3:17002.
4. Gilron I, Baron R, Jensen T. Neuropathic pain: principles of diagnosis and treatment. *Mayo Clin Proc.* 2015;90:532-545.
5. Weerink MAS, Struys M, Hannivoort LN, Barends CRM, Absalom AR, Colin P. Clinical Pharmacokinetics and Pharmacodynamics of Dexmedetomidine. *Clin Pharmacokinet.* 2017;56:893-913.
6. Keating GM. Dexmedetomidine: A Review of Its Use for Sedation in the Intensive Care Setting. *Drugs.* 2015;75:1119-1130.

7. Jessen Lundorf L, Korvenius Nedergaard H, Moller AM. Perioperative dexmedetomidine for acute pain after abdominal surgery in adults. *Cochrane Database Syst Rev.* 2016;2:CD010358.
8. Lin JP, Chen CQ, Huang LE, Li NN, Yang Y, Zhu SM, et al. Dexmedetomidine Attenuates Neuropathic Pain by Inhibiting P2X7R Expression and ERK Phosphorylation in Rats. *Exp Neurobiol.* 2018;27:267-276.
9. Jo EK, Kim JK, Shin DM, Sasakawa C. Molecular mechanisms regulating NLRP3 inflammasome activation. *Cell Mol Immunol.* 2016;13:148-159.
10. He Y, Hara H, Nunez G. Mechanism and Regulation of NLRP3 Inflammasome Activation. *Trends Biochem Sci.* 2016;41:1012-1021.
11. Coll RC, Robertson AA, Chae JJ, Higgins SC, Munoz-Planillo R, Inserra MC, et al. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat Med.* 2015;21:248-255.
12. Grace PM, Strand KA, Galer EL, Urban DJ, Wang X, Baratta MV, et al. Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation. *Proc Natl Acad Sci U S A.* 2016;113:E3441-3450.
13. Ahmed SM, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: Pivotal roles in inflammation. *Biochim Biophys Acta Mol Basis Dis.* 2017;1863:585-597.
14. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, et al. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. *J Clin Invest.* 2006;116:984-995.
15. Arruri V, Komirishetty P, Areti A, Dungavath SKN, Kumar A. Nrf2 and NF-kappaB modulation by Plumbagin attenuates functional, behavioural and biochemical deficits in rat model of neuropathic pain. *Pharmacol Rep.* 2017;69:625-632.
16. Du L, Wang J, Chen Y, Li X, Wang L, Li Y, et al. Novel biphenyl diester derivative AB-38b inhibits NLRP3 inflammasome through Nrf2 activation in diabetic nephropathy. *Cell Biol Toxicol.* 2019;
17. Li F, Wang X, Zhang Z, Zhang X, Gao P. Dexmedetomidine Attenuates Neuroinflammatory-Induced Apoptosis after Traumatic Brain Injury via Nrf2 signaling pathway. *Ann Clin Transl Neurol.* 2019;6:1825-1835.
18. Attal N, Bouhassira D. Pharmacotherapy of neuropathic pain: which drugs, which treatment algorithms? *Pain.* 2015;156 Suppl 1:S104-114.
19. Liang F, Liu M, Fu X, Zhou X, Chen P, Han F. Dexmedetomidine attenuates neuropathic pain in chronic constriction injury by suppressing NR2B, NF-kappaB, and iNOS activation. *Saudi Pharm J.* 2017;25:649-654.
20. Farghaly HS, Mahmoud AM, Abdel-Sater KA. Effect of dexmedetomidine and cold stress in a rat model of neuropathic pain: Role of interleukin-6 and tumor necrosis factor-alpha. *Eur J Pharmacol.* 2016;776:139-145.
21. Xie XJ, Ma LG, Xi K, Fan DM, Li JG, Zhang Q, et al. Effects of microRNA-223 on morphine analgesic tolerance by targeting NLRP3 in a rat model of neuropathic pain. *Mol Pain.*

2017;13:1744806917706582.

22. Jia M, Wu C, Gao F, Xiang H, Sun N, Peng P, et al. Activation of NLRP3 inflammasome in peripheral nerve contributes to paclitaxel-induced neuropathic pain. *Mol Pain*. 2017;13:1744806917719804.
23. Li S, Yang C, Fang X, Zhan G, Huang N, Gao J, et al. Role of Keap1-Nrf2 Signaling in Anhedonia Symptoms in a Rat Model of Chronic Neuropathic Pain: Improvement With Sulforaphane. *Front Pharmacol*. 2018;9:887.
24. Yang Y, Luo L, Cai X, Fang Y, Wang J, Chen G, et al. Nrf2 inhibits oxaliplatin-induced peripheral neuropathy via protection of mitochondrial function. *Free Radic Biol Med*. 2018;120:13-24.
25. Pan CW, Pan ZZ, Hu JJ, Chen WL, Zhou GY, Lin W, et al. Mangiferin alleviates lipopolysaccharide and D-galactosamine-induced acute liver injury by activating the Nrf2 pathway and inhibiting NLRP3 inflammasome activation. *Eur J Pharmacol*. 2016;770:85-91.
26. Liu X, Zhou W, Zhang X, Lu P, Du Q, Tao L, et al. Dimethyl fumarate ameliorates dextran sulfate sodium-induced murine experimental colitis by activating Nrf2 and suppressing NLRP3 inflammasome activation. *Biochem Pharmacol*. 2016;112:37-49.
27. Castejon ML, Sanchez-Hidalgo M, Aparicio-Soto M, Montoya T, Martin-LaCave I, Fernandez-Bolanos JG, et al. Dietary oleuropein and its new acyl-derivate attenuate murine lupus nephritis through HO-1/Nrf2 activation and suppressing JAK/STAT, NF-kappaB, MAPK and NLRP3 inflammasome signaling pathways. *J Nutr Biochem*. 2019;74:108229.

## Figures

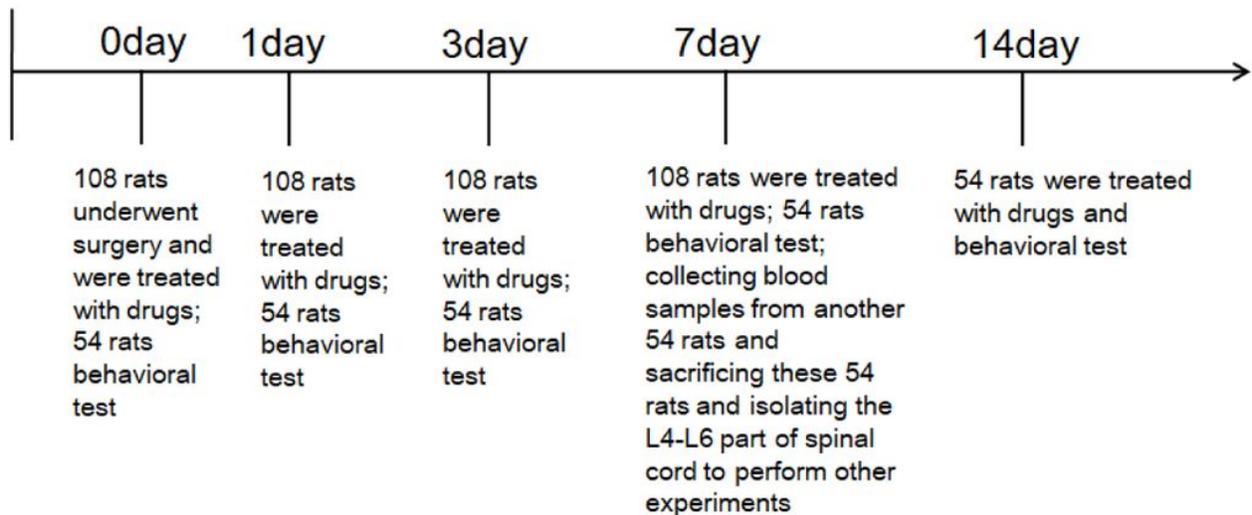


Figure 1

## Experiment schedule

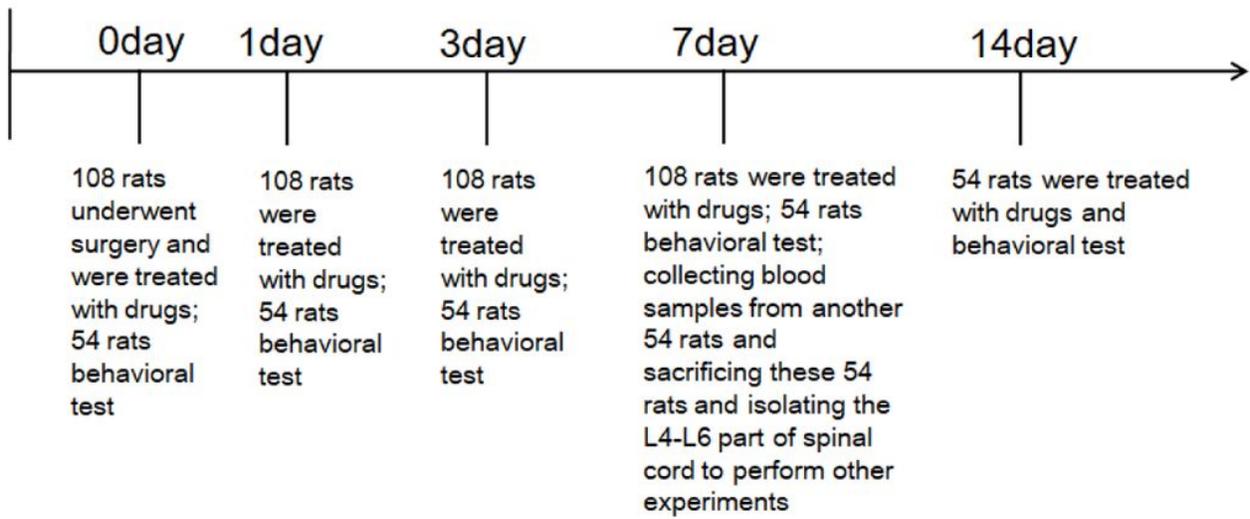


Figure 1

## Experiment schedule

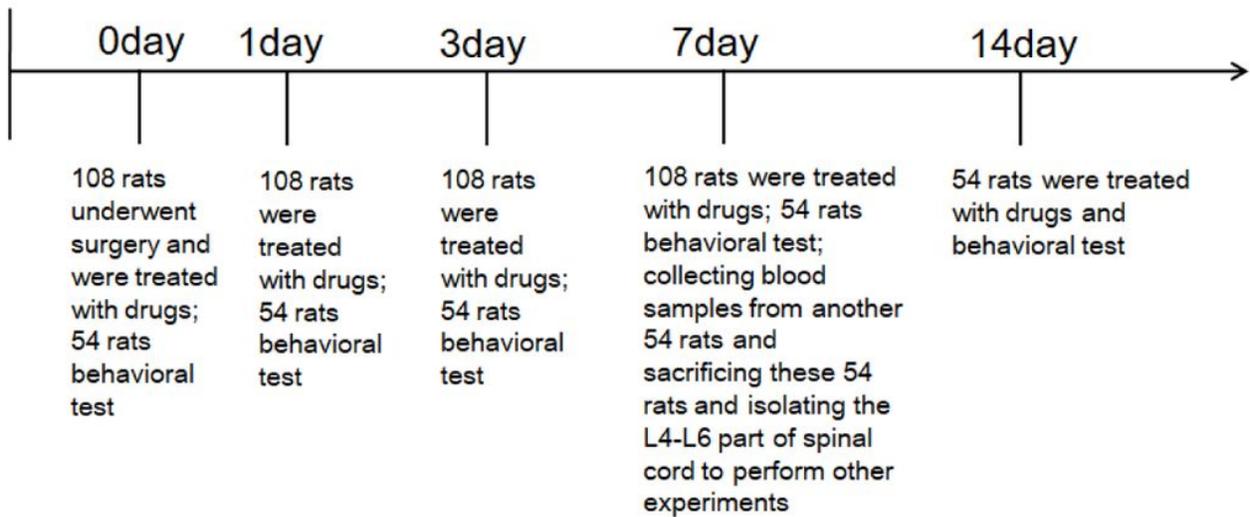
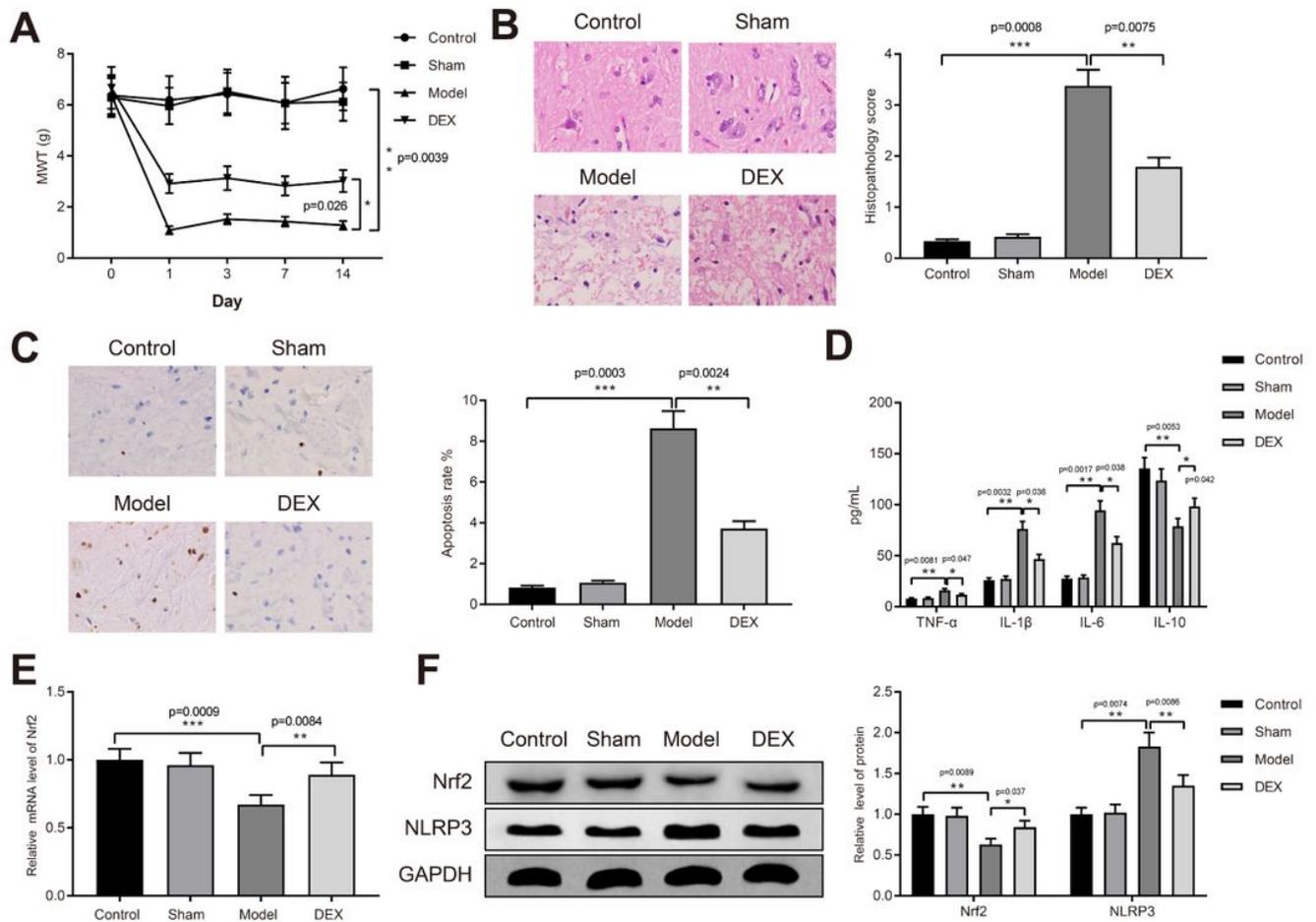


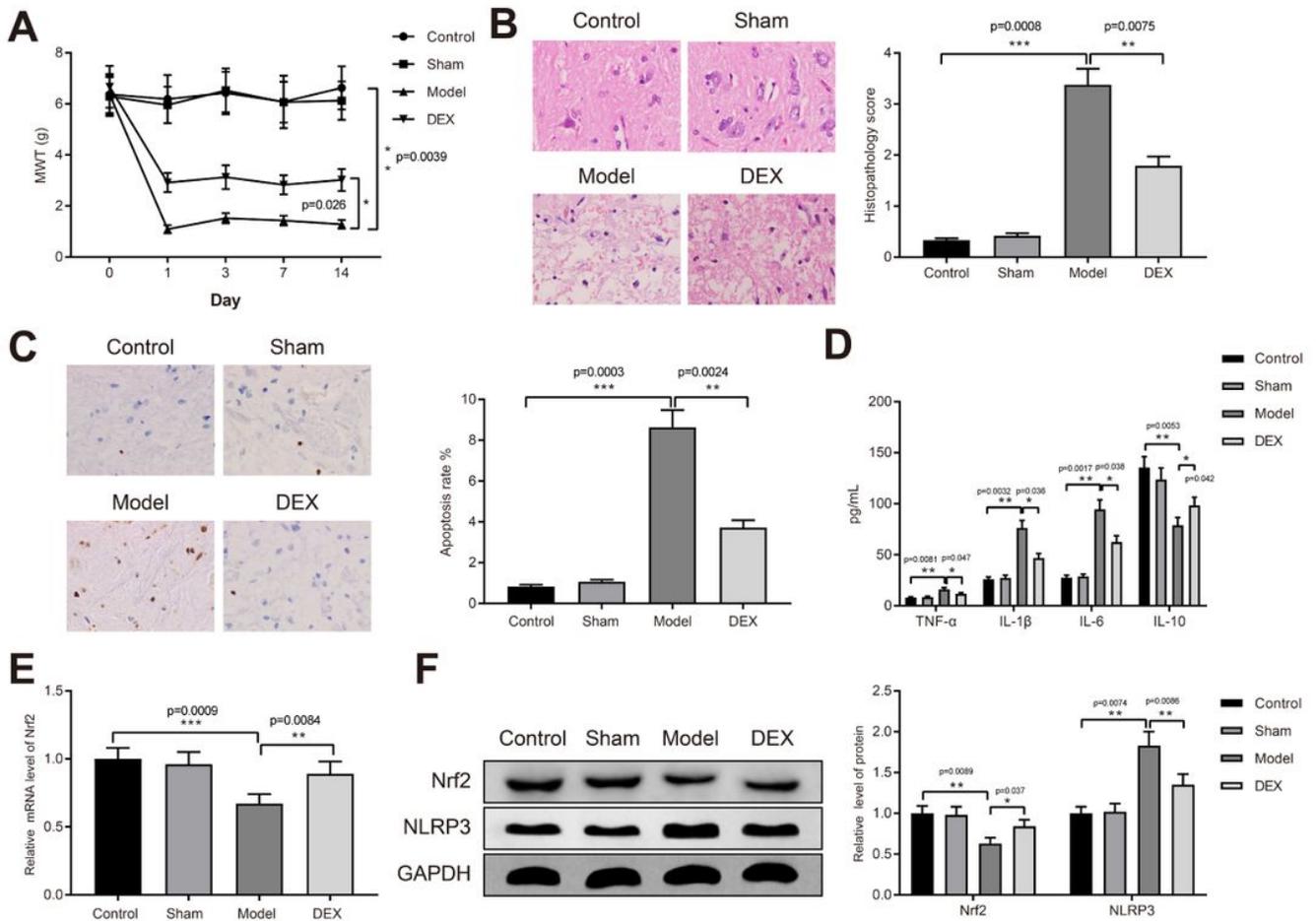
Figure 1

## Experiment schedule



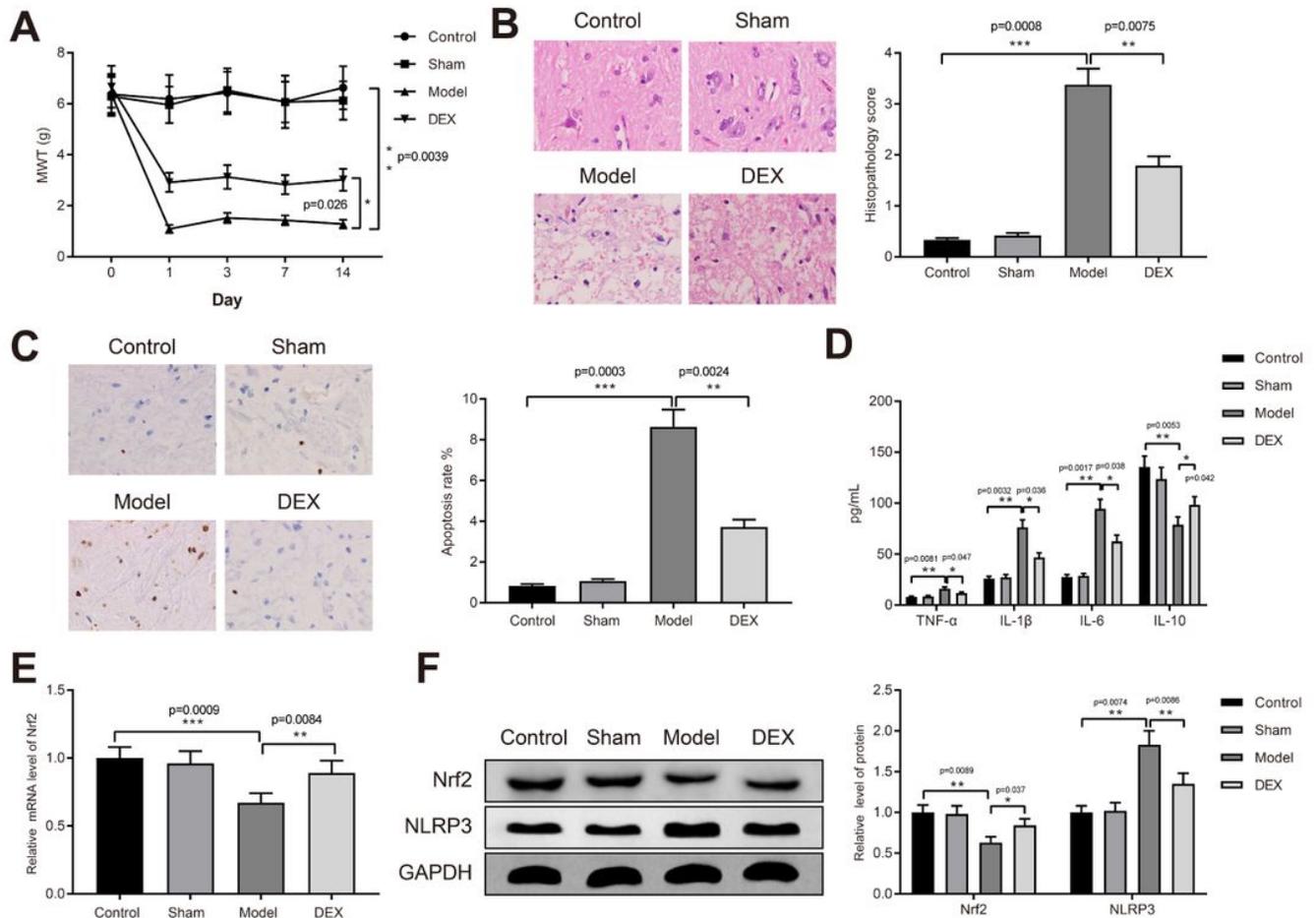
**Figure 2**

DEX reduces neuropathic pain of CCI rats Notes: CCI rats were injected with DEX before MWT (A) and spinal cords injury (B) were assessed ( $\times 400$ ). (C) TUNEL staining tested apoptosis in spinal cords ( $\times 400$ ). (D) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. (E) qRT-PCR detected the expression of Nrf2 in the spinal cords. (F) Western blot detected the protein levels of Nrf2 and NLRP3. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared to Control group or Model group; data are presented as mean  $\pm$  standard deviation (SD); n = 6. DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



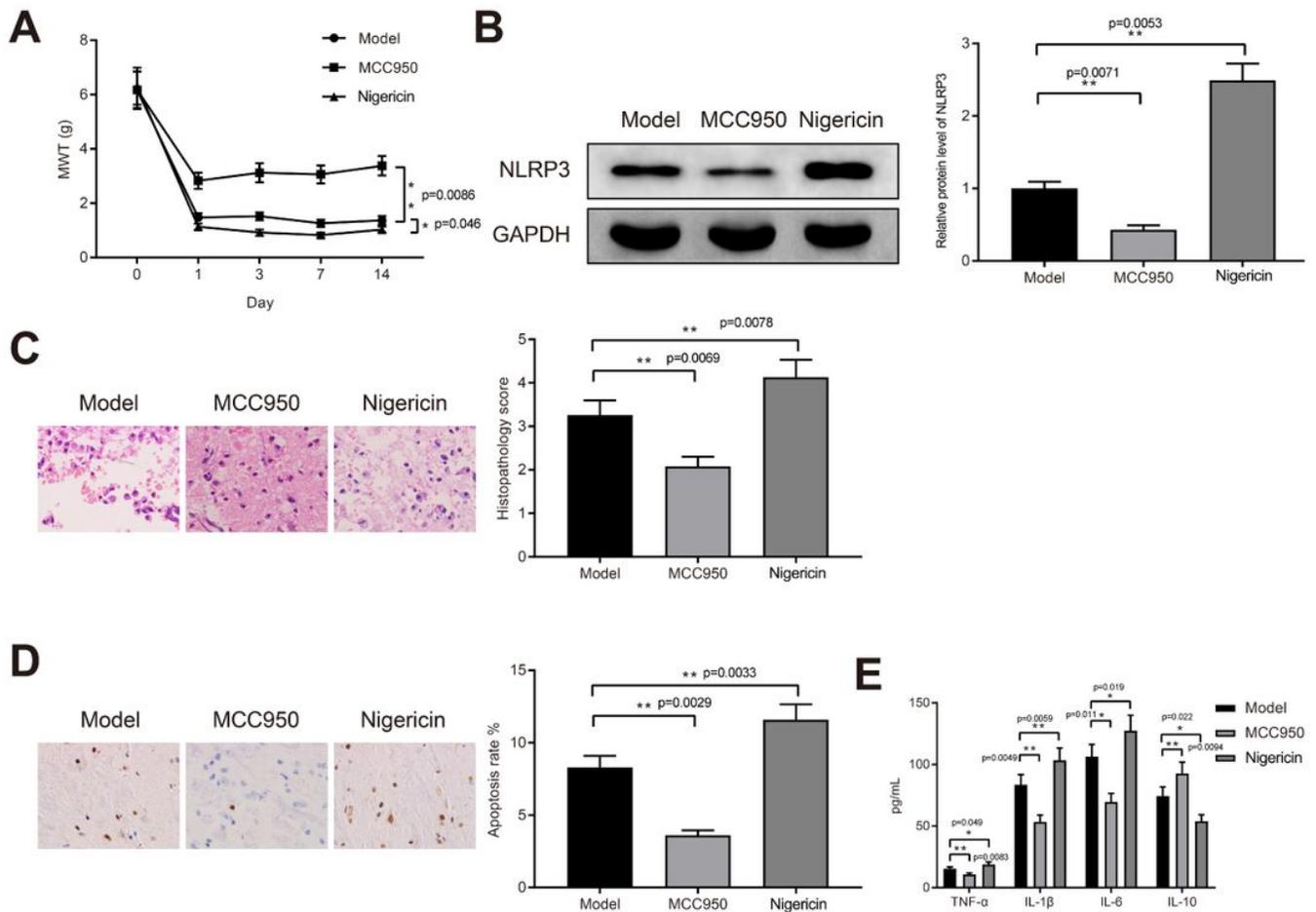
**Figure 2**

DEX reduces neuropathic pain of CCI rats Notes: CCI rats were injected with DEX before MWT (A) and spinal cords injury (B) were assessed ( $\times 400$ ). (C) TUNEL staining tested apoptosis in spinal cords ( $\times 400$ ). (D) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. (E) qRT-PCR detected the expression of Nrf2 in the spinal cords. (F) Western blot detected the protein levels of Nrf2 and NLRP3. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared to Control group or Model group; data are presented as mean  $\pm$  standard deviation (SD); n = 6. DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



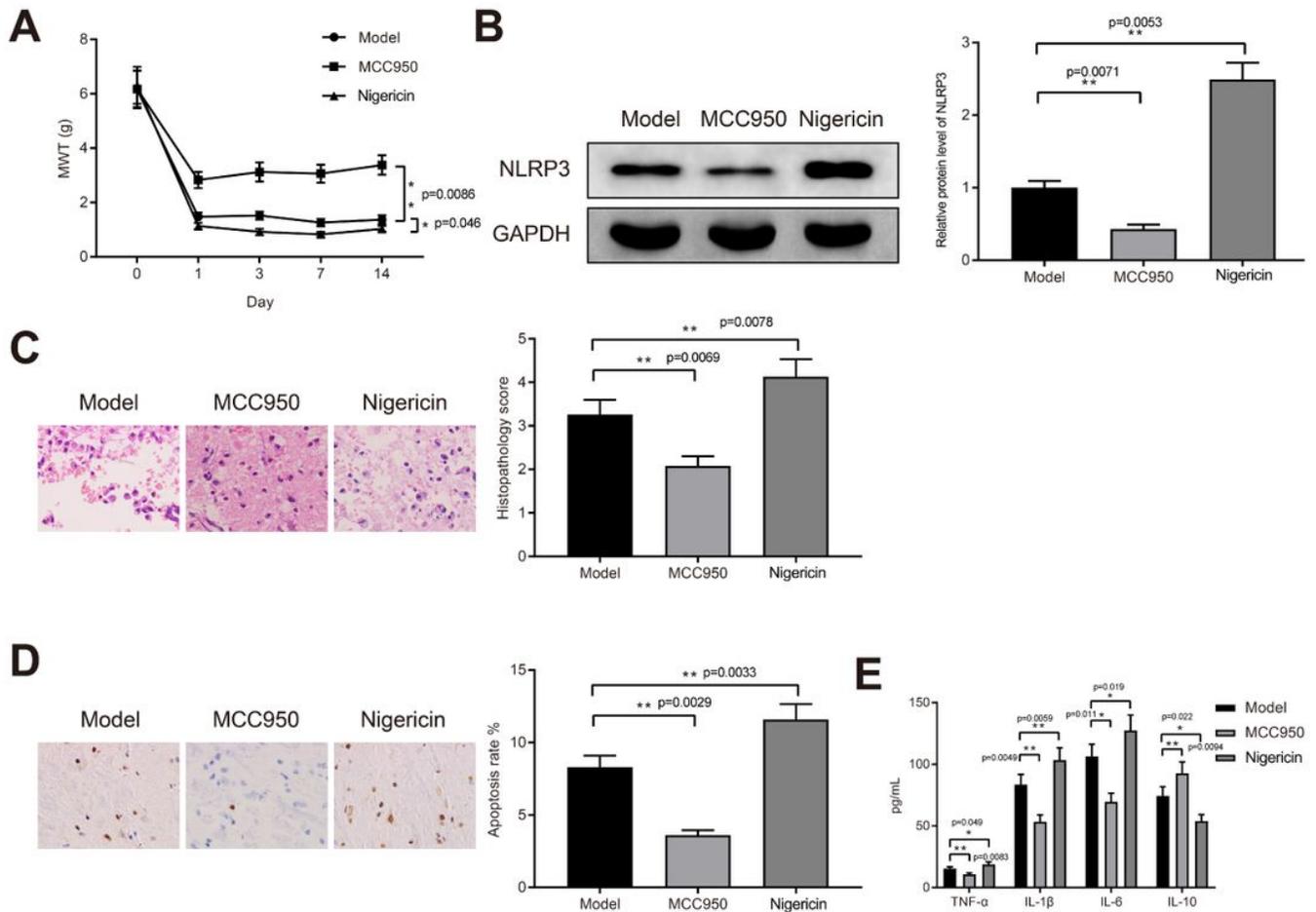
**Figure 2**

DEX reduces neuropathic pain of CCI rats Notes: CCI rats were injected with DEX before MWT (A) and spinal cords injury (B) were assessed ( $\times 400$ ). (C) TUNEL staining tested apoptosis in spinal cords ( $\times 400$ ). (D) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. (E) qRT-PCR detected the expression of Nrf2 in the spinal cords. (F) Western blot detected the protein levels of Nrf2 and NLRP3. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared to Control group or Model group; data are presented as mean  $\pm$  standard deviation (SD); n = 6. DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



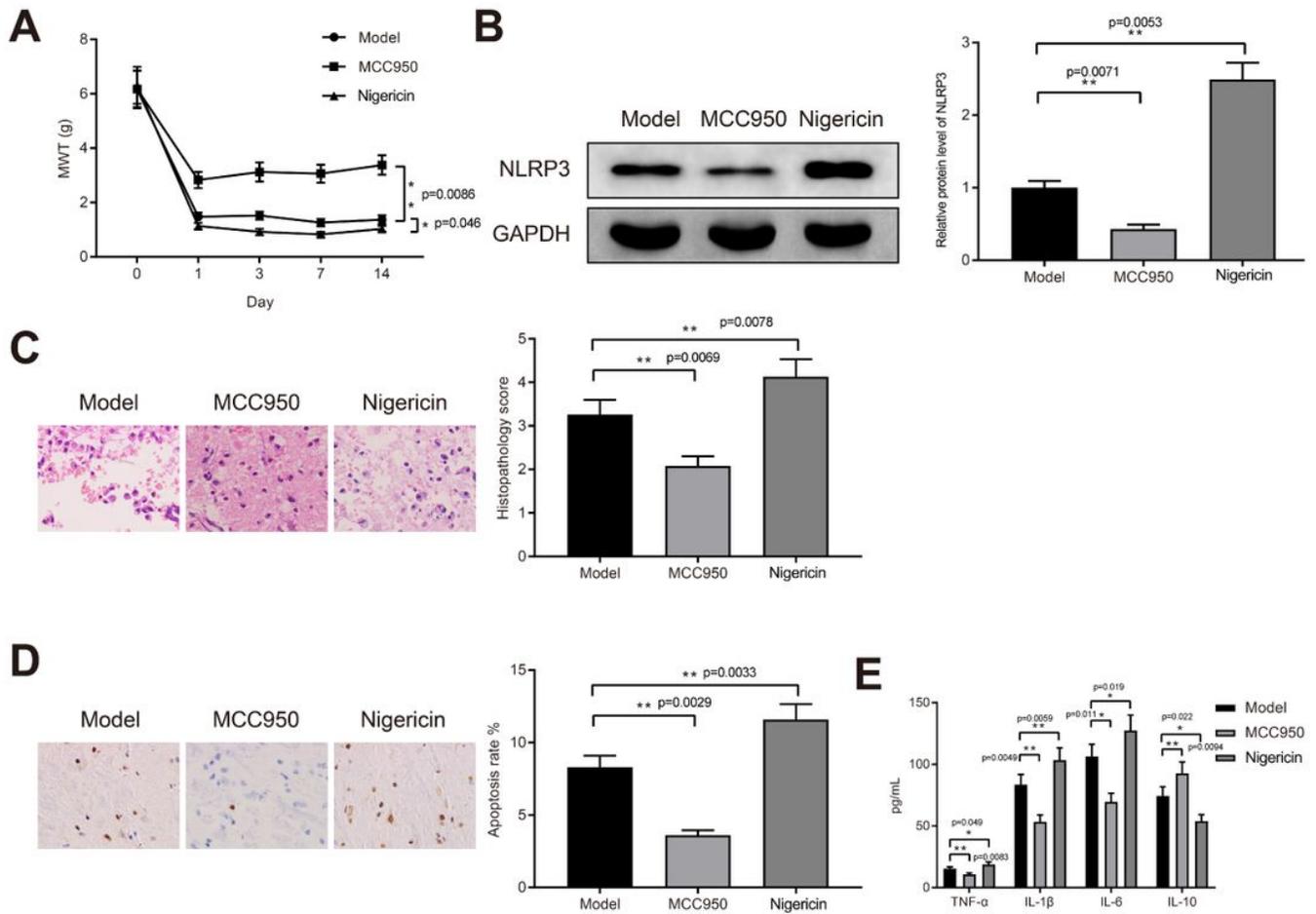
**Figure 3**

NLRP3 increases neuropathic pain of CCI rats Notes: CCI rats were injected with NLRP3 antagonist MCC950 or NLRP3 activator Nigericin. (A) MWT of the rats was tested in response to mechanical stimulation. (B) Western blot tested the expression of NLRP3 in spinal cords. (C) H&E staining tested spinal cords injury ( $\times 400$ ). (D) TUNEL staining tested apoptosis in the spinal cords ( $\times 400$ ). (E) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. \*P < 0.05, \*\*P < 0.01, compared to Model group; data are presented as mean  $\pm$  SD; n = 6. CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



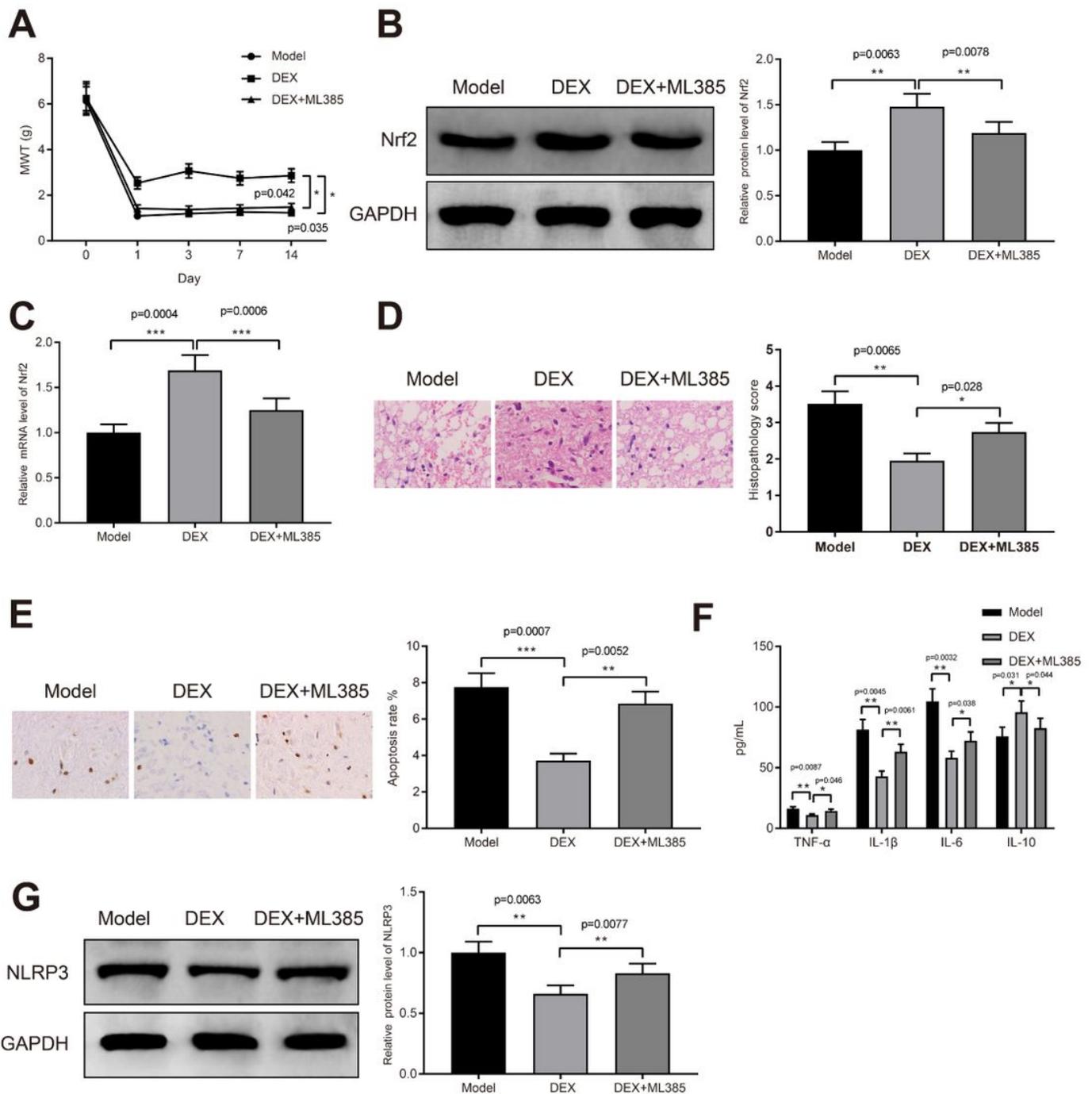
**Figure 3**

NLRP3 increases neuropathic pain of CCI rats Notes: CCI rats were injected with NLRP3 antagonist MCC950 or NLRP3 activator Nigericin. (A) MWT of the rats was tested in response to mechanical stimulation. (B) Western blot tested the expression of NLRP3 in spinal cords. (C) H&E staining tested spinal cords injury ( $\times 400$ ). (D) TUNEL staining tested apoptosis in the spinal cords ( $\times 400$ ). (E) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. \*P < 0.05, \*\*P < 0.01, compared to Model group; data are presented as mean  $\pm$  SD; n = 6. CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



**Figure 3**

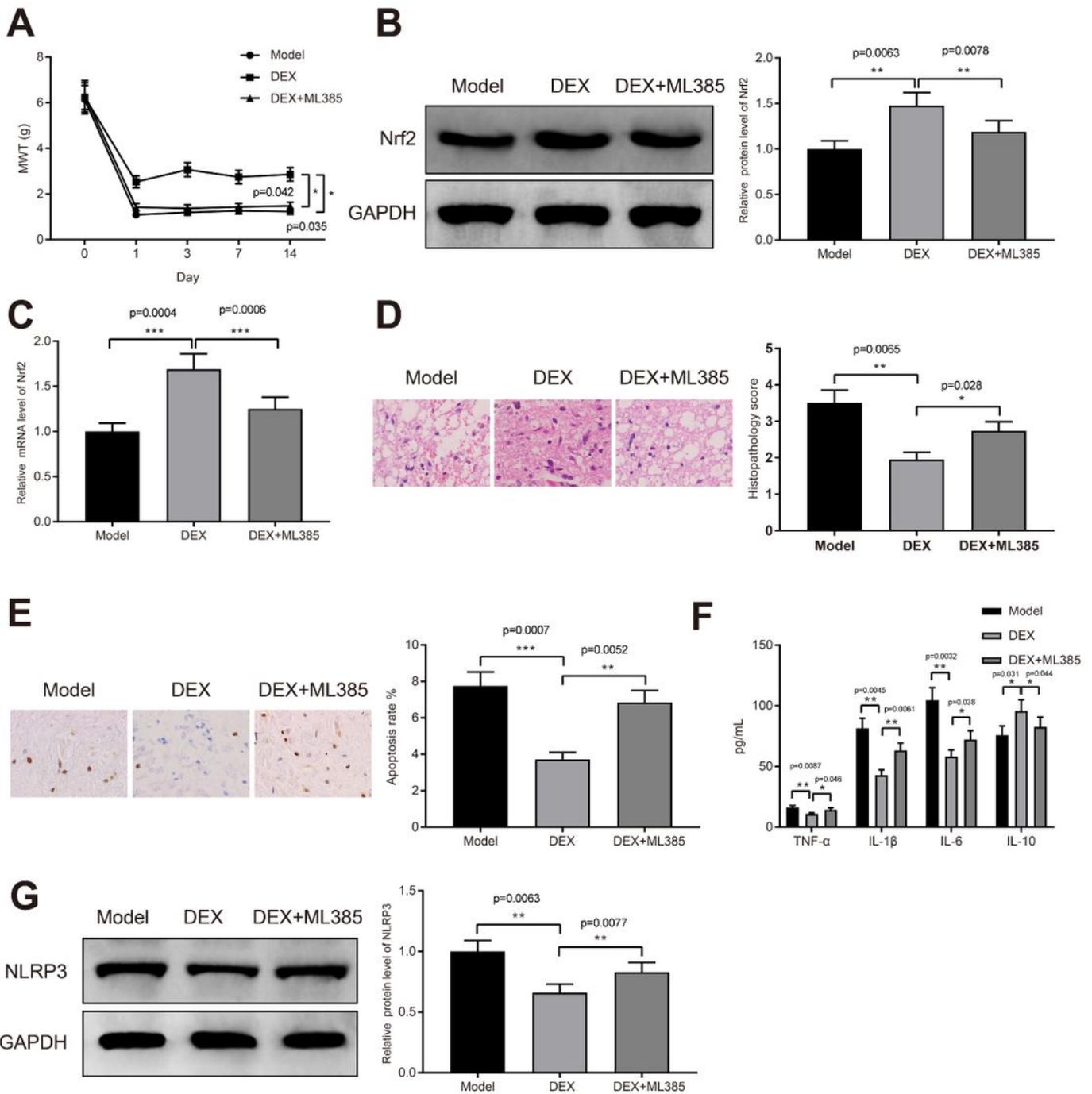
NLRP3 increases neuropathic pain of CCI rats Notes: CCI rats were injected with NLRP3 antagonist MCC950 or NLRP3 activator Nigericin. (A) MWT of the rats was tested in response to mechanical stimulation. (B) Western blot tested the expression of NLRP3 in spinal cords. (C) H&E staining tested spinal cords injury ( $\times 400$ ). (D) TUNEL staining tested apoptosis in the spinal cords ( $\times 400$ ). (E) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. \*P < 0.05, \*\*P < 0.01, compared to Model group; data are presented as mean  $\pm$  SD; n = 6. CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



**Figure 4**

DEX reduces neuropathic pain by activating Nrf2 in CCI rats Notes: CCI rats were injected with DEX and Nrf2 inhibitor ML385. (A) MWT of the rats was tested in response to mechanical stimulation. Western blot (B) and qRT-PCR (C) detected the expression of Nrf2 in spinal cords. (D) H&E staining tested spinal cords injury ( $\times 400$ ). (E) TUNEL staining tested apoptosis in the spinal cords ( $\times 400$ ). (F) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. (G) Western blot detected the expression of NLRP3 in the spinal cords. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared to Model group or DEX group; data are

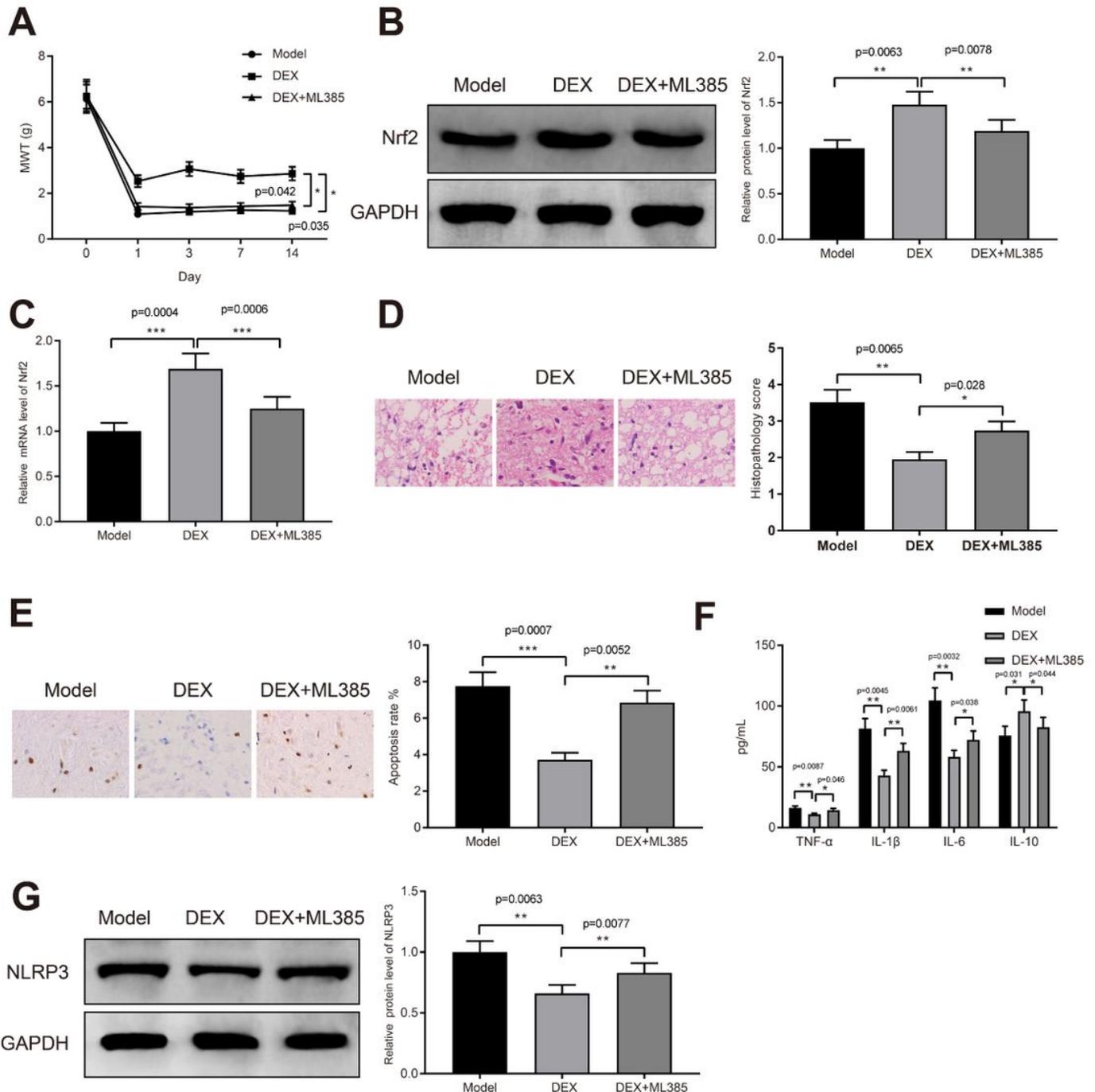
presented as mean  $\pm$  SD; n = 6. DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



**Figure 4**

DEX reduces neuropathic pain by activating Nrf2 in CCI rats Notes: CCI rats were injected with DEX and Nrf2 inhibitor ML385. (A) MWT of the rats was tested in response to mechanical stimulation. Western blot (B) and qRT-PCR (C) detected the expression of Nrf2 in spinal cords. (D) H&E staining tested spinal cords injury ( $\times$  400). (E) TUNEL staining tested apoptosis in the spinal cords ( $\times$  400). (F) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. (G) Western blot detected the expression of NLRP3 in the

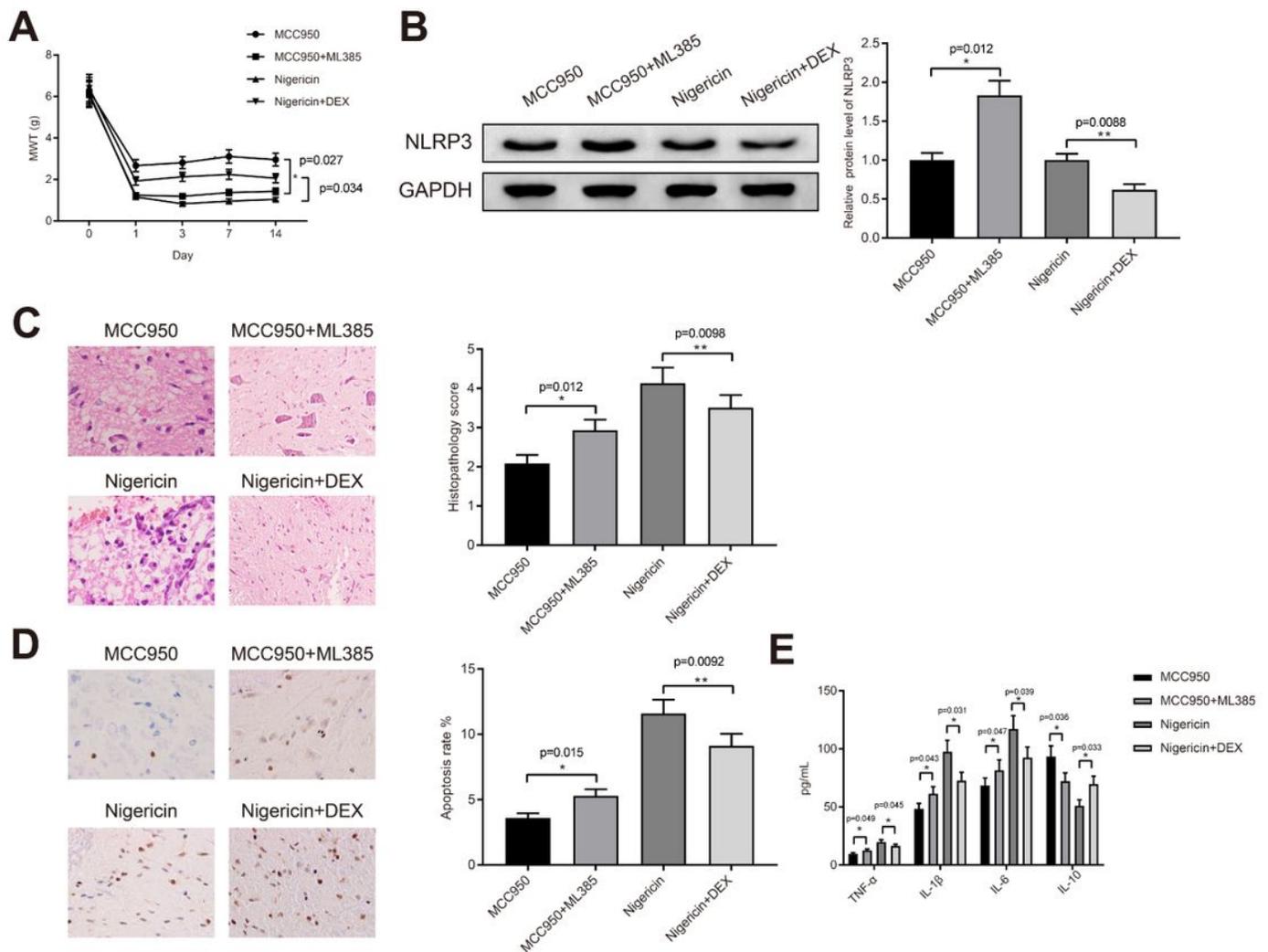
spinal cords. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared to Model group or DEX group; data are presented as mean ± SD; n = 6. DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



**Figure 4**

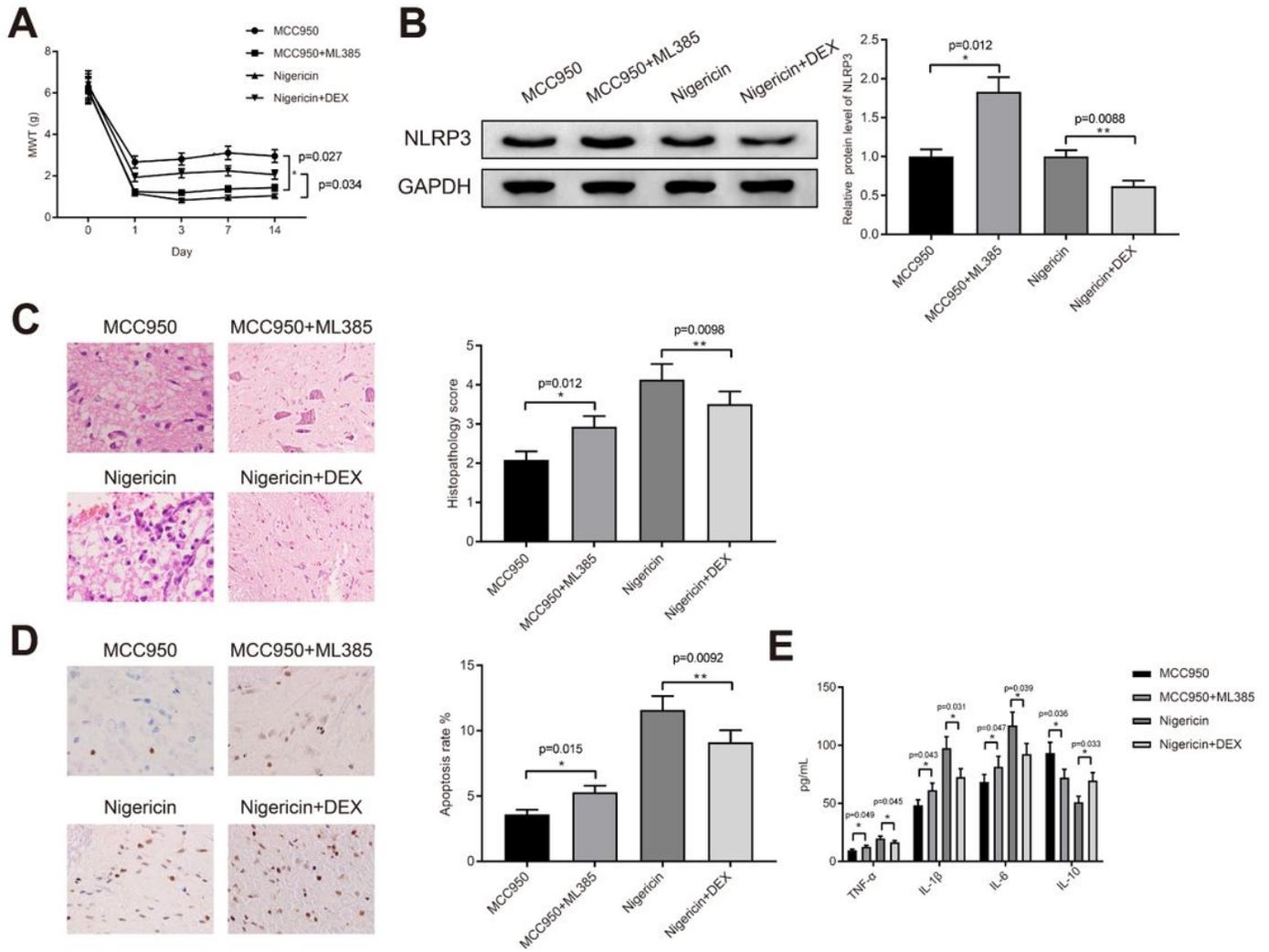
DEX reduces neuropathic pain by activating Nrf2 in CCI rats Notes: CCI rats were injected with DEX and Nrf2 inhibitor ML385. (A) MWT of the rats was tested in response to mechanical stimulation. Western blot (B) and qRT-PCR (C) detected the expression of Nrf2 in spinal cords. (D) H&E staining tested spinal cords injury (× 400). (E) TUNEL staining tested apoptosis in the spinal cords (× 400). (F) ELISA measured

the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. (G) Western blot detected the expression of NLRP3 in the spinal cords. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared to Model group or DEX group; data are presented as mean  $\pm$  SD; n = 6. DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



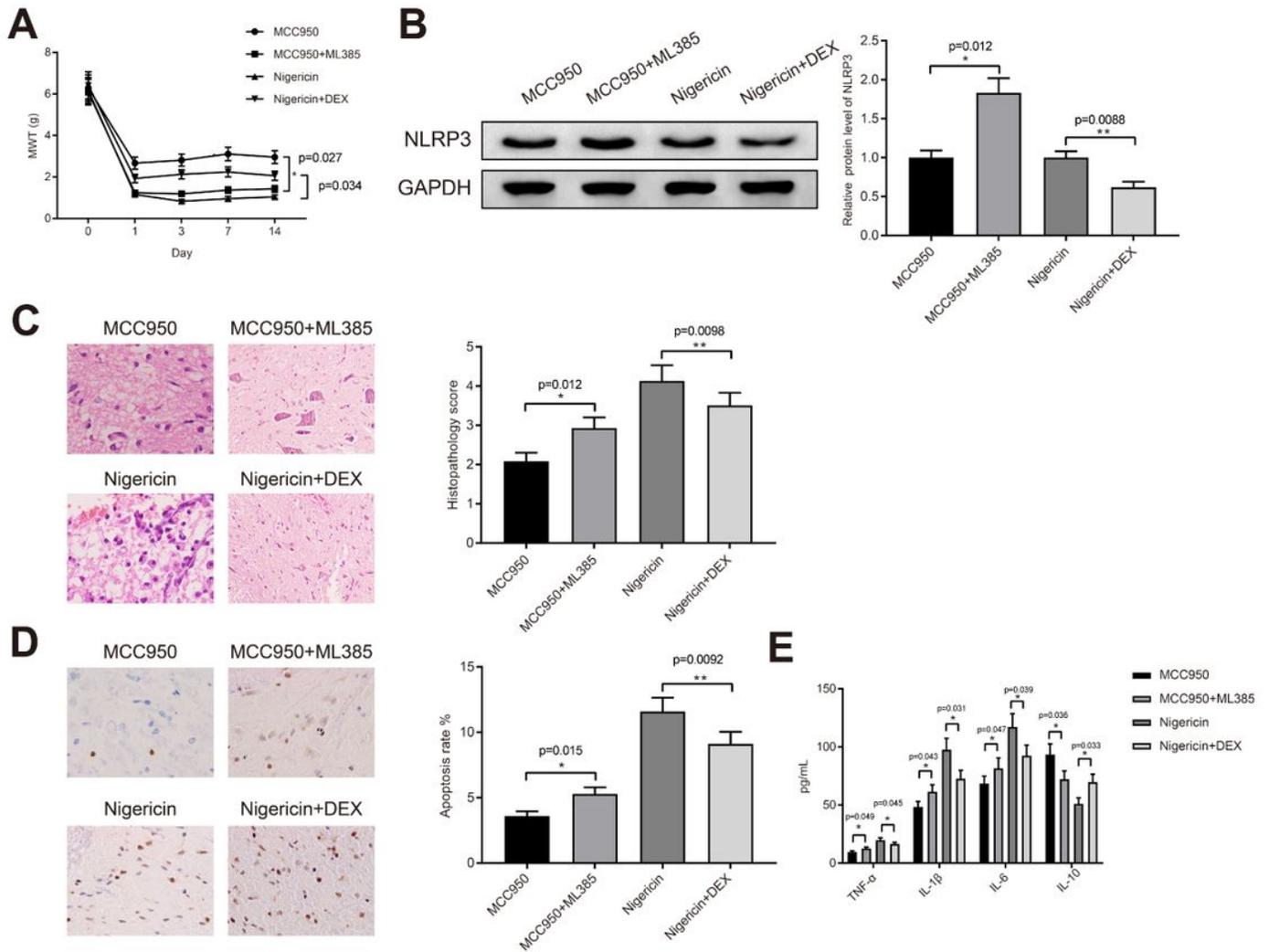
**Figure 5**

DEX releases neuropathic pain of CCI rats by suppressing NLRP3 via activation of Nrf2. Notes: CCI rats were injected with MCC950+ML385 or Nigericin+DEX. (A) MWT of the rats was tested in response to mechanical stimulation. (B) Western blot detected the expression of NLRP3 in spinal cords. (C) H&E staining tested spinal cords injury ( $\times$  400). (D) TUNEL staining tested apoptosis in the spinal cords ( $\times$  400). (E) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared to MCC950 group or Nigericin group; data are presented as mean  $\pm$  SD; n = 6. DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



**Figure 5**

DEX releases neuropathic pain of CCI rats by suppressing NLRP3 via activation of Nrf2. Notes: CCI rats were injected with MCC950+ML385 or Nigericin+DEX. (A) MWT of the rats was tested in response to mechanical stimulation. (B) Western blot detected the expression of NLRP3 in spinal cords. (C) H&E staining tested spinal cords injury ( $\times 400$ ). (D) TUNEL staining tested apoptosis in the spinal cords ( $\times 400$ ). (E) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared to MCC950 group or Nigericin group; data are presented as mean  $\pm$  SD; n = 6. DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold



**Figure 5**

DEX releases neuropathic pain of CCI rats by suppressing NLRP3 via activation of Nrf2. Notes: CCI rats were injected with MCC950+ML385 or Nigericin+DEX. (A) MWT of the rats was tested in response to mechanical stimulation. (B) Western blot detected the expression of NLRP3 in spinal cords. (C) H&E staining tested spinal cords injury ( $\times 400$ ). (D) TUNEL staining tested apoptosis in the spinal cords ( $\times 400$ ). (E) ELISA measured the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , compared to MCC950 group or Nigericin group; data are presented as mean  $\pm$  SD;  $n = 6$ . DEX, dexmedetomidine; CCI, chronic constriction injury; MWT, mechanical withdrawal threshold