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Asiaticoside Alleviates Cerebral Ischemia-Reperfusion Injury via NOD2/Mitogen-Activated Protein Kinase (MAPK)/Nuclear Factor kappa B (NF-κB) Signaling Pathway

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 Chunhui Zhang
BCDF 2 Suyan Chen
BCG 3 Zhenxiang Zhang
CDF 3 Hui Xu
CFG 4 Weihong Zhang
CDF 5 Dongqing Xu
BDF 1 Beilei Lin
DF 2 Yongxia Mei

1 Department of Clinical Nursing, Nursing and Health College of Zhengzhou University, Zhengzhou, Henan, P.R. China
2 Department of Public Nursing, Nursing and Health College of Zhengzhou University, Zhengzhou, Henan, P.R. China
3 Department of Fundamental Nursing, Nursing and Health College of Zhengzhou University, Zhengzhou, Henan, P.R. China
4 Department of Basic Medicine, Nursing and Health College of Zhengzhou University, Zhengzhou, Henan, P.R. China
5 Rehabilitation Department, Henan Province Hospital of Traditional Chinese Medicine (TCM), Zhengzhou, Henan, P.R. China

Corresponding Author: Hui Xu, e-mail: huixuzz@163.com
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Background: Cerebral ischemia-reperfusion injury (CIRI) remains a serious health problem. *Centella asiatica* formulations are used to treat central nervous system disorders. In the present study, asiaticoside, an extract of the plant *Centella asiatica,* was investigated in CIRI *in vivo* and *vitro.*

Material/Methods: We made a CIRI model *in vivo* in SD rats treated by middle cerebral artery occlusion, and a cell model of ischemia-reperfusion injury was made in PC12 cells treated by deprivation of oxygen and glucose/restoration. CIRI *in vivo* was assessed by scores of neurological functions, encephaledema, and cerebral infarction area. Inflammation level and oxidative stress level were detected by the appropriate kits. TUNEL assay was performed for assessment of cell apoptosis and Western blot analysis was performed to assess protein expression levels. CCK8 assay was performed for evaluation of cell survival and flow cytometer was used to detect cell apoptosis *in vitro*.

Results: Nervous function injury, brain edema, cell apoptosis, infarct size, apoptosis-related protein expressions, and protein expressions of the NOD2/MAPK/NF-κB signaling pathway in the CIRI model were all reversed by asiaticoside in rats. The cell apoptosis, inflammation level, and oxidative stress level in the model of cerebral ischemia-reperfusion injury were reduced by asiaticoside. The effects of asiaticoside on CIRI were reversed by NOD 2 agonists.

Conclusions: Asiaticoside showed a protective effect against cerebral ischemia-reperfusion injury via the NOD2/MAPK/NF-κB signaling pathway. These findings are vital for future research on use of asiaticoside in CIRI, providing a new avenue for alleviating CIRI.

MeSH Keywords: Hypoxia-Ischemia, Brain • Medicine, Chinese Traditional • Stroke

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Background

Cerebral ischemia-reperfusion injury occurs as a secondary injury during treatment of ischemic stroke. Ischemic stroke is a disease with high morbidity and disability [1,2]. Timely recovery of blood flow is the most effective method for avoiding loss of brain function. Current treatment methods include drug thrombolysis, endovascular treatment, and mechanical thrombectomy [3–7], but the therapeutic effects are not satisfactory. There are still many unresolved questions about therapy of ischemic stroke. The time window of mechanical thrombectomy is only 6 h and is not sufficient. The timely restoration of blood flow results in oxidative stress, inflammatory responses, and endoplasmic reticulum stress [8–10]. Therefore, it is crucial to find a way to avoid or attenuate cerebral ischemia-reperfusion injury to improve treatment of ischemia stroke. Adjuvant therapy via an effective drug may be a new strategy to improve treatment for ischemic stroke.

Many studies have been conducted to discover a new effective drug to reduce ischemia-reperfusion injury, revealing many promising natural products that protect against cerebral ischemia-reperfusion injury. *Dorema aucheri* extract was reported to possess neuroprotective effects in ischemia-reperfusion injury in rats [11]. Ginkgolides and bilobalide were reported to have antioxidant effects in cerebral ischemia injury via activation of the Akt/Nrf2 pathway [12]. Methyl protodioscin was found to show protective effects in ischemia/reperfusion injury in rats [13]. Natural products with different structures and various bioactivities may be a promising source for discovery of a new drug.

Asiaticoside is the extract of *Centella asiatica*. Asiatica is commonly used in Ayurvedic formulations for therapy of central nervous system disorders [14]. Asiaticoside, as the main component of *Centella asiatica,* has great research value. A previous study showed that asiaticoside has preventive effects in experimental migraine [15]. Asiaticoside was also reported to inhibit amyloidogenesis in Alzheimer's disease patients and to possess neuroprotective effects in ischemia-hypoxia induced rat cortex neurons [16,17]. These previous studies suggest that asiaticoside is a promising agent in treatment for brain disease and exerts neuroprotective effects in ischemia-hypoxia injury. We speculated that asiaticoside may play a vital role in cerebral ischemia-reperfusion injury. In the present research, the effect and mechanism of asiaticoside on cerebral ischemia-reperfusion injury were comprehensively investigated.

Material and Methods

Cell culture and treatment

PC cells (CRL-1721.1) were purchased from the American Type Culture Collection. The PC cells (1×10^4) were cultured in DEME medium containing 10% fetal ovine serum in a 96-well plate in a 5% CO_2 incubator. The cells were divided into an AS+MDP group, experimental groups, an oxygen-glucose deprivation/reperfusion (OGD/R) group, and a control group. The experimental groups were pretreated with different concentrations of asiaticoside (10 nmol, 50 nmol, and 100 nmol) for 30 min. Cells in the AS+MDP group were pretreated with MDP (2 μg/ml) and asiaticoside (100 nmol). Then the OG/R was performed. The process of OG/R was conducted as follows: The cells were cultured in medium with glucose deprivation in an atmosphere of 5% CO_2 and 95% N_2. One hour later, the medium with glucose deprivation was replaced by normal medium, the atmosphere was changed to normal atmosphere, and the cells were cultured for 12 h. The cells treated with OGD/R without asiaticoside were in the OGD/R group, and the cells without OGD/R treatment or asiaticoside treatment were in the control group.

Animal and treatments

The Sprague-Dawley rats (weight, 300–350 g) were obtained from Zhengzhou University. The animal experiments were performed in line with the ethics guidelines of Zhengzhou University. The rats were housed in a standard environment and had free access to water and food. The rats were assigned to experimental groups treated orally with different concentrations of asiaticoside (20, 40, and 60 mg/kg), a positive contrast group treated with nimodipine (20 mg/kg), a sham group, and a model control group. The sham group and model control group were treated with same volume of saline.

Establishment of the middle cerebral artery occlusion model

The middle cerebral artery occlusion (MCAO) model was established by the reforming longa method. We administered 10% chloral hydrate by intraperitoneal injection for anesthetization. The rats were placed in supine position and a midline incision was made in the neck. The internal carotid artery, common carotid artery, and external carotid artery of right side were isolated. The proximal end of the external carotid artery and the common carotid artery was ligated, and the internal carotid artery was occluded. A V-shaped oblique incision was made at the bifurcation of the external carotid artery and the internal carotid artery. An occlusion line was inserted. Then, the internal carotid artery was released, and the suppository line was slowly pushed into the internal carotid artery through the bifurcation until the occlusion line had crossed the artery in the brain (MCA) to the beginning of the anterior cerebral artery. Then, we recorded the time, ligated the top of the common carotid artery, and then suturing was immediately performed. The reperfusion was performed 1.5 h later and the occlusion line was gently pulled back to the incision in the external carotid artery. The detections in this study were conducted after

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24-h reperfusion. The operation for the sham group was without the occlusion of the arteries and the remaining was the same as with the model group.

Scores of neurological functions

After 24-h reperfusion, the neurological symptoms were observed and scored as follows: 0 indicated no neurological symptoms, 1 indicated unable to extend the contralateral forepaw, 2 indicated decreased forelimb resistance to contralateral thrust, and 3 indicated circling to the contralateral side plus the symptoms in 1 and 2.

Measurement of encephaledema

After 24-h reperfusion, body weights of all rats were recorded (M). All rats were sacrificed and the brains removed. The brains were rinsed with saline solution and dried with filter paper. The brain wet weight was measured using an electronic balance and recorded (m_2). After that, the brains of all rats were put into an oven to dry to a constant weight and the dry weight was recorded (m_1). The formulas are shown as follows:

$$\text{Brain water content } \% = \frac{m_2 - m_1}{m_2}; \quad \text{Cerebral index } \% = \frac{m_2}{M}$$

Measurement of cerebral infarction area

After reperfusion for 24 h, the rats were sacrificed and the brains were taken out. The residual blood was washed with normal saline and brains were dried with filter paper. The cerebellum and lower brainstem were removed and frozen at –20°C for 20 min. Then, uniform coronal sections were made. The sections were quickly immersed in 1% 2, 3, 5-chlorinated triphenyltetrazolium (TTC) solution and incubated at 37°C for 20 min. Then, sections were fixed by 4% paraformaldehyde solution. The sections were taken out 6 h later and the surface liquid was removed by blotting with filter paper. Images were taken by using a digital camera. The cerebral infarction area was measured using the Med Brain 2.0 system.

Detection of inflammation level and oxidative stress level

We assessed the levels of inflammatory factors MCP-1, IL-6, IL-1β, and TNF-α. The plasma of rats in different groups was centrifuged at 3000 rpm for 15 min and cryopreserved. The levels of MCP-1, IL-6, IL-1β, and TNF-α were detected according to the instructions of the corresponding kits. ROS, MDA, and LDH, as indicators of oxidative stress, were assessed using the corresponding kits following the kit instructions. The kits mentioned above were purchased form MSKBIO (Wuhan, China)

TUNEL assay

Cell apoptosis was detected by TUNEL assay as described before [18]. Briefly, the In-Situ Cell Death Detection kit (#11684817910, MERCK) was used for assessment. The TUNEL assay was performed according to the manufacturer's specifications. Cell apoptosis in different groups was observed by fluorescence microscope (Olympus Corporation, Japan) with magnification ×400.

Western blot

The lysates of brain tissue or cell lysates in different groups were submitted to 10% SDS-PAGE gels for separation, and then were transferred to nitrocellulose membranes. Membranes were blocked with 5% skim milk, and then incubated overnight with the primary antibodies at 4°C. After the membranes were rinsed with TBST, the membrane was incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies for 1 h. The blots were imaged using the ECL Plus chemiluminescence reagent kit (Thermo, USA). The density of the bands was calculated using Image Quant_LAS500 (GE Healthcare).

CCK8 assay

The PC12 cells were divided into a control group, an OGD/R group, experiment groups, and an MDP + Asiaticoside (AS) group. The cells of different group were cultured in a 96-well plate with a density of 1×10^4. In the experimental groups, the cells were treated with different concentrations of asiaticoside (10 nmol, 50 nmol, and 100 nmol) for 30 min. In the MDP+Asiaticoside (AS) group, the cells were treated with MDP (2 μg/ml) and asiaticoside (100 nmol). Then, OGD/R was performed. The Counting Kit-8 (CCK8) detection kit (Donghuan biotech) was used for assessment of cell viability. In each well, 10 μL CCK8 agent was added and the cells were cultured for 2 h. Cell viability was assessed by absorbance at 450 nm.

Cell apoptosis assessment

The cells of different groups were seeded in a 6-well plate at a density of 5×10^5. After the pretreatment and OGD/R, the cells were harvested. Then, the cells were treated by Annexin V FITC and propidium iodide (PI) for 16 min in the dark. Flow cytometry (BD Biosciences) was used to assess cell apoptosis in the different groups.

Statistical analysis

GraphPad Prism (GraphPad Software, Inc, La Jolla, CA) and SPSS were used for processing the data. Data are presented as mean±SD. One-way or two-way analysis of variance (ANOVA) was used to analyze the data.

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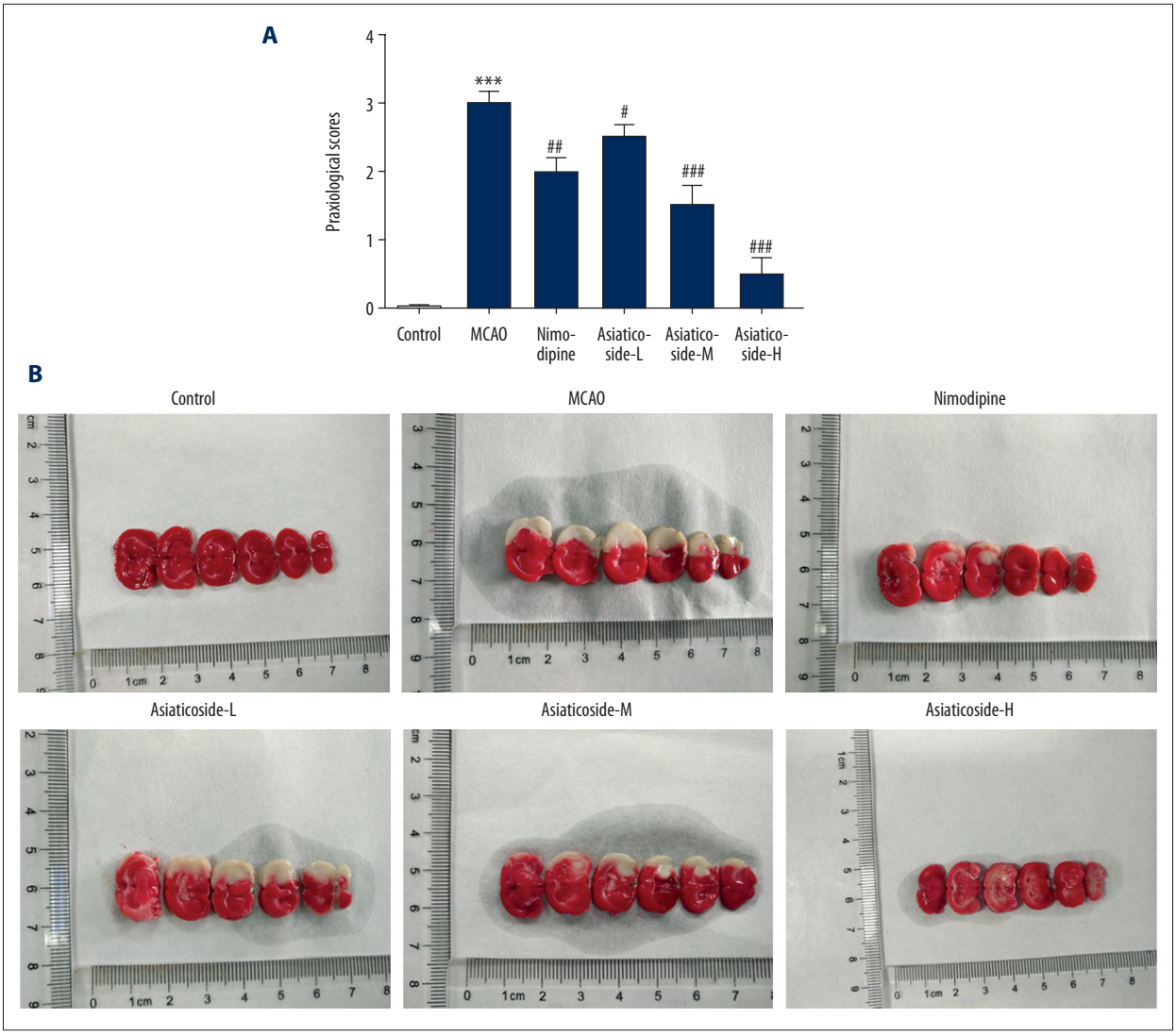

Figure 1. (**A, B**) The effects of asiaticoside on nervous function score and infarct size in cerebral ischemia-reperfusion injury in rats. The nervous function score and infarct size in different groups. The infarct size in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus MCAO group *** P<0.001 versus control group.

Results

Asiaticoside in rats alleviated nervous function injury, brain edema, and infarct size in cerebral ischemia-reperfusion injury

As shown by Figure 1A, the score of nervous function was elevated in the MCAO group and compared with the control group. The score of nervous function in rats of MCAO was reduced by nimodipine and, more obviously, by asiaticoside at high concentration. The brain water (Table 1), cerebral index (Table 1), and infarct size (Figure 1B) were elevated in the MCAO group compared with the control group, and the water content/cerebral index/infarct size induced by MCAO was reduced by nimodipine and, more obviously, by asiaticoside. These results

Table 1. The effects of asiaticoside on cerebral index and brain water.

Group	Cerebral index/%	Brain water content/%
Control	0.59±0.01	81.44±0.48
MCAO	0.72±0.02	85.08±0.61
nimodipine	0.61±0.02**	82.66±0.21
Asiaticoside-L	0.67±0.01**	83.61±0.81
Asiaticoside-M	0.63±0.02**	83.38±0.38
Asiaticoside-H	0.61±0.02	83.08±0.45

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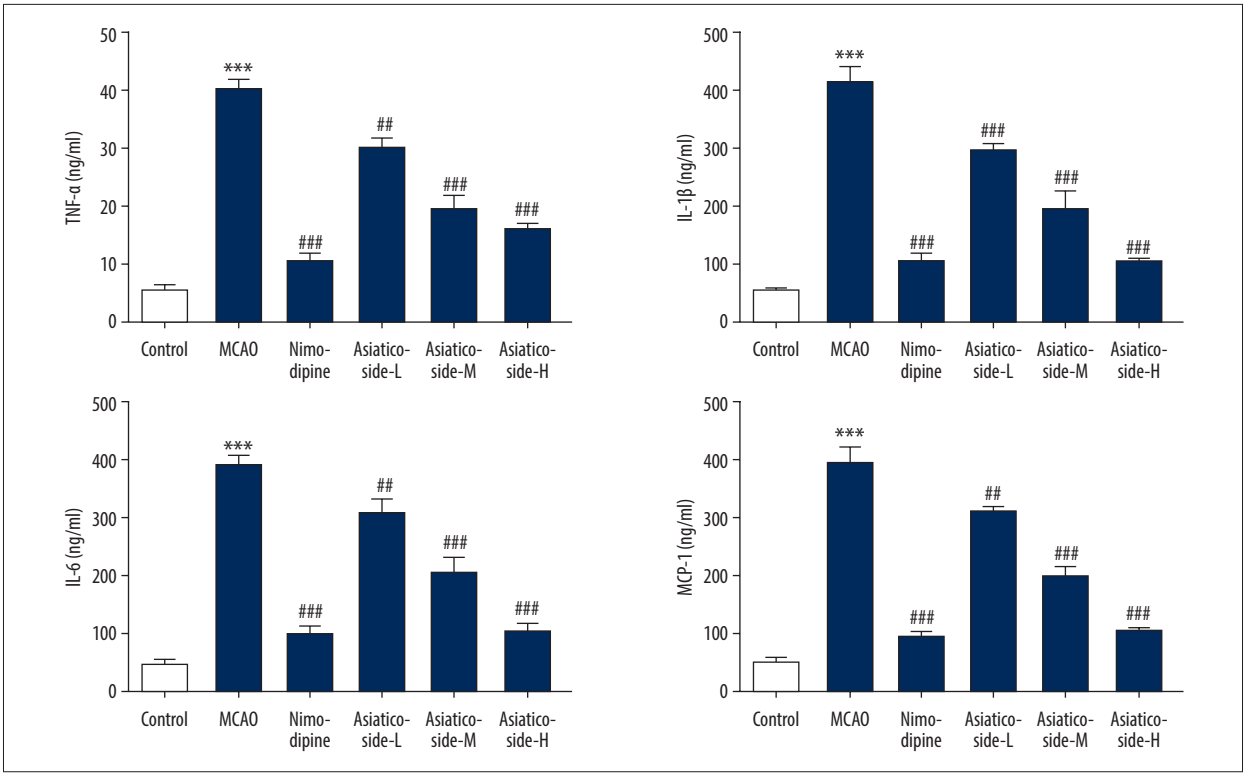

Figure 2. The effect of asiaticoside on inflammation level in cerebral ischemia-reperfusion injury in rats. The levels of MCP-1, IL-6, IL-1β and TNF-α in different groups. ### P<0.001 and ## P<0.01 versus MCAO group *** P<0.001 versus control group.

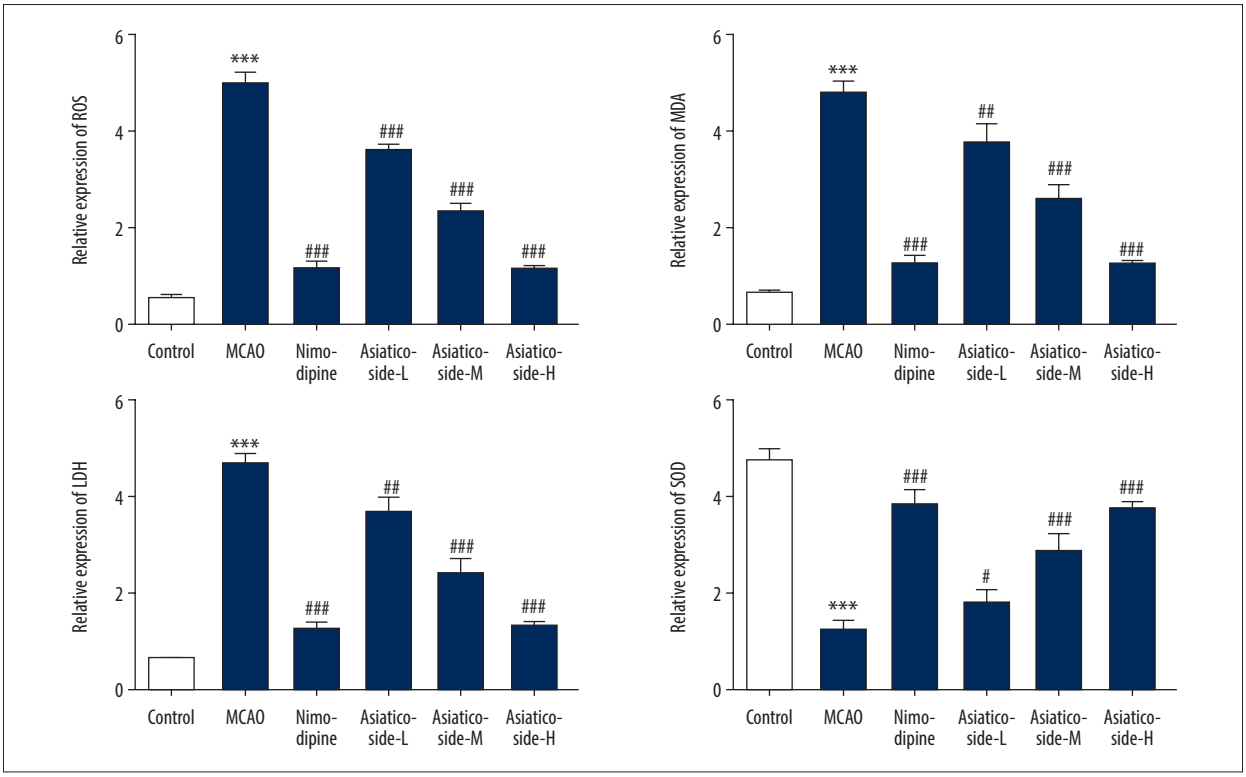

Figure 3. The role of asiaticoside on oxidant stress in cerebral ischemia-reperfusion injury in rats. The levels of SOD, LDH, MDA and ROS in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus MCAO group *** P<0.001 versus control group.

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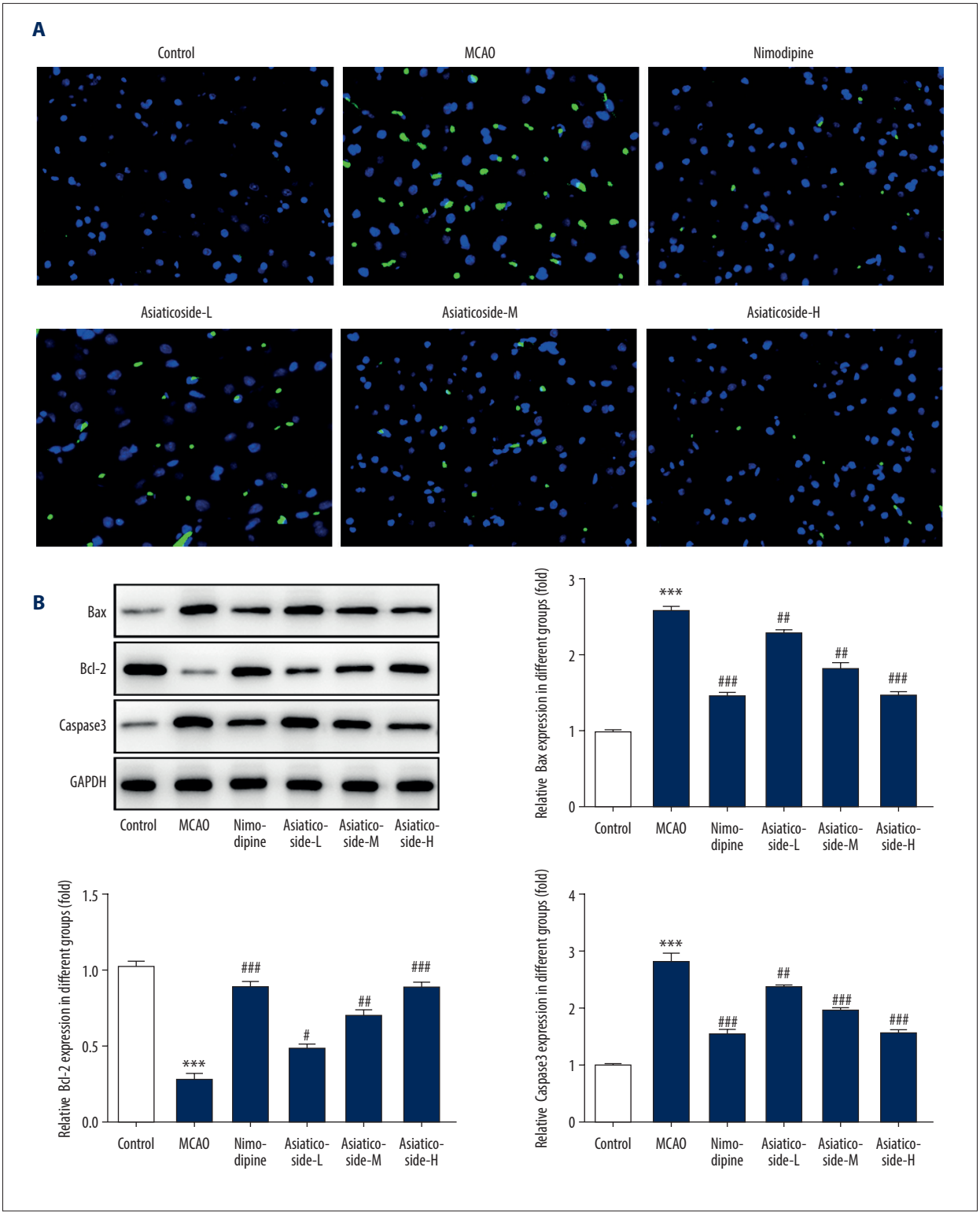

Figure 4. (**A, B**) The effect of asiaticoside on cell apoptosis and apoptosis-related protein expressions in cerebral ischemia-reperfusion injury in rats. The cell apoptosis level and apoptosis-related protein expressions in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus MCAO group *** P<0.001 versus control group.

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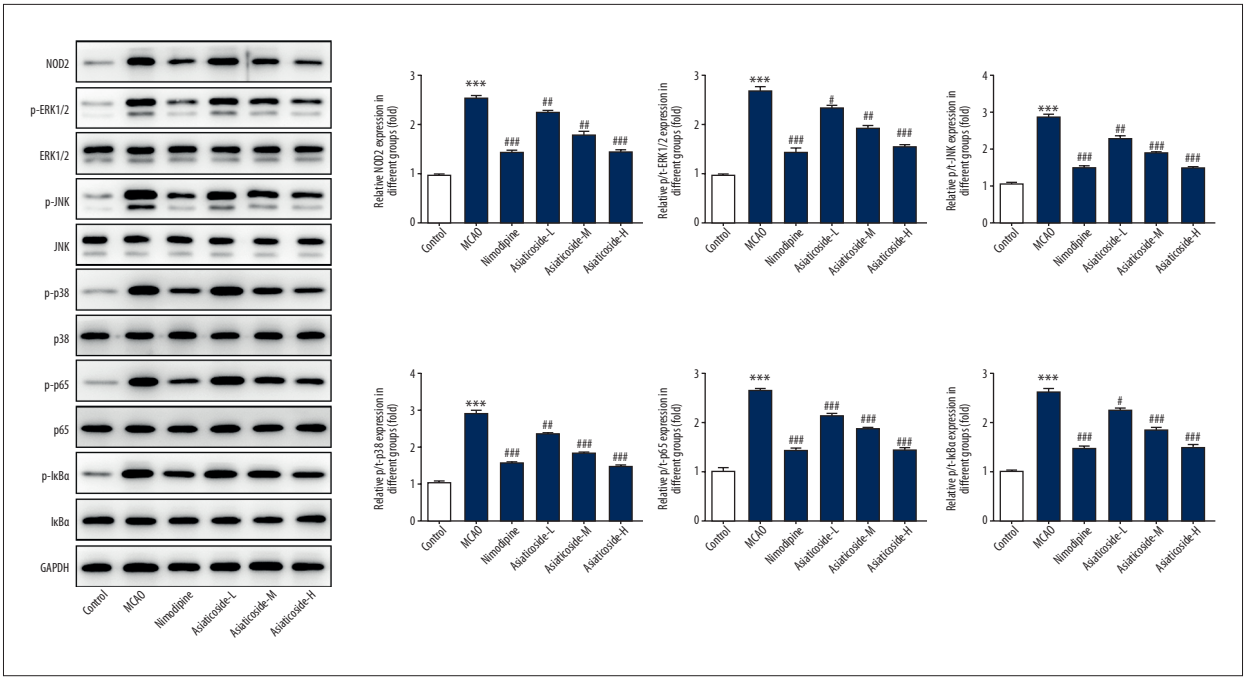

Figure 5. The effect of asiaticoside on protein expressions of NOD2/MAPK/NF-κB signaling pathway in cerebral ischemia-reperfusion injury in rats. The expressions of NOD2, P-ERK1/2, P-JNK, P-p38, P-65, and p-IkBa in NOD2/MAPK/NF-κB signaling pathway in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus MCAO group *** P<0.001 versus control group.

show that asiaticoside is superior to nimodipine in reducing nervous function injury, brain edema, and infarct size in rats.

Asiaticoside in rats inhibited the inflammation and oxidative stress in cerebral ischemia-reperfusion injury

The inflammatory factors MCP-1, IL-6, IL-1β, and TNF-α were higher in the MCAO group compared to the control group (Figure 2). The levels of MCP-1, IL-6, IL-1β, and TNF-α induced by MCAO were reduced by MCAO in a dose-dependent manner. ROS, MDA, LDH, and SOD are indicators of oxidative stress. Levels of ROS, MDA, and LDH were higher in the MCAO group than in the control group. SOD level, an indicator of anti-oxidative stress, was decreased by MDA in contrast with the control group. The levels of SOD, LDH, MDA, and ROS induced by MCAO were reversed by MCAO in a dose-dependent manner (Figure 3). The effect of MCAO at high concentration on inflammatory factor level and oxidative stress were similar with the effect of the drug. The findings suggest that asiaticoside alleviated cerebral ischemia-reperfusion injury through inhibition of inflammation and oxidative stress.

Asiaticoside attenuated the cell apoptosis in cerebral ischemia-reperfusion injury in rats

There was very little cell apoptosis in the control group, and after MCAO, the cell apoptosis was increased (Figure 4A). The cell apoptosis induced by MCAO was reduced by asiaticoside in a dose-dependent manner. Compared with the control group, the cell apoptosis level in the asiaticoside-H group and drug group was the lowest among all groups, indicating that asiaticoside reduced cell apoptosis in rats with cerebral ischemia-reperfusion injury. The apoptosis-related proteins Bcl2, bax, and caspase3 were assessed (Figure 4B), showing that, compared with the control group, bax and caspase3 pro-apoptosis proteins were obviously higher in the MCAO group and Bcl-2 was significantly decreased in the MCAO group. The levels of caspase3, bax, and bcl-2 induced by MCAO were reversed by asiaticoside in a dose-dependent manner and this reversing effect was similar to that of nimodipine. All these results indicated that asiaticoside attenuated cell apoptosis through upregulation of Bcl-2 and downregulation of bax and caspase3.

Asiaticoside treatment of rats with cerebral ischemia-reperfusion injury reduced the expressions of NOD2, P-ERK1/2, P-JNK, P-p38, P-65, and p-IkBa via the NOD2/MAPK/NF-κB signaling pathway.

We investigated the role of the NOD2/MAPK/NF-κB signaling pathway in inflammation (Figure 5). The protein expressions of the NOD2/MAPK/NF-κB signaling pathway were higher in the MCAO group than in any other group. The protein expressions induced by MCAO were reduced by asiaticoside in a dose-dependent manner. MCAO at high concentrations achieved nearly the same effect on protein expressions of the NOD2/MAPK/NF-κB signaling pathway with nimodipine. These results show

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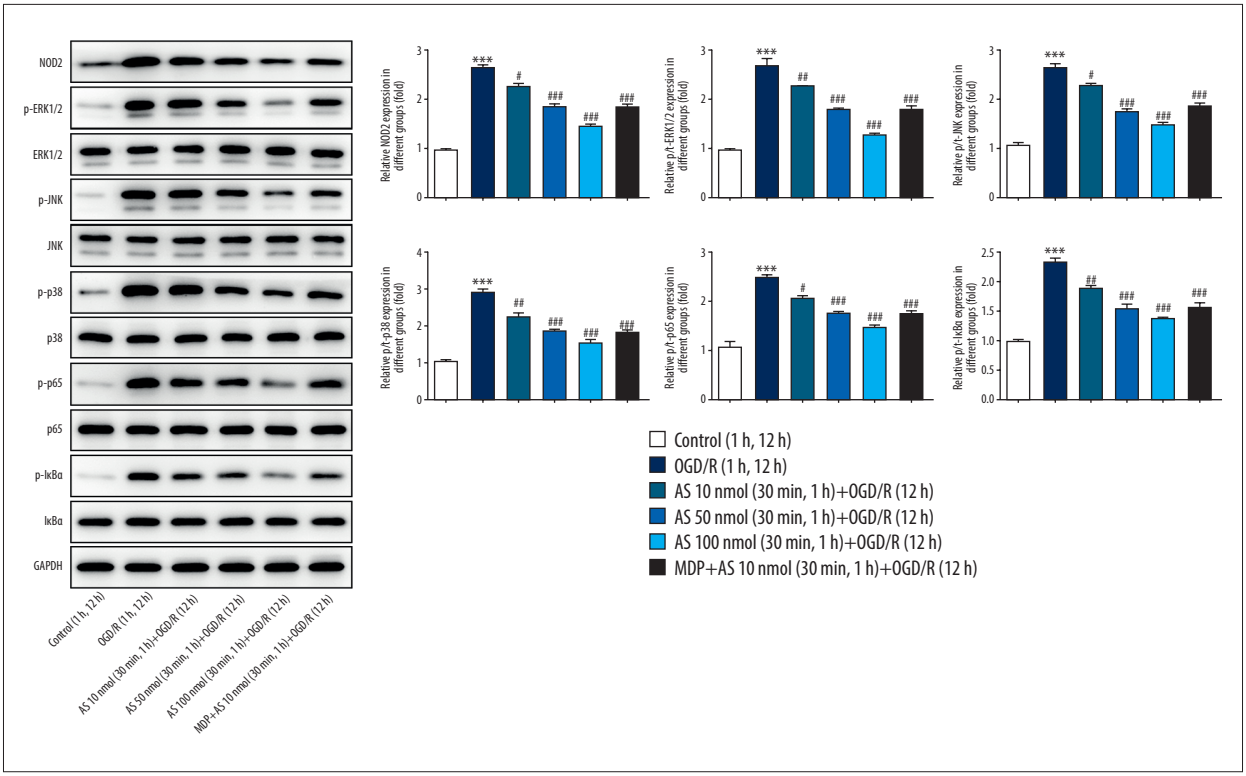

Figure 6. Asiaticoside and NOD 2 agonists (MDP) effects on protein expressions of NOD2/MAPK/NF-κB signaling pathway in OGD/R treated cells. Expressions of NOD2, P-ERK1/2, P-JNK, P-p38, P-65, and p-IkBa in NOD2/MAPK/NF-κB signaling pathway in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus OGD/R group *** P<0.001 versus control group.

that the effect of asiaticoside occurred via the NOD2/MAPK/NF-κB signaling pathway.

Asiaticoside increased cell survival and reduced cell apoptosis in OGD/R-induced cells via the NOD2/MAPK/NF-κB signaling pathway

To further verify the role of the signaling pathway *in vitro*, the effect of NOD 2 agonists (MDP) was evaluated. As shown by Figure 6, the protein expressions of the NOD2/MAPK/NF-κB signaling pathway were higher in the OGD/R group and the OGD/R-induced proteins expressions were alleviated by asiaticoside in a dose-dependent manner. The effect of asiaticoside on protein expressions of the NOD2/MAPK/NF-κB signaling pathway in the OGD/R treatment group was reversed by NOD 2 agonists (MDP), demonstrating that the effect of asiaticoside may be through the NOD2/MAPK/NF-κB signaling pathway. Cell survival was decreased by OGD/R in contrast to the control group (Figure 7). The cell survival in the OGD/R group with asiaticoside pretreatment was higher than in the OGD/R group. The OGD/R-induced cell survival was increased with the concentration of asiaticoside pretreatment and was decreased by NOD 2 agonists together with asiaticoside (100 nmol), indicating that the effect of asiaticoside on cell survival was through blocking the NOD2/MAPK/NF-κB

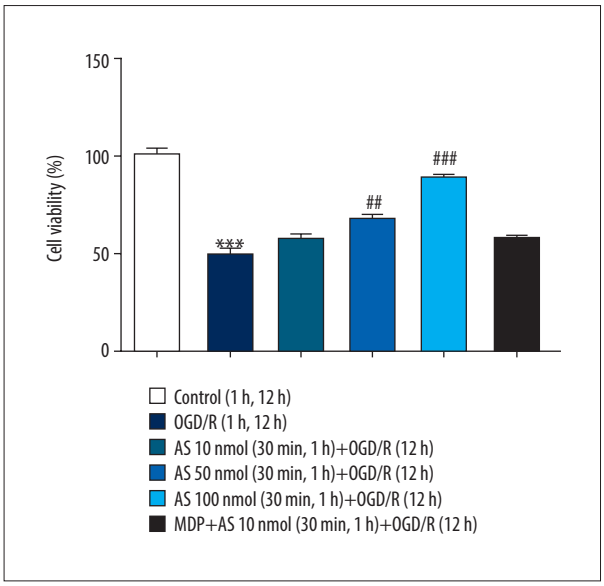

Figure 7. Effects of asiaticoside and NOD 2 agonists (MDP) on cell viability in OGD/R induced cells. The cell viability in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus OGD/R group *** P<0.001 versus control group.

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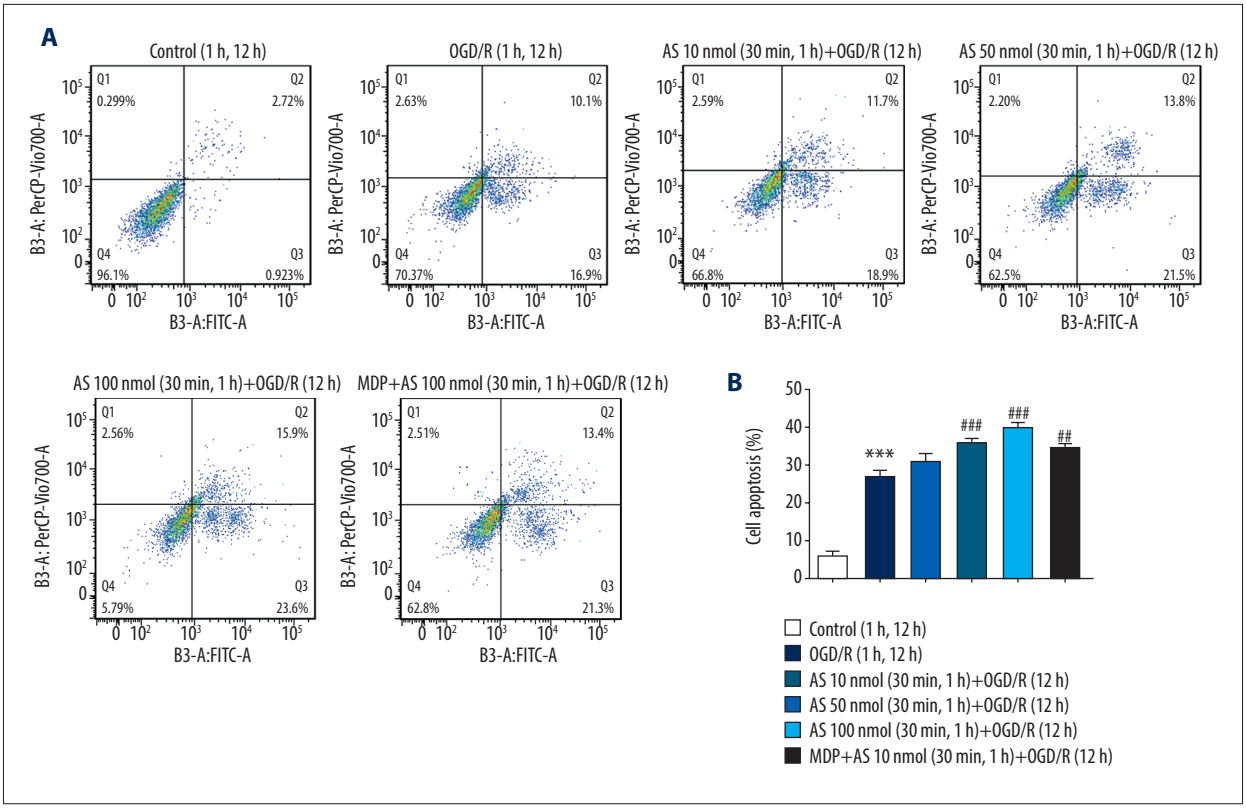

Figure 8. Asiaticoside and NOD 2 agonists (MDP) effects on cell apoptosis in OGD/R-induced cells. The cell apoptosis in different groups (**A, B**). ### P<0.001, ## P<0.01 and # P<0.05 versus OGD/R group *** P<0.001 versus control group.

signaling pathway. In the OGD/R group, the cell apoptosis was higher than in any other group (Figure 8A, 8B). The cell apoptosis induced by OGD/R was reduced by asiaticoside pretreatment and this effect was reversed by NOD 2 agonists together with asiaticoside (100 nmol). Furthermore, compared with the control group, the pro-apoptosis proteins, including bax and caspase3, were upregulated by OGD/R, and the change trend for bcl-2, which is the anti-apoptosis protein, was contrary to that of pro-apoptosis proteins (Figure 9). The effects were attenuated by asiaticoside in a dose-dependent manner and the effects of asiaticoside were reduced in the presence of NOD 2 agonists together with asiaticoside (100 nmol), demonstrating that asiaticoside reduced cell apoptosis through the NOD2/MAPK/NF-κB signaling pathway.

Asiaticoside reduced the levels of inflammation and oxidative stress in OGD/R induced cells via NOD2/MAPK/NF-κB signaling pathway

The levels of MCP-1, IL-6, IL-1β, and TNF-α were higher in the OGD/R group compared with the control group, and the OGD/R-induced inflammation was reduced by asiaticoside in a dose-dependent manner (Figure 10). The inhibition effect of asiaticoside on inflammation level was reduced by NOD 2 agonists together with asiaticoside (100 nmol), confirming that the inhibition effect of asiaticoside on inflammation level was through the NOD2/MAPK/NF-κB signaling pathway. In contrast to the control group, ROS, MDA, and LDH induced by OGD/R were higher and SOD was lower (Figure 11). The oxidative stress level induced by OGD/R was reduced by asiaticoside in a dose-dependent manner and NOD 2 agonists reversed this effect, indicating that asiaticoside inhibited oxidative stress in OGD/R-induced cells via the NOD2/MAPK/NF-κB signaling pathway.

Discussion

Centella asiatica has been applied as a folk medicine for neurological disorders in China [19]. Asiaticoside is an extract of the plant *Centella asiatica* and is used as a psychoactive drug in India [20]. Asiaticoside has been reported to have numerous biology activities, including anti-inflammation, anti-oxidative stress, anti-tumor promoting, and wound healing [21–25]. Increasing evidence has confirmed that asiaticoside protects neurological function [17,23,26], but there have been few *in vivo* and *in vitro* studies on the effects of asiaticoside in cerebral ischemia-reperfusion injury. Here, we explored the role of asiaticoside *in vivo/vitro* in cerebral ischemia-reperfusion injury and investigated the possible signaling pathway mechanism. The results

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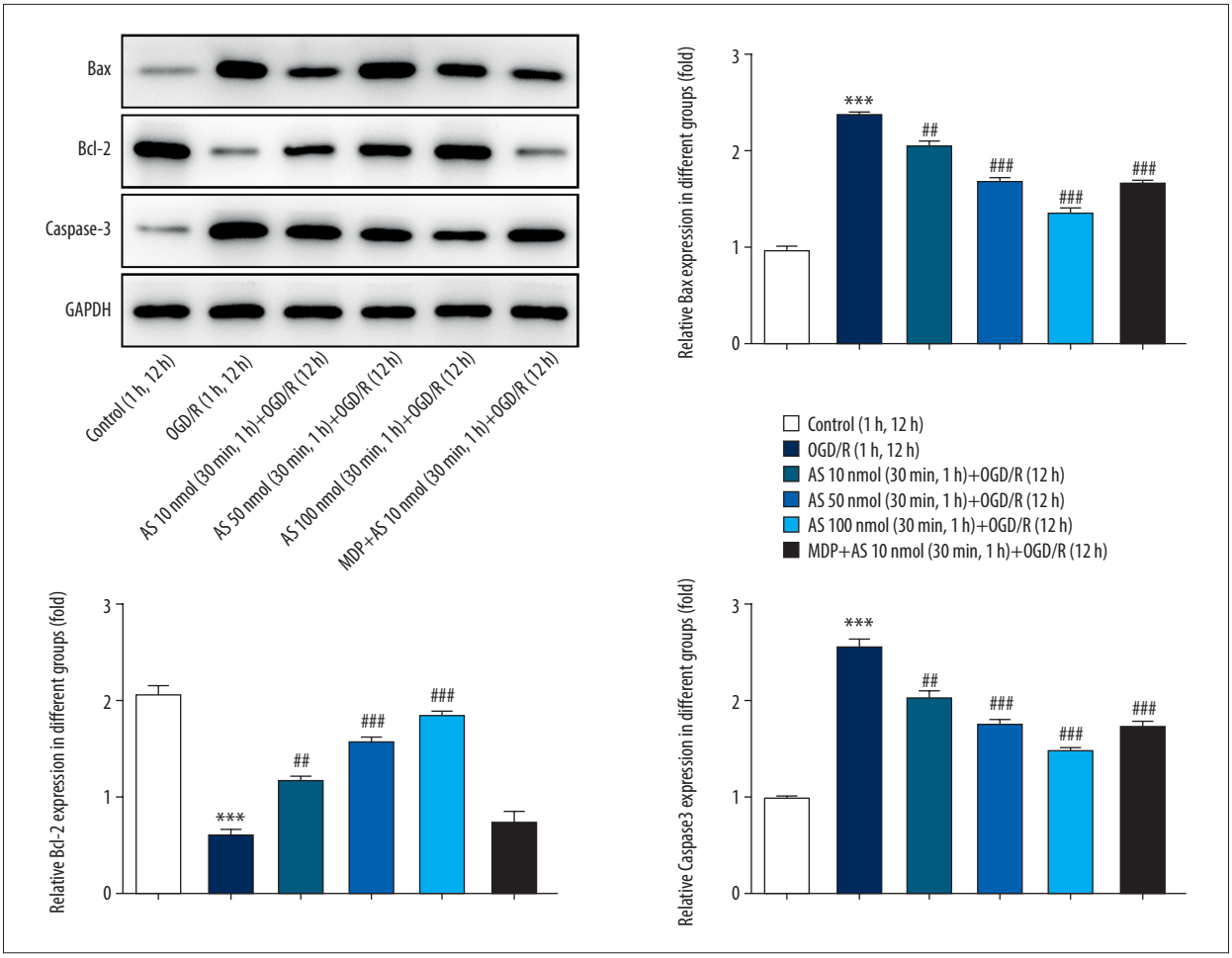

Figure 9. Asiaticoside and NOD 2 agonists (MDP) effects on expressions of apoptosis-related proteins in cells treated by OGD/R. The expressions of apoptosis-related proteins in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus OGD/R group *** P<0.001 versus control group.

demonstrated that asiaticoside has a protective effect on cerebral ischemia-reperfusion injury. Asiaticoside alleviated nervous function injury, brain edema, and infarct size in rats with cerebral ischemia-reperfusion injury. Asiaticoside was confirmed to possess many protective effects on cerebral ischemia-reperfusion injury, including increasing cell survival, reducing cell apoptosis, and inhibiting inflammation and oxidative stress.

Ischemic stroke, which is a major cause of death and disability, remains a serious health problem. Quickly restoring the blood flow limits brain function losses. However, at present the therapeutic effect is not ideal. The therapeutic window for ischemic stroke is limited and the recovery of blood flow creates a secondary injury at the same time. There is a pressing need for a new drug that can prolong the "time window" of therapeutic cerebral ischemia and reduce the injuries from ischemia-reperfusion. In this study, as shown by *in vivo* experiments, asiaticoside reduced neurological function loss, brain edema, and infarct size, as well as cell apoptosis. The protective effect of asiaticoside on ischemia-reperfusion makes it a promising drug.

In addition, inflammation and oxidative stress are known as major causes of ischemia-reperfusion injury. In the current research, the pro-inflammation factors MCP-1, IL-6, IL-1β, and TNF-α induced by MCAO and OGD/R were obviously reduced by asiaticoside. Levels of ROS, MDA, LDH, and SOD, which are indicators of oxidative stress, are increased by MCAO and OGD/R. The levels of ROS, MDA, LDH, and SOD were reversed by asiaticoside. These findings strongly support that asiaticoside, by virtue of its anti-inflammation and anti-oxidative effects, is a promising agent in prevention of ischemia-reperfusion injury. Cell apoptosis is one of the pathophysiological events in ischemia-reperfusion injury, and reducing cell apoptosis is another way to alleviate ischemia-reperfusion injury. In this study, the cell survival was increased by asiaticoside and cell apoptosis was decreased by asiaticoside in a rat model of ischemia-reperfusion injury.

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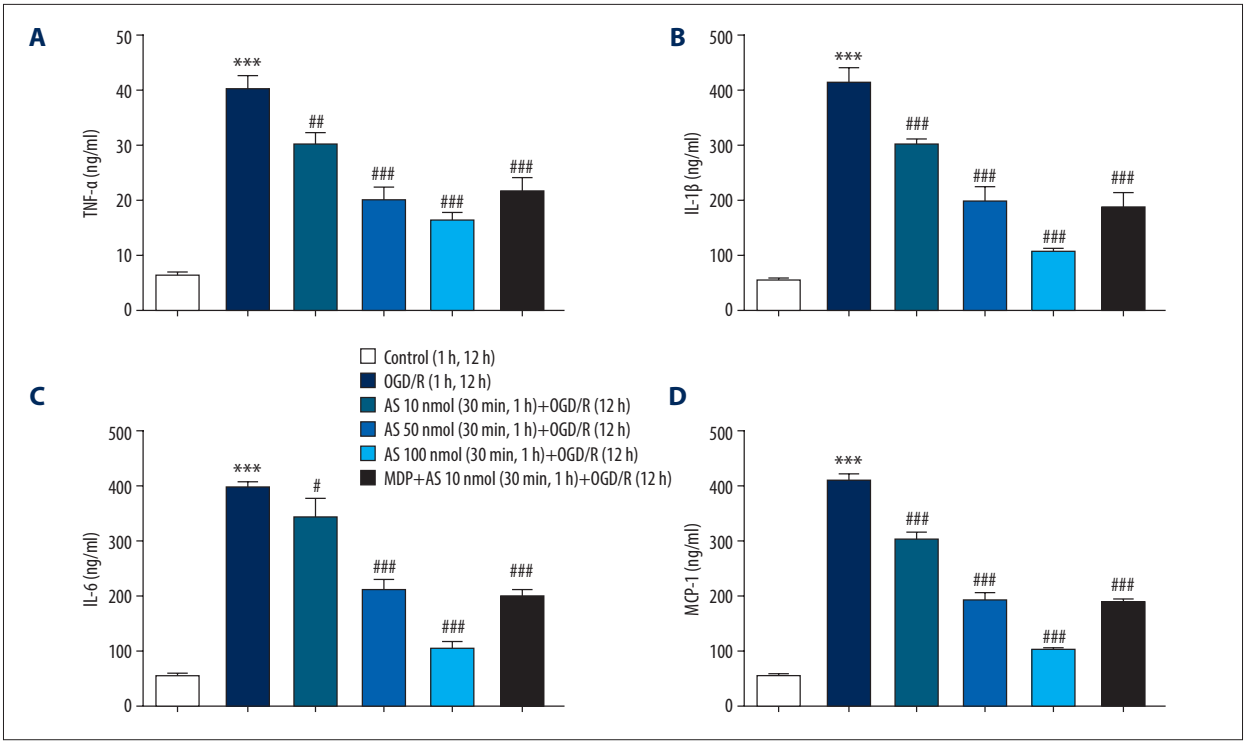

Figure 10. (**A–D**) Asiaticoside and NOD 2 agonists (MDP) effects on inflammation level in cells treated by OGD/R. The levels of MCP-1, IL-6, IL-1β, and TNF-α in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus OGD/R group *** P<0.001 versus control group.

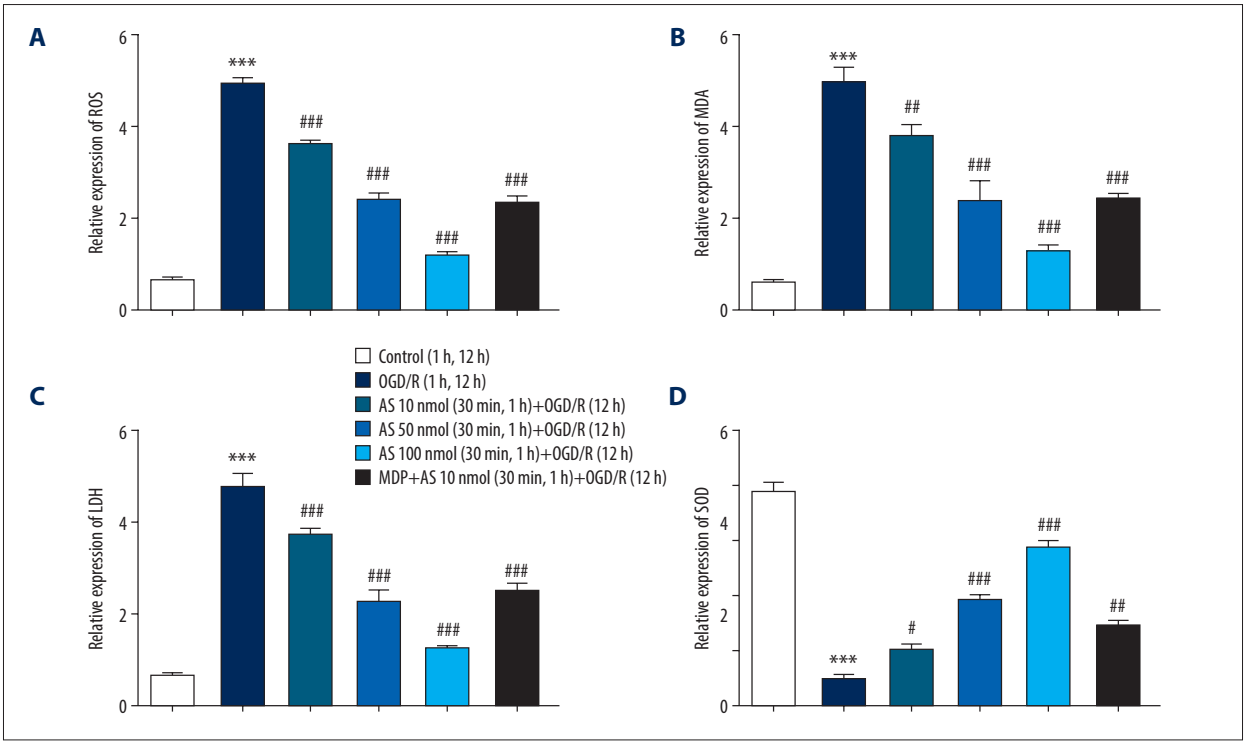

Figure 11. (**A–D**) Asiaticoside and NOD 2 agonists (MDP) effects on oxidative stress level in OGD/R-induced cells. The levels of SOD, LDH, MDA, and ROS in different groups. ### P<0.001, ## P<0.01 and # P<0.05 versus OGD/R group *** P<0.001 versus control group.

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The protective mechanism of asiaticoside was further investigated in ischemia-reperfusion injury. The MAPK signal pathway was involved in many cellular processes, including cell differentiation, cell apoptosis, and cell proliferation. The MAPK pathway was reported to be activated by oxidative stress and inflammation [27–29]. NF-κB is a vital pathway for regulation of the release of inflammation factors. MAPK is a positive regulator of the NF-κB signaling pathway [30]. Furthermore, NF-κB was triggered by NOD2, which was reported to promote the innate inflammatory response by activating NF-κB signaling. Our results showed that proteins of the NOD2/MAPK/NF-κB signaling pathway were upregulated *in vivo* and *in vitro* in a rat model ischemia-reperfusion injury in contrast to the control group. The proteins of the NOD2/MAPK/NF-κB signaling pathway induced by MCAO and OGD/R were reduced by asiaticoside. The effect of asiaticoside on cell apoptosis, inflammation, and oxidative stress were reduced by NOD 2 agonists, confirming that the effects of asiaticoside were achieved via the NOD2/MAPK/NF-κB signaling pathway.

Conclusions

Asiaticoside, the main extract of *Centella asiatica,* showed protective effects in ischemia-reperfusion injury *in vivo* and *vitro* via the NOD2/MAPK/NF-κB signaling pathway, and shows great potential in treatment of ischemia-reperfusion injury. Our findings provide a new approach to treatment of ischemia-reperfusion injury and pave the way for future research.

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