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Corynoline alleviates hepatic ischemia–reperfusion injury by inhibiting NLRP3 inflammasome activation through enhancing Nrf2/HO-1 signaling

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Abstract

Objective Corynoline has displayed pharmacological effects in reducing oxidative stress and inflammatory responses in many disorders. However, its effects on hepatic ischemia–reperfusion (I/R) injury remain unclear. This study aimed to investigate the protective effects of corynoline against hepatic I/R injury and the underlying mechanisms.

Methods Rat models with hepatic I/R injury and BRL-3A cell models with hypoxia/reoxygenation (H/R) insult were constructed. Models were pretreated with corynoline and/or other inhibitors for functional and mechanistic examination.

Results Corynoline pretreatment effectively mitigated hepatic I/R injury verified by reduced serum transaminase levels, improved histological damage scores, and decreased apoptosis rates. Additionally, corynoline pretreatment significantly inhibited I/R-triggered oxidative stress and inflammatory responses, as indicated by enhanced mitochondrial function, reduced levels of ROS and MDA, reduced neutrophil infiltration and suppressed proinflammatory cytokine release. In vitro experiments further showed that corynoline pretreatment increased cellular viability, decreased LDH activity, reduced cellular apoptosis, and inhibited oxidative stress and inflammatory injury in H/R-induced BRL-3A cells. Mechanistically, corynoline significantly increased Nrf2 nuclear translocation and expression levels of its target gene, HO-1. It also blocked NLRP3 inflammasome activation both in vivo and in vitro. Furthermore, pretreatment with Nrf2 inhibitor ML-385 counteracted the protective effect of corynoline on hepatic I/R injury. Ultimately, in vitro studies revealed that the NLRP3 activator nigericin could also nullified the protective effects of corynoline in BRL-3A cells, but had minimal impact on Nrf2 nuclear translocation.

Conclusions Corynoline can exert protective effects against hepatic I/R injury by inhibiting oxidative stress, inflammatory responses, and apoptosis. These effects may be associated with inhibiting ROS-induced NLRP3 inflammasome activation by enhancing Nrf2/HO-1 signaling. These data provide new understanding about the mechanism of corynoline action, suggesting it is a potential drug applied for the treatment and prevention of hepatic I/R injury.

Keywords Corynoline · Liver · Ischemia–reperfusion injury · Nrf2 · NLRP3 inflammasome

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Introduction

Hepatic ischemia reperfusion (I/R) injury is an inevitable pathological occurrence during numerous liver surgeries, particularly in liver transplantation [1]. Uncontrollable hepatic I/R injury significantly contributes to severe postoperative complications and potential transplantation failure [1, 2]. There is currently a limited array of drugs or strategies available for alleviating hepatic I/R injury, but they are not satisfactory. Therefore, it is critical to investigate an effective therapeutic strategy for clinically treating hepatic I/R injury. The underlying pathophysiology of hepatic I/R injury is intricate, with mounting evidence indicating that hepatic I/R can induce elevated levels of pro-inflammatory cytokines and reactive oxygen species (ROS) production, both of which have the potential to cause liver damage [3]. Accordingly, identifying an effective method to reduce the inflammatory response and oxidative stress may potentially serve as a viable strategy for preventing and treating hepatic I/R injury.

Inflammation is one of the pathological processes essential to hepatic I/R injury, with the multi-protein inflammasome complexes playing a main part in the inflammatory responses following I/R insult [4]. Among these inflammasomes, the nod-like receptor pyrin-containing pyrin domain 3 (NLRP3) inflammasome has been extensively studied and consists of a nucleotide-binding domain, an apoptosis-associated speck-like protein (ASC), and pro-caspase1 [5]. Following stimulation, NLRP3 inflammasome assembly and subsequent activation of caspase-1 lead to the cleavage of pro-interleukin (IL)-1β and pro-IL-18 into their mature forms. The release of mature IL-1β and IL-18 into the serum initiates an inflammatory response [5]. Numerous studies have confirmed that hepatic I/R activates the NLRP3 inflammasome, and inhibition of this inflammasome could significantly alleviate hepatic I/R injury [6, 7]. Although the precise regulatory mechanism governing NLRP3 inflammasome activation remains uncertain, numerous studies have linked this activation with ROS levels [8, 9]. ROS are highly reactive and unstable molecules generated primarily through mitochondrial oxidative phosphorylation by reducing oxygen [10]. Excessive levels of ROS can cause damage to cells and lead to tissue dysfunction by oxidizing lipids, proteins, mitochondria, and DNA. Furthermore, ROS have been associated with the development of various diseases, including hepatic I/R injury [10, 11]. The excessive release of ROS induced by hepatic I/R is also reportedly an important trigger for NLRP3 inflammasome formation and activation [11]. Hence, preventing ROS production or increasing endogenous antioxidant levels could significantly contribute to inhibiting NLRP3 inflammasome activation.

Nuclear factor erythroid-2 related factor 2 (Nrf2) serves as an intrinsic regulator of antioxidants, playing a crucial role in maintaining cellular defense mechanisms by exerting its antioxidant, anti-inflammatory, and cytoprotective properties [12, 13]. Upon activation, Nrf2 can detach from Kelch-like epichlorohydrin-1 (Keap1), subsequently relocating to the nucleus, where it binds to antioxidant response elements (AREs). This binding event enables Nrf2 to effectively govern the expression of genes involved in antioxidation, such as heme oxygenase-1 (HO-1) and superoxide dismutase (SOD) [13]. In a previous investigation, we established that activation of Nrf2/HO-1 signaling plays a crucial role in mitigating oxidative damage during hepatic I/R injury [14]. While Nrf2 acts as an extremely sensitive signal for scavenging ROS to combat oxidative stress, it also participates in safeguarding cells against the inflammatory response [15]. Recent research has suggested that by activating Nrf2, excessive ROS production can be hindered, subsequently leading to inhibited NLRP3 inflammasome activation [16, 17]. Therefore, the negative regulatory effects of Nrf2 on NLRP3 inflammasome activation may be a valuable tool for mitigating hepatic I/R injury.

Corynoline (Fig. 1a), a compound derived from Corydalis bungeana Turcz., has garnered significant attention for its potential therapeutic applications in clinical settings. It exhibits noteworthy anti-inflammatory, antioxidative, and antitumor properties [18–20]. Several studies have shown that corynoline could markedly inhibit lipopolysaccharide (LPS)-induced inflammatory cytokine production and inflammatory injury in a series of disease models [21–23]. Moreover, corynoline can attenuate the IL-1β treatment-induced inflammatory response and oxidative stress in chondrocytes through inhibition of IL-6, tumor necrosis factor (TNF)-α, and ROS production via activating the Nrf2/NF-kB pathway [24]. This modulation of the Nrf2/NF-kB pathway by corynoline has also been shown to ameliorate DSS-induced colonic injury [25]. Notably, recent research has suggested that corynoline may alleviate zearalenone-induced liver injury by activating the SIRT1/Nrf2 pathway and inhibiting the production of inflammatory mediators [26]. Although numerous studies have established the pharmacological properties of corynoline in various diseases, its impacts on hepatic I/R injury and the associated mechanisms remain poorly understood. Therefore, in the present study, we used in vivo and in vitro experiments to explore if corynoline can alleviate hepatic I/R injury. We also examined the connections between its effects and inhibition of NLRP3 inflammasome activation by upregulating Nrf2.

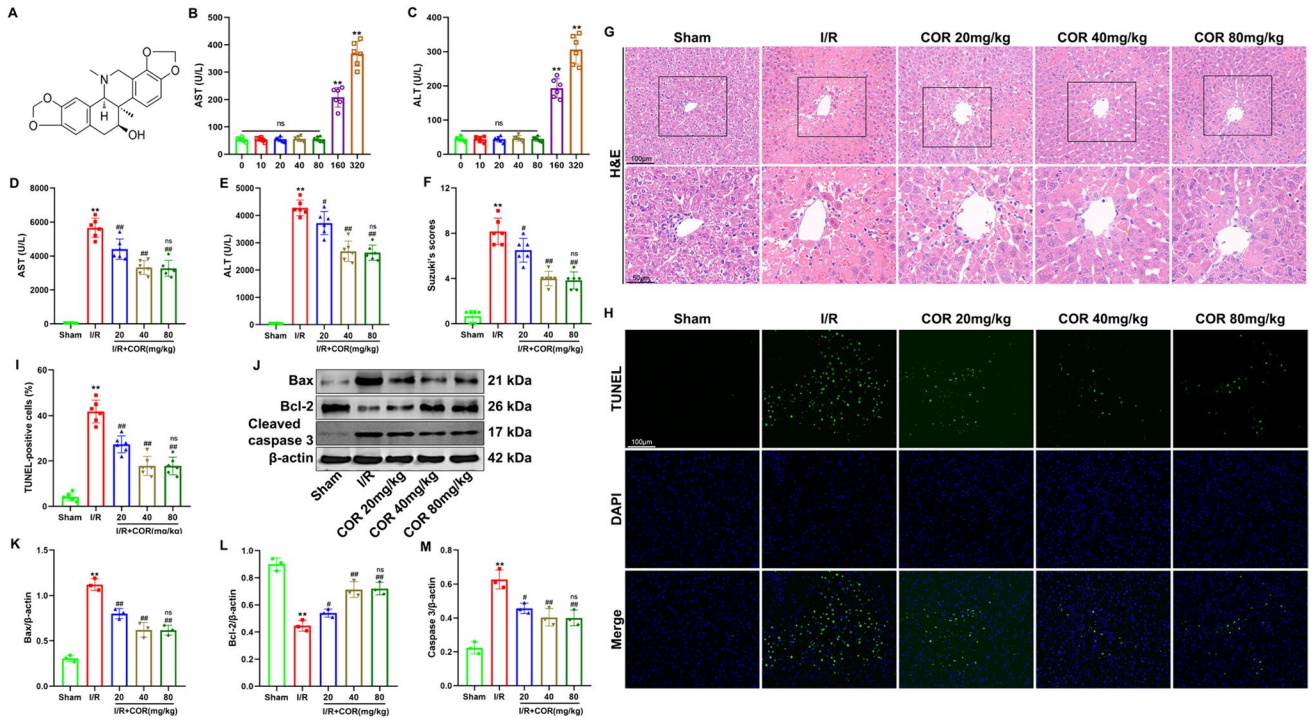

Fig. 1 Corynoline alleviates the hepatocellular damage and apoptosis induced by I/R in rats. **a**. The corynoline chemical structure; **b**–**c**. Rats in the sham group were pretreated with vehicle or different doses of corynoline, as indicated; **d**–**m**. Rats underwent I/R and were pretreated with vehicle or different doses of corynoline, as indicated; **b**–**e**. Serum levels of ALT and AST; **f**–**g**. Representative images of H&E-stained liver tissues and degree of hepatic injury represented by the Suzuki score. Scale bar = 100 μm (upper panels) and 50 μm (lower panels); **h**–**i**. Representative TUNEL staining images in liver sections and quantitative analysis of TUNEL-positive cells. Scale bar = 100 μm; J-M. Western blot analysis of Bax, Bcl-2, and cleaved caspase3 protein expression levels. The band density was measured and normalized to β-actin. The experiments were repeated at least three times. All data are presented as the mean ± standard deviation (n = 6 per group). ns, not significant versus the Sham group or I/R + COR (40 mg/kg); $^{**}P < 0.01$ versus the Sham group; $\#P < 0.05$ and $\#\#P < 0.01$ versus the I/R group

Materials and methods

Animals

Male Sprague–Dawley rats, weighing between 200 and 250 g, were sourced from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). These rats were housed in a controlled environment with regulated temperature and humidity, following a light–dark cycle of 12 h each. They had unrestricted access to both food and water. All animal care and experimental procedures strictly adhered to the guidelines approved by the Committee for Animal Research at Zhengzhou University (Approval No.: 2023-KY-0761-002).

Model establishment and experimental design

A nonfatal model of 70% hepatic warm ischemia and reperfusion was established using a previously described protocol [27]. In brief, rats were subjected to midline laparotomy under anesthesia with 40 mg/kg pentobarbital sodium to expose the hepatic hilar structure. Subsequently, a no-damage vascular clip was used to clamp the hepatic artery, portal veins, and bile ducts supporting the three upper liver lobes to induce segmental hepatic ischemia. Following a 1-h occlusion period, the clip was released and reperfusion was conducted for 6 h. The rats were randomly allocated into the following groups: Sham group (Sham, six rats), where the rats underwent laparotomy and hepatic hilum mobilization without vascular occlusion; Sham + corynoline group (COR, six groups with six rats per group), where the rats were pretreated with corynoline (purity > 98%, HY-N0705, MedChemExpress (MCE), Monmouth Junction, NJ, USA) at 10, 20, 40, 80, 160, and 320 mg/kg concentrations 1 h prior to laparotomy without vascular occlusion; I/R group (I/R, six rats), where the rats were pretreated with vehicle, then with vascular occlusion; I/R + corynoline group (I/R + COR, three groups with six rats per group), where the rats were pretreated with corynoline at selective concentrations (20, 40, and 80 mg/kg) 1 h prior to laparotomy, then with vascular occlusion; I/R + ML385 group (I/R + ML385, six rats), where the rats were pretreated with ML385 (HY-100523,

MCE, 30 mg/kg), a selective Nrf2 inhibitor, 30 min prior to laparotomy, then with vascular occlusion; I/R + corynoline + ML385 group (I/R + COR + ML385, six rats), where the rats were pretreated with corynoline (30 mg/kg) and ML385 (30 mg/kg) as described above, then with vascular occlusion. Corynoline and ML385 were solubilized in a normal saline solution containing 5% DMSO and stored at 4 °C. The rats were euthanized in a humane manner following a 6-h reperfusion period. Blood samples and the three upper lobes of the liver were gathered and preserved for subsequent analysis.

Biochemical analysis and enzyme-linked immunosorbent assays (ELISAs)

The activities of AST, ALT, and LDH in serum samples or cell culture supernatants were quantified using an automated biochemical analyzer (Rayto, Shenzhen, China) following the respective manufacturer's protocols. The levels of IL-6, TNF-α, and IL-1β in serum samples or cell culture supernatants were determined using ELISA kits. Additionally, the SOD and MDA activities in cell or tissue lysates were assessed using colorimetric assay kits. All kits used in this study were procured from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Histological analysis and liver injure evaluation

The liver samples were preserved using formalin and then embedded in paraffin. Thin sections of the tissue (5 μm) were subjected to staining with hematoxylin and eosin (H&E), followed by examination under a light microscope. The assessment of hepatic tissue damage was conducted based on Suzuki's criteria [28]. The histological score was graded from 0 to 12 depending on the severity of cellular vacuolization (score: 0–4), hepatic sinusoid congestion (score: 0–4), and hepatocyte necrosis (score: 0–4).

TUNEL, immunohistochemistry (IHC), and immunofluorescence (IF) assays

Hepatic macrophages and neutrophils were identified through IHC staining. In brief, tissue Sects. (5 μm) were incubated overnight at 4 °C with a primary antibody against CD68 (1:100, Abcam, ab283654) or MPO (1:1000, Abcam, ab208670), followed by incubation with a horseradish peroxidase (HRP)-labeled secondary antibody. Subsequently, the tissue sections were treated with 3,3'-diaminobenzidine tetrahydrochloride (DAB) and visualized using Leica Microsystems at a magnification of 200×. The Nrf2 and NLRP3 protein expression patterns in liver tissues were detected using IF staining. Briefly, tissue Sects. (5 μm) were incubated overnight at 4 °C with a primary antibody against Nrf2 (1:500, Abcam, ab313825) or NLRP3 (1:100, ABclonal, A5652), washed, and then incubated with an Alexa Fluor 555-conjugated secondary antibody (1:500; Abcam, ab150078). Finally, the slides were counterstained with DAPI and analyzed with a fluorescence microscope (Olympus). Cell apoptosis levels in liver tissues were assessed using the TUNEL assay, along with the In Situ Cell Death Detection Kit (Roche, Basel, Switzerland). Six randomly selected fields from each slide were examined at a magnification of 200×. Image-pro plus 6.0 software was employed for image analysis purposes.

ROS measurement

ROS levels were assessed using dihydroethidium (DHE, Servicebo, Wuhan, China), a fluorescent probe that exhibits sensitivity towards ROS. In brief, frozen liver Sects. (4 μm) or BRL-3A cells were incubated with 10 μM DHE in the dark at 37 °C for 30 min. Subsequently, counterstaining was performed by treating the samples with DAPI at room temperature for 10 min. Following three washes with PBS, fluorescence microscopy was employed to visualize the ROS generation in cells or tissues. Image density analysis was conducted utilizing Image J software (NIH, Bethesda, MD, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) was used to extract total RNA from tissue and cell samples following the manufacturer's protocol. Subsequently, a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was employed to reverse transcribe the total RNA into cDNA. For gene expression analysis, Applied Biosystems SYBR Green Mix kits (Thermo Fisher Scientific) were used for qRT-PCR analysis. The primer sequences can be found in Table 1. To determine relative expression levels with the $2^{-\Delta\Delta CT}$ method, data normalization was performed using β-actin expression levels.

Western blot analysis

The Whole Protein Extraction Kit (KeyGEN BioTECH) was used to extract total proteins from tissues and cells. In addition, the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Shanghai, China) was used to extract nuclear and cytoplasmic proteins from tissues and cells according to the manufacturer's instructions. Briefly, the liver tissue or cells were lysed with protein extractor A containing PMSF (Biosharp, China) on an ice for 15 min. Next, 10 μL B agent was added to the samples, which were then stirred and centrifuged at 4 °C. The resulting supernatants then included

Table 1 The primer sequences for qRT-PCR

Gene	Forward (5′-3′)	Reverse (5′-3′)
TNF-α	ATGGGCTCCCTCTCATCAGT	GCTTGGTGGTTTGCTACGAC
IL-6	CTGGTCTTCTGGAGTTCCGTT	GCATTGGAAGTTGGGGTAGGA
IL-1β	CGTGGG ATGATGACGACCTG	GCCACAGGGATTTTGTCGTT
β-actin	AGATCAAGATCATTGCTCCTCCT	ACGCAGCTCAGTAACAGTCC

the extracted cytoplasmic protein. For precipitation, the remaining supernatant was completely removed, then 50 μL nuclear protein extractant containing PMSF was added for precipitation. After 30 min of intermittent stirring on ice, the supernatant obtained by centrifugation was the extracted nuclear protein. The BCA Protein Assay Kit (Beyotime) was then used to determine the protein concentrations of the extracted samples. Following denaturation, equivalent amounts of total protein were separated through 10%–12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride membrane. Subsequently, the membranes were blocked with 5% skim milk at room temperature for 2 h before being incubated overnight with primary antibodies at 4 °C. The primary antibodies used in this study were as follows: anti-NLRP3 antibody (1:2000, ABclonal, A5652), anti-ASC antibody (1:1000, Abcam, ab309497), anti-cleaved caspase1 p20 (Asp296, 1:1000, Affinity, AF4005), anti-cleaved IL-1β (Asp116, 1:1000, Affinity, AF4006), anti-IL-18 (1:1000, Proteintech, 10,663–1-AP), anti-Nrf2 (1:500, Abcam, ab313825), anti-HO-1 (1:1000, Proteintech, 10,701–1-AP), anti-Bcl-2 (1:1000, Proteintech, 26,593–1-AP), anti-Bax (1:5000, Proteintech, 50,599–2-Ig), anti-cleaved caspase3 (1:1000, Proteintech, 25,128–1-AP), anti-β-actin (1:5000, Proteintech, 66,009–1-Ig), and anti-Lamin B1 (1:5000, Proteintech, 12,987–1-AP). After three washes in TBST, the membranes were incubated with an HRP-conjugated secondary antibody (1:5000, Proteintech, SA00001-1/SA00001-2) at room temperature for 2 h. Subsequently, visualization was performed using the ECL Plus Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK). Densitometry analysis utilizing Image J software was employed to determine the relative protein expression levels. Cytoplasmic proteins were normalized to β-actin, while nuclear proteins were normalized to Lamin B1.

Transmission *electron* microscopy (TEM) analysis

Liver tissue fragments (1–2 mm^3) were promptly immersed in 2.5% glutaraldehyde, then incubated at 4 °C overnight. The tissues were then fixed in 2% osmium tetroxide for 2 h. Subsequently, the samples underwent dehydration and infiltration processes before being sectioned into ultra-thin slices (70–90 mm). These sections were then stained with 2% uranyl acetate and subjected to TEM (Tecnai G2 20 TWIN, FEI, Hillsboro, OR, USA) analysis with an acceleration voltage of 200 kV.

Cell H/R model and cell viability assay

BRL-3A cells were acquired from the Cell Resource Center of the Chinese Academy of Sciences (Shanghai, China). These cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2. The H/R cell model, mimicking hepatic I/R, has been described previously [28]. In brief, the cells were cultured in DMEM without serum (glucose-free) and then subjected to hypoxia by placing them inside a tri-gas incubator saturated with an atmosphere consisting of 94% N_2, 5% CO_2, and 1% O_2 for 12 h. Following this treatment, the glucose-free DMEM was removed and replaced with complete medium. The cells were subsequently incubated for 6 h in the presence of oxygen to facilitate reoxygenation. Corynoline was administered at different concentrations (1, 2.5, 5, 10, 20, 40, or 80 μM) 24 h prior to cell pretreatment. The viability of BRL-3A cells was assessed using CCK-8 assays (Sigma-Aldrich, St. Louis, MO, USA). In brief, the cells were cultured in 96-well plates. Following pretreatment, 10 μL CCK-8 solution was added to each well and incubated at 37 °C for 1 h. The absorbance values at 450 nm were then measured using a microplate reader (Molecular Devices, CA, USA). From the above results, the following cell culture groups were established: sham group, H/R group, H/R + COR group, and H/R + COR + nigericin group. Cells in the sham group were cultured in complete culture medium under normal conditions, while cells in the H/R group were subjected to H/R. Corynoline (10 μM) was added into the culture medium 24 h prior to H/R in the H/R + COR and H/R + COR + nigericin groups. In addition, nigericin (10 μM, MCE, HY-100381), an NLRP3 activator, was added 30 min before H/R in the H/R + COR + nigericin group.

Flow cytometry analysis

BRL-3A cell apoptosis levels were assessed using flow cytometry with the Annexin V-FITC/PI apoptosis kit (BD, Pharmingen, UK) following the manufacturer's instructions. In brief, after pretreatment, 20 μL PI and 10 μL Annexin

V-FITC were added to the cells. Subsequently, the cells were incubated at 37 °C in the dark for 15 min. The apoptotic cells were then analyzed using a FACS flow cytometer (Becton Dickinson, CA, USA). FlowJo 7.6 software (Tree Star, Inc.) was used for data analysis.

Statistical analysis

All data were analyzed using SPSS version 21.0 (SPSS, Chicago, IL, USA) and presented as the mean ± standard deviation (SD). The unpaired t-test was employed for comparing two groups, while one-way ANOVA followed by a Tukey post hoc test was used for analyzing comparisons among multiple groups. P-values < 0.05 were considered statistically significant.

Results

Corynoline alleviates hepatocellular damage and apoptosis during hepatic I/R injury in rats

To determine the appropriate corynoline dosage to administer to rats, we first used a concentration gradient to assess its impact on liver function. The preliminary test results indicated that corynoline did not significantly affect liver function at an 80 mg/kg dose, as demonstrated by AST and ALT levels in COR-pretreated rats (Fig. 1b and c). As depicted in Fig. 1d and e, the serum ALT and AST levels of rats subjected to I/R were significantly elevated compared with those in the sham group. However, pretreatment with corynoline at doses of 20 mg/kg, 40 mg/kg, and 80 mg/kg effectively reduced the serum ALT and AST levels in rats undergoing I/R. Consistent with these results, histological examination using H&E staining revealed noticeable liver injury in the I/R group compared with the sham group. This damage presented as extensive sinusoidal congestion, hepatocyte necrosis, vacuolar degeneration, inflammatory cell infiltration, and higher Suzuki scores. In contrast, pretreatment with corynoline at 20 mg/kg, 40 mg/kg, and 80 mg/kg doses resulted in clear reductions in hepatic injury and Suzuki scores in rats undergoing I/R (Fig. 1f and g). Additionally, the I/R group exhibited a significant increase in the number of TUNEL-positive cells (apoptotic cells) compared with the sham group. However, pretreatment with corynoline at doses of 20 mg/kg, 40 mg/kg, and 80 mg/kg resulted in a notable reduction in the apoptotic cell count (Fig. 1h and i). To further validate these findings, we used western blot analysis to assess the liver tissue expression levels of Bcl-2, an anti-apoptotic protein, as well as cleaved caspase3 and Bax, both pro-apoptotic proteins. Compared with the sham group, hepatic I/R induced a significant upregulation of cleaved caspase3 and Bax protein expression, while downregulating Bcl-2 protein expression. However, corynoline pretreatment at doses of 20 mg/kg, 40 mg/kg, and 80 mg/kg effectively reversed these effects (Fig. 1j–m). No significant differences were found between the 40 mg/kg and 80 mg/kg corynoline pretreatment doses in the AST and ALT levels, tissue histology injury, or cell apoptosis rates during hepatic I/R injury in these rats. Overall, these results indicate that corynoline could alleviate I/R-induced liver injury. The optimal corynoline pretreatment dose in the rats was 40 mg/kg, which was used for subsequent experiments.

Corynoline attenuates I/R-induced oxidative stress and promotes Nrf2/HO-1 expression in rats

During liver I/R injury, hepatocytes experience mitochondrial damage, resulting in increased ROS and MDA levels, as well as decreased SOD levels. These changes serve as crucial indicators for assessing oxidative damage [14]. To investigate whether the protective effects of corynoline on hepatic I/R injury in rats are associated with its antioxidant properties, we initially conducted TEM analysis of the liver tissues. This analysis showed that corynoline pretreatment had little effect on the hepatocyte mitochondria morphology in sham-treated rats. However, liver I/R injury in rats resulted in the development of mitochondrial morphological abnormalities, including swelling of mitochondria, reduction or loss of mitochondrial ridges, formation of cavities, and accumulation of lipid droplets. After corynoline pretreatment, these changes were clearly alleviated (Fig. 2a). Next, we performed ROS staining and determined the MDA and SOD levels in the liver tissues. Corynoline pretreatment had no effects on the ROS, SOD, or MDA levels in sham-treated rats. However, the hepatic tissues of the I/R group showed significantly decreased SOD levels and increased MDA concentrations compared with those of the sham group. Conversely, the I/R + COR group exhibited significantly higher SOD levels and lower MDA concentrations than the I/R group (Fig. 2b and c). Furthermore, there was a marked increase in ROS accumulation in the I/R group compared with the sham group, while this was notably decreased following corynoline pretreatment (Fig. 2d and e).

Nrf2 is a redox-sensitive transcription factor that is activated and translocates into the nucleus in response to oxidative stress [12]. Thus, we further detected the effect of corynoline treatment on Nrf2 expression patterns. IF staining demonstrated that Nrf2 protein was primarily localized in the cytoplasm after hepatic I/R injury in the rats, yet its expression levels were remarkably increased in the nuclei after corynoline treatment (Fig. 2f and g). These data were supported by the western blot analysis results. As shown in Fig. 2h–k, corynoline pretreatment increased Nrf2 nuclear translocation in rats after hepatic I/R injury. Moreover, the expression levels of HO-1, an Nrf2 target gene, were also

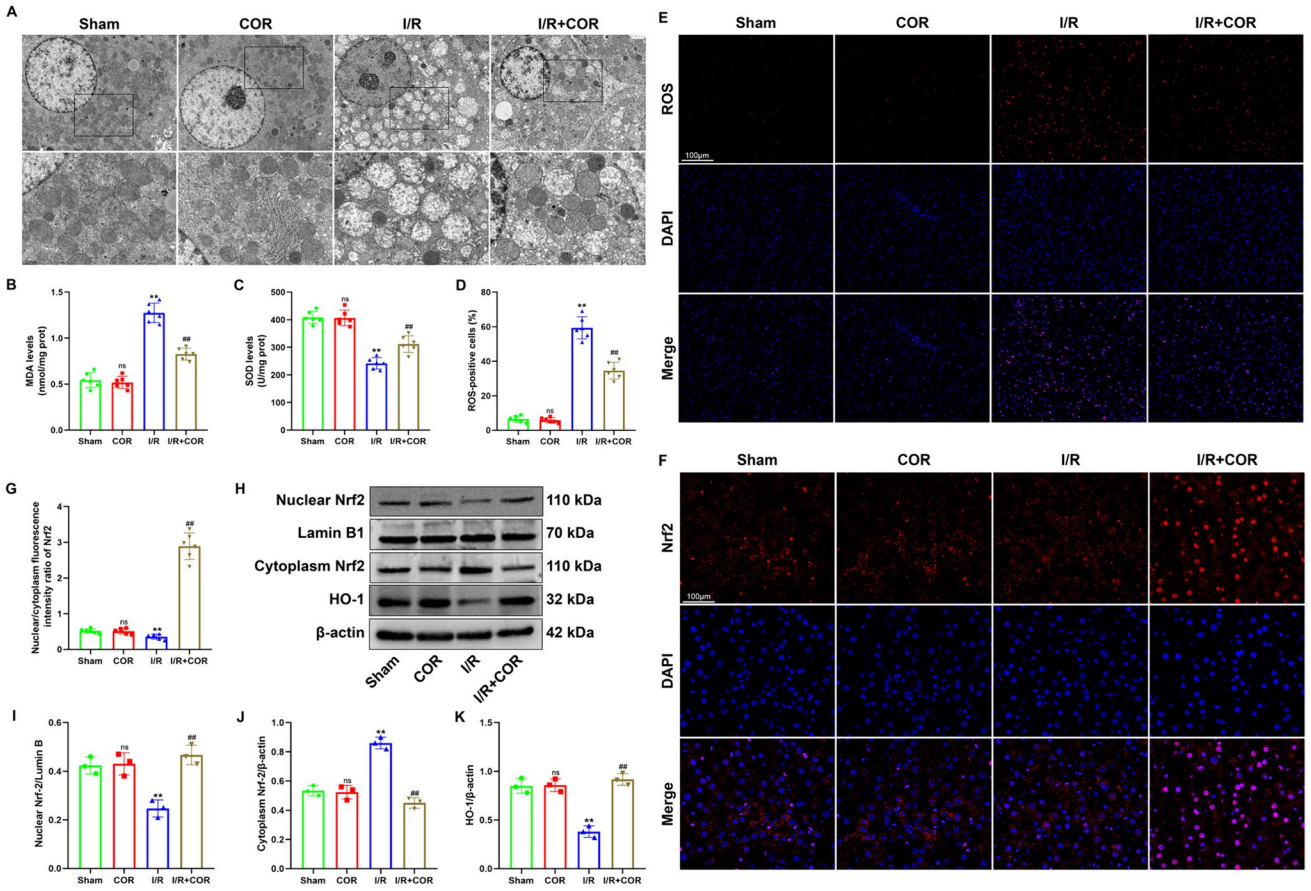

Fig. 2 Corynoline attenuates I/R-induced oxidative stress and promotes Nrf2/HO-1 expression in rats. **a**. Representative TEM images of mitochondrial morphology. Scale bar = 2 μm (upper panels) and 500 nm (lower panels). **b**–**c**. MDA and SOD concentrations in liver tissues; **d**–**e**. Representative images of ROS staining in liver tissues and quantitative analysis of ROS-positive cells. Scale bar = 100 μm; **f**–**g**. Representative images of Nrf2 immunofluorescence staining and quantitative analysis of the ratio of nuclear Nrf2 to cytoplasmic Nrf2. Scale bar = 100 μm; **h**–**k**. Western blot analysis of nuclear Nrf2, cytoplasmic Nrf2, and HO-1 protein expression levels. The band density was measured and normalized to Lamin B1 or β-actin. The experiments were repeated at least three times. All data are presented as the mean ± standard deviation (n = 6 per group). ns, not significant versus the Sham group or I/R + COR (40 mg/kg); $^{**}P < 0.01$ versus the Sham group; $\#P < 0.05$ and $\#\#P < 0.01$ versus the I/R group

enhanced after corynoline pretreatment. Taken together, our results reveal that corynoline treatment could alleviate the liver oxidative stress injury induced by I/R, as well as promote Nrf2 nuclear translocation and its downstream target gene expression.

Corynoline inhibits I/R-induced NLRP3 inflammasome activation in rats

The NLRP3 inflammasome is another important participant in hepatic I/R injury-related mechanisms. It can be activated in an ROS-dependent manner, linking cellular oxidative stress to the inflammatory response [6]. We next used IF staining and western blot analysis to explore if corynoline treatment could influence NLRP3 inflammasome activation in rat liver I/R injury. As shown in Fig. 3a and b, the IF staining data suggested a higher distribution of NLPR3-positive cells in the liver after I/R stimulation compared with the sham group. However, pretreatment with corynoline significantly reduced the NLPR3 expression levels. Western blot analysis showed that hepatic I/R injury led to NLRP3 inflammasome activation, as evidenced by increased protein expression levels of NLRP3, ASC, cleaved caspase1, IL-1β, and IL-18 in the I/R group compared with the sham group. Following corynoline pretreatment, there was a significant decrease in these protein levels (Fig. 3c–h). These findings suggest that corynoline pretreatment can effectively inhibit NLRP3 inflammasome activation induced by I/R in rats.

Corynoline attenuates I/R-induced inflammatory responses in rats

Activation of the NLRP3 inflammasome can trigger and amplify the inflammatory response [5]. Next, we examined

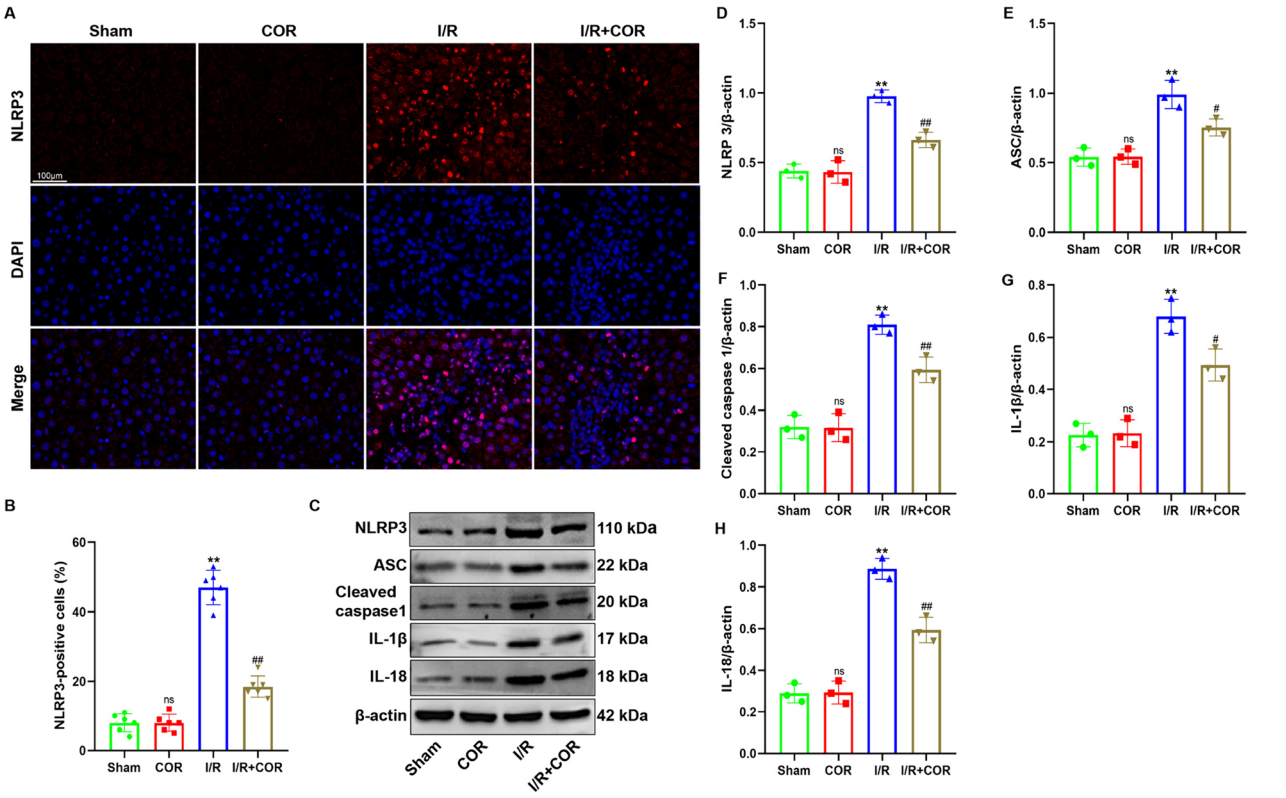

Fig. 3 Corynoline inhibits I/R-induced NLRP3 inflammasome activation in rats. **a**–**b**. Representative images of NLRP3 immunofluorescence staining and quantitative analysis of NLRP3-positive cells. Scale bar = 100 μm; **c**–**h**. Western blot analysis of NLRP3, ASC, cleaved caspase1, IL-1β, and IL-18 protein expression levels. The band density was measured and normalized to β-actin. The experiments were repeated at least three times. All data are presented as the mean ± standard deviation (n = 6 per group). ns, not significant versus the Sham group; $^{**}P < 0.01$ versus the COR group; $\#P < 0.05$ and $\#\#P < 0.01$ versus the I/R group

the impact of corynoline treatment on hepatic inflammatory responses induced by I/R in rats. To evaluate changes in pro-inflammatory mediator expression patterns, we firstly conducted ELISAs to detect the serum levels of IL-6, TNF-α, and IL-1β. The results depicted in Fig. 4a–c indicate that I/R injury led to a significant elevation in pro-inflammatory cytokine levels compared with the sham group. However, pretreatment with corynoline substantially decreased the levels of these pro-inflammatory cytokines. Furthermore, these protein trends were consistent with those observed in liver tissues at the mRNA level (Fig. 4d–f). Subsequently, we assessed MPO and CD68 protein expression by IHC staining to investigate the effects of corynoline on I/R-induced inflammatory cell infiltration in rat livers. These cells included neutrophils (MPO-positive) and macrophages (CD68-positive). I/R significantly upregulated CD68 and MPO expression levels, which was effectively abated by corynoline pretreatment (Fig. 4g–i). Collectively, these data indicate that corynoline treatment can attenuate I/R-induced inflammatory responses in rats.

Corynoline suppresses NLRP3 inflammasome activation through Nrf2/HO-1 activation during hepatic I/R injury in rats

Because corynoline exerted both antioxidant and anti-inflammatory effects, we further explored if a relationship exists between Nrf2 activation and NLRP3 inflammasome inhibition through corynoline pretreatment. Western blot analysis revealed that pretreatment with corynoline led to a significant enhancement in Nrf2 translocation into the nucleus and increased HO-1 protein expression levels. Conversely, corynoline pretreatment resulted in decreased protein expression levels of NLRP3, ASC, cleaved caspase1, IL-1β, and IL-18 within rat livers experiencing I/R injury. However, these observed alterations were nullified upon administration of ML385, a selective Nrf2 inhibitor (Figs. 5a–i). These findings strongly suggest that corynoline can impede NLRP3 inflammasome activation by stimulating Nrf2/HO-1 signaling.

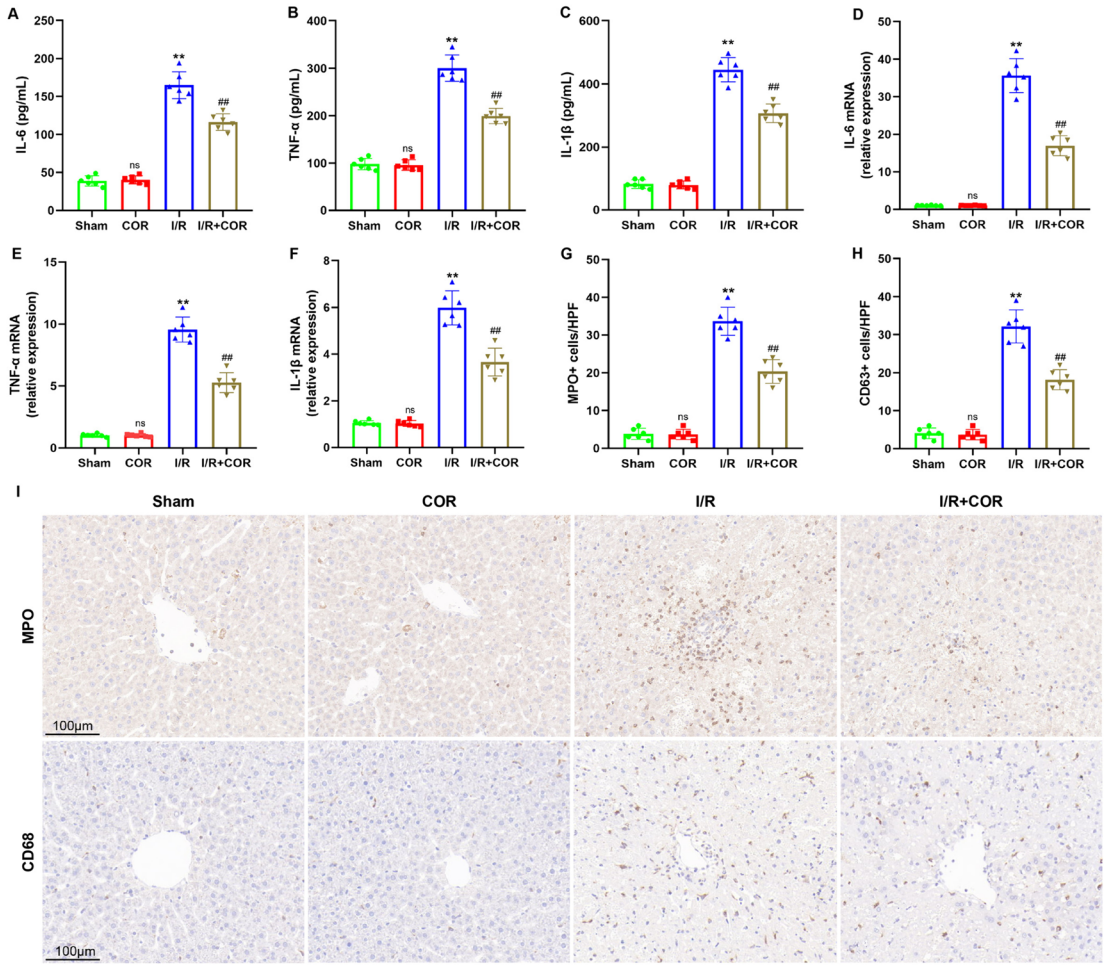

Fig. 4 Corynoline attenuates I/R-induced inflammatory responses in rats. **a**–**c**. Serum levels of IL-6, TNF-α, and IL-1β; **d**–**f**. The relative mRNA expression levels of IL-6, TNF-α, and IL-1β in liver tissues; **g**–**i**. Representative images of MPO and CD68 immunohistochemistry staining and quantification analysis of MPO-positive neutrophils and CD68-positive macrophages. Scale bar = 100 μm. The experiments were repeated at least three times. All data are presented as the mean ± standard deviation (n = 6 per group). ns, not significant versus the Sham group; $^{**}P < 0.01$ versus the COR group; $^{\#\#}P < 0.01$ versus the I/R group

Blocking the Nrf2/HO-1 pathway abolishes the antioxidant, anti-inflammatory, and hepatoprotective activities of corynoline during hepatic I/R injury in rats

Because corynoline treatment could mitigate hepatic I/R injury by inhibiting oxidative stress, inflammation, and apoptosis, as well as by upregulating Nrf2/HO-1 pathway components expression levels, we further explored whether blocking the Nrf2/HO-1 pathway with ML385 treatment could impact the protective effects exerted by corynoline. As depicted in Fig. 6a–f, the I/R + COR + ML385 group exhibited a significant exacerbation of liver injury compared with the I/R + COR group. This was evident from the higher levels of AST, ALT, TUNEL-positive cells, and Suzuki scores observed in the former group. These findings indicate that pretreatment with ML385 could counteract the hepatoprotective effects of corynoline against liver damage. Furthermore, ML385 treatment effectively counteracted the corynoline-induced elevation of SOD levels and reduction in MDA levels. Additionally, the inhibited release of IL-6, TNF-α, and IL-1β and neutrophil and macrophage recruitment by corynoline treatment were nullified by adding ML385. These findings indicate that the Nrf2/HO-1 signaling pathway contributes to the antioxidant, anti-inflammatory, and hepatoprotective activities of corynoline in hepatic I/R injury.

Corynoline protects against H/R-induced apoptosis and activates the Nrf2/HO-1/NLRP3 signaling pathway in vitro

From these in vivo results, we constructed an in vitro H/R model using BRL-3A cells, aiming to further validate the

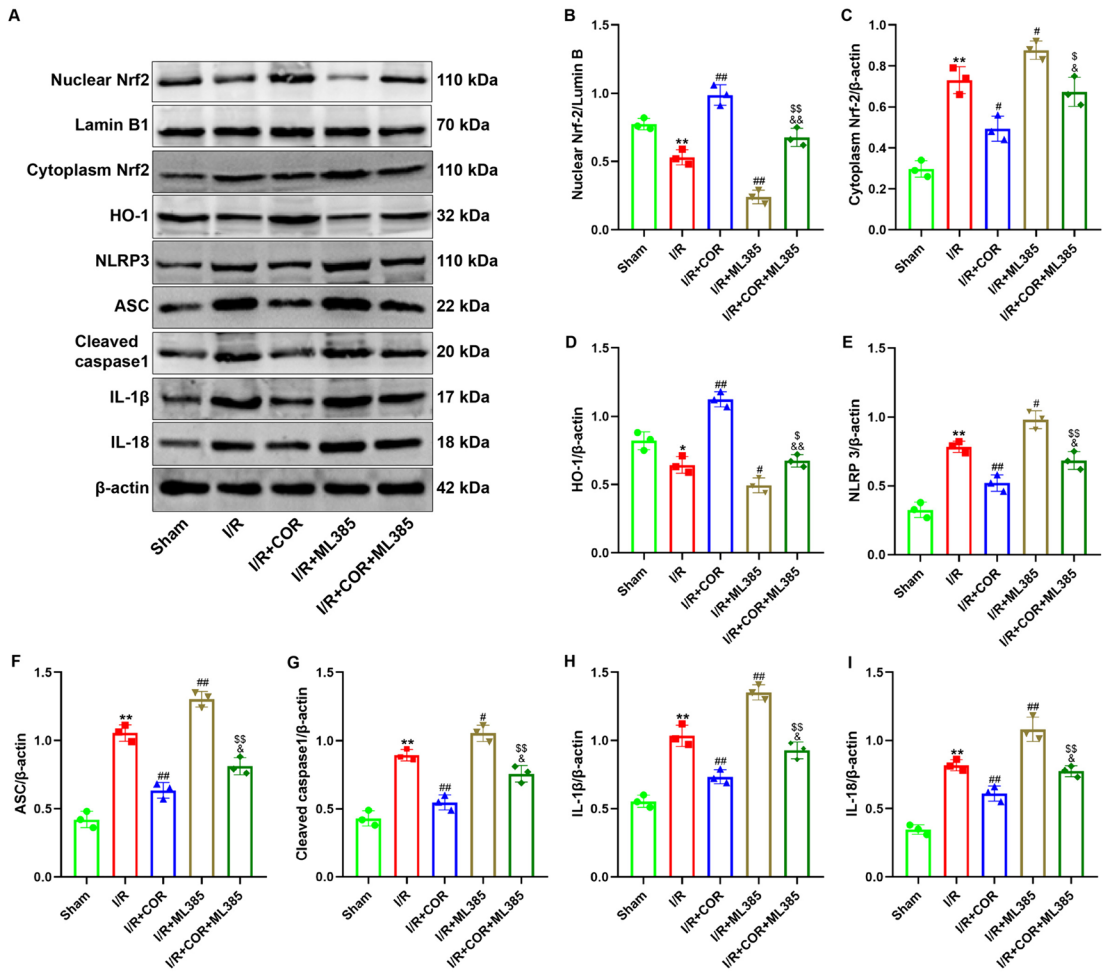

Fig. 5 Corynoline suppresses NLRP3 inflammasome activation through Nrf2/HO-1 activation during hepatic I/R injury in rats. **a–i**. Western blot analysis of nuclear Nrf2, cytoplasm Nrf2, HO-1, NLRP3, ASC, cleaved caspase1, IL-1β, and IL-18 protein expression levels. The band density was measured and normalized to Lamin B1 or β-actin. The experiments were repeated at least three times. All data are presented as the mean ± standard deviation. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the Sham group; $\#P < 0.05$ and $\#\#P < 0.01$ versus the I/R group; $\&P < 0.05$ and $\&\&P < 0.01$ versus the I/R + COR group; $\$P < 0.05$ and $\$\$P < 0.01$ versus the I/R + ML385 group

protective properties of corynoline against hepatic I/R injury. We first employed CCK-8 assays to investigate the impact of various corynoline concentrations on BRL-3A cell viability, seeking to identify the optimal concentration for pretreatment. As shown in Fig. 7a, the cell viability remained largely unaffected when exposed to corynoline concentrations of 20 μM or lower. However, a significant decrease in cell viability was observed when the concentration reached 40 μM, suggesting potential toxicity at concentrations exceeding 40 μM. Subsequently, BRL-3A cells were incubated with varying concentrations of corynoline (1, 2.5, 5, 10, or 20 μM) and subjected to H/R exposure for cell viability assessment. The results indicated that H/R exposure led to a substantial reduction in cell viability, but this reduction was significantly attenuated by corynoline concentrations of 2.5, 5, 10, and 20 μM (Fig. 7b). However, pretreatment with corynoline at a dose of either 20 or 40 mg/kg did not yield any significant changes in BRL-3A cell viability when exposed to H/R. From these findings, the subsequent experiments were conducted using a 20 μM corynoline concentration.

We next investigated the effect of corynoline pretreatment on hepatocyte apoptosis levels and the underlying mechanism. Nigericin, an NLRP3 activator, was used to stimulate NLRP3 expression. As illustrated in Fig. 7c–f, corynoline pretreatment effectively enhanced the expression levels of proteins that promote apoptosis, such as Bax and cleaved caspase3, while suppressing the expression levels of the anti-apoptotic protein Bcl-2, in H/R-induced BRL-3A cells. After nigericin administration, the protective effect of corynoline was diminished. Flow cytometry analysis clearly demonstrated that BRL-3A cell apoptosis levels increased considerably during H/R injury, but were significantly reversed following corynoline pretreatment. However,

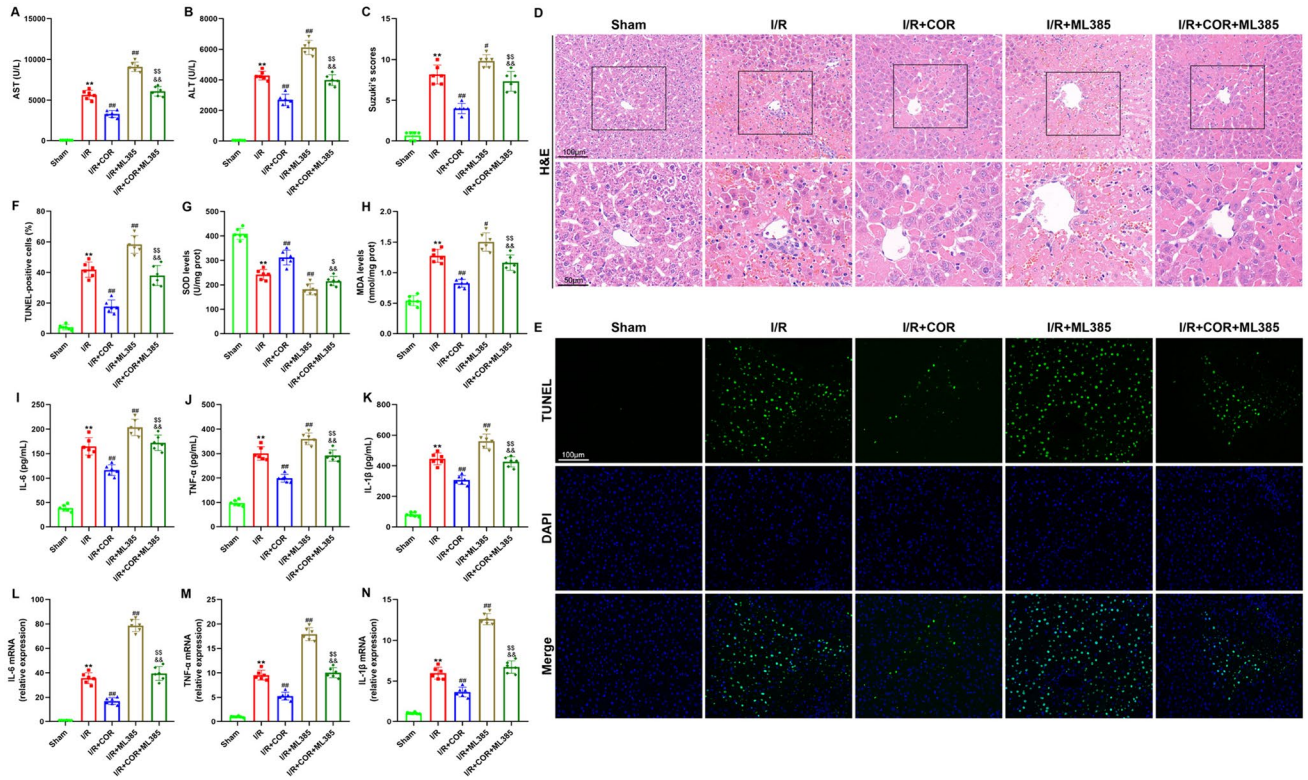

Fig. 6 ML385 abolishes the antioxidative, anti-inflammatory, and hepatoprotective activities of corynoline during hepatic I/R injury in rats. **a**–**b**. Serum ALT and AST levels; **c**–**d**. Representative images of H&E-stained liver tissues and degree of hepatic injury represented by the Suzuki score. Scale bar = 100 μm (upper panels) and 50 μm (lower panels); **e**–**f**. Representative TUNEL staining images in liver sections and quantitative analysis of TUNEL-positive cells. Scale bar = 100 μm; G-H. MDA and SOD concentrations in liver tissues; **i**–**k**. Serum levels of IL-6, TNF-α, and IL-1β; L-N. The relative mRNA expression levels of IL-6, TNF-α, and IL-1β in liver tissues. The experiments were repeated at least three times. All data are presented as the mean ± standard deviation. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the Sham group; $\#P < 0.05$ and $\#\#P < 0.01$ versus the I/R group; $\&P < 0.05$ and $\&\&P < 0.01$ versus the I/R + COR group; $\$P < 0.05$ and $\$\$P < 0.01$ versus the I/R + ML385 group

the anti-apoptotic effect of corynoline was diminished by nigericin administration (Fig. 7g and h). Additionally, the western blot data in Fig. 7i–m demonstrate that the Nrf2/HO-1 protein expression levels were suppressed in BRL-3A cells induced by H/R. This is evident from the reduced Nrf2 nuclear translocation and HO-1 expression levels observed in the H/R group compared with the control group. However, upon pretreatment with corynoline, both Nrf2 nuclear translocation and HO-1 expression levels were significantly enhanced in the H/R + COR group. In addition, the NLRP3 inflammasome component proteins NLRP3, ASC, cleaved caspase1, IL-1β, and IL-18 were markedly elevated in the H/R group relative to the control group. Corynoline pretreatment notably suppressed the expression levels of these NLRP3 inflammasome component proteins. As anticipated, pretreatment with nigericin did not affect Nrf2/HO-1 expression levels, but markedly increased those of NLRP3 inflammasome component proteins. Collectively, these results indicate that corynoline pretreatment can protect against H/R-induced injury in BRL-3A cells. Additionally, Nrf2/HO-1/NLRP3 pathway activation may be imperative for corynoline to exert its optimal functionality.

NLRP3 inflammasome activation abrogates the protective effects of corynoline on H/R injury in BRL-3A cells

To determine if the NLRP3 inflammasome does mechanistically participate in the effect of corynoline on H/R injury in BRL-3A cells, the cells were treated with nigericin to stimulate the NLRP3 inflammasome. This nigericin treatment did reverse the protective effects of corynoline. As demonstrated in Fig. 8a–c, corynoline pretreatment substantially decreased the released AST, ALT, and LDH levels with H/R injury compared with the H/R group. After activation of NLRP3 by nigericin, the protective effect of corynoline on H/R injury was counteracted. Moreover, BRL-3A cells exposed to H/R showed greatly increased oxidative stress injury and pro-inflammatory cytokine release compared with the control group, as shown by higher ROS, MDA, IL-6, TNF-α, and

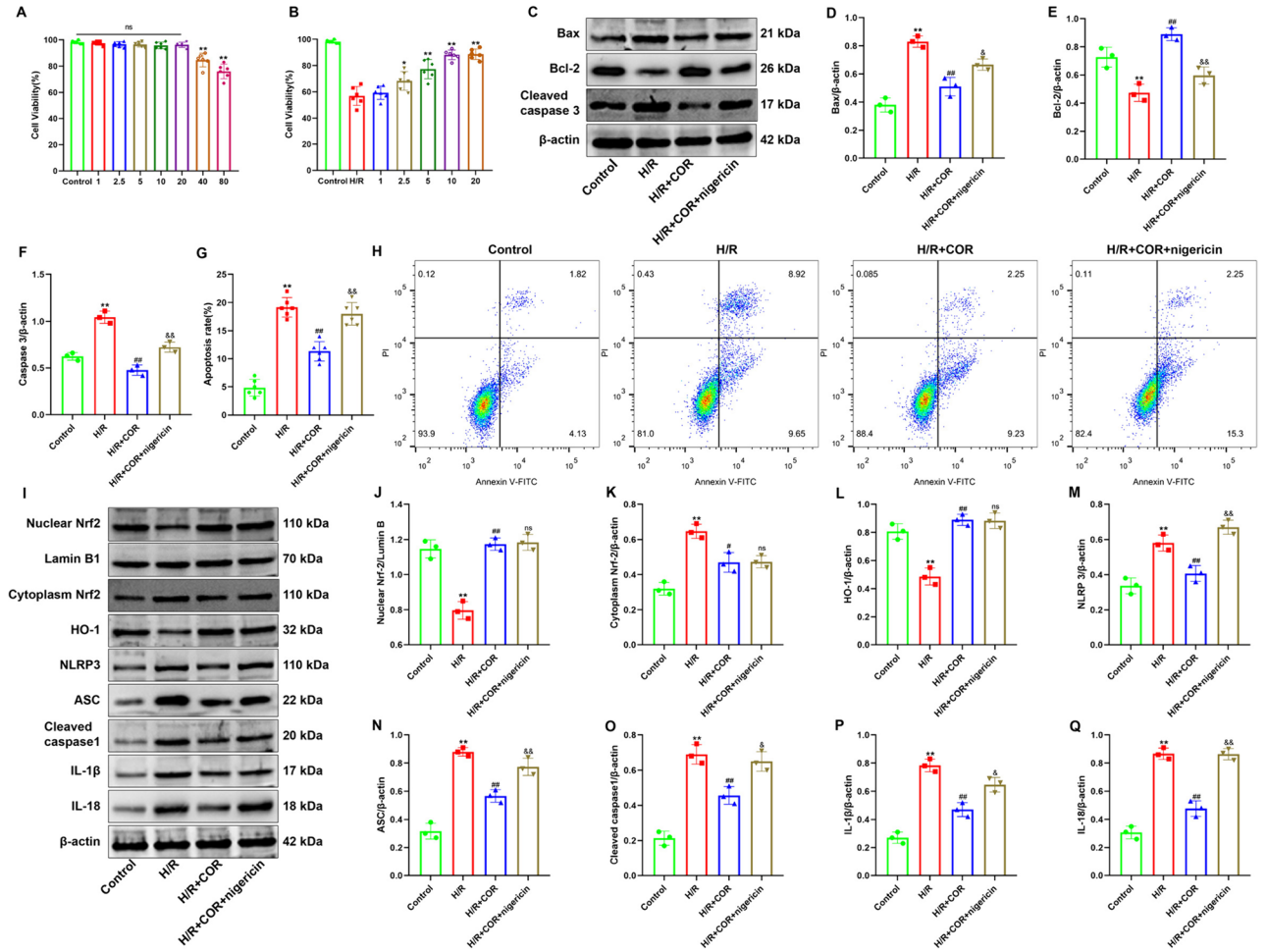

Fig. 7 Corynoline protects against H/R-induced apoptosis and activates Nrf2/HO-1/NLRP3 signaling in vitro. **a**–**b**. BRL-3A cells were incubated with various corynoline concentrations for 24 h, then treated with or without H/R. The cell viability was determined by CCK-8 assays; **c**–**f**. Western blot analysis of Bax, Bcl-2, and cleaved caspase3 protein expression levels. The band density was measured and normalized to β-actin. **g**–**h**. BRL-3A cell apoptosis rates were assessed and quantitatively analyzed by flow cytometry; **i**–**q**. Western blot analysis of nuclear Nrf2, cytoplasmic Nrf2, HO-1, NLRP3, ASC, cleaved caspase1, IL-1β, and IL-18 protein expression levels. The band density was measured and normalized to Lamin B1 or β-actin. The experiments were repeated at least three times. All data are presented as the mean ± standard deviation. ns, not significant versus the Control group or the H/R + COR group; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the Control group; $\#P < 0.01$ and $\#\#P < 0.01$ versus the H/R group; $\$P < 0.05$ and $\$\$P < 0.01$versus the H/R + COR group

IL-1β levels and lower SOD levels in the H/R group. Corynoline pretreatment substantially alleviated the oxidative stress injury and inflammatory response induced by H/R in BRL-3A cells. However, nicomycin could eliminate these corynoline-mediated anti-inflammatory and antioxidant effects in BRL-3A cells (Fig. 8d–m). These results suggest that corynoline treatment can protect BRL-3A cells against H/R-induced injury in an NLRP3-dependent manner.

Discussion

Hepatic I/R injury has attracted considerable research attention as a significant obstacle in liver surgical procedures. Excessive oxidative stress and/or overwhelmed inflammatory responses are believed to play essential roles in hepatic I/R injury pathogenesis [3]. Accordingly,

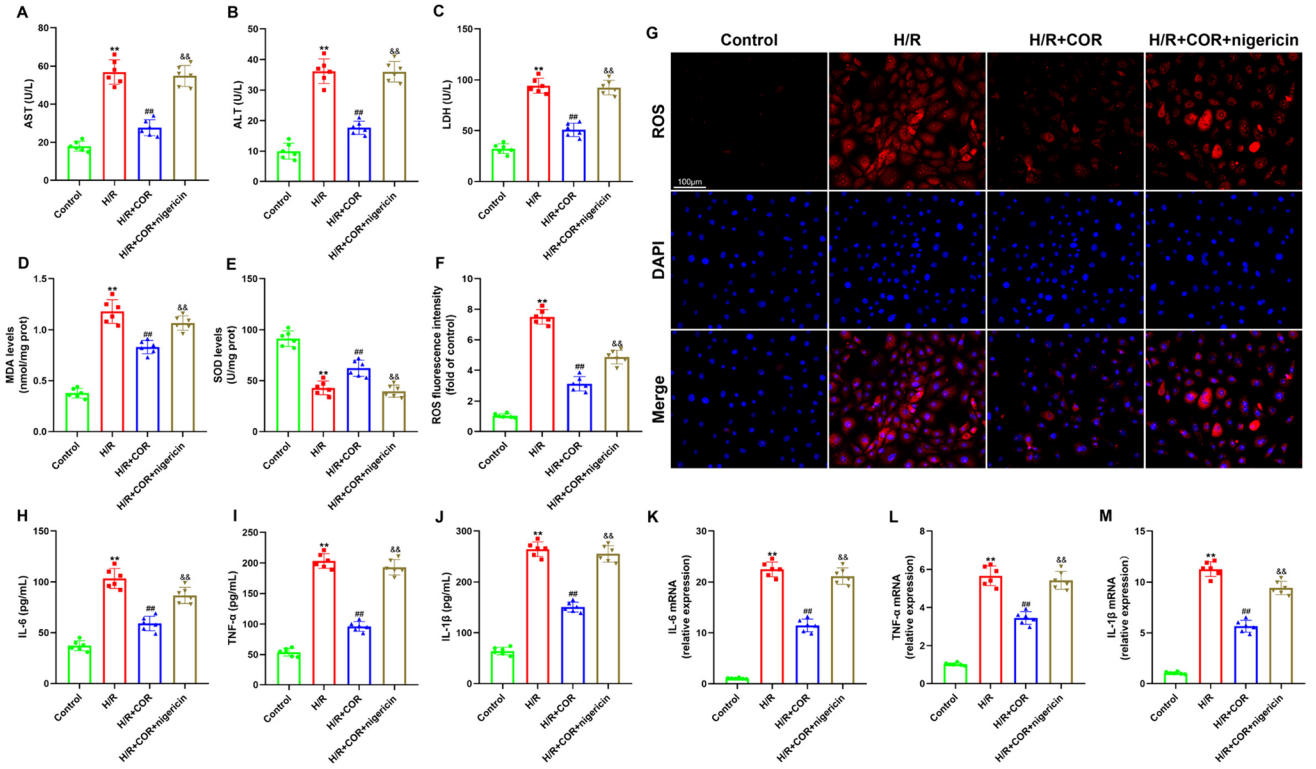

Fig. 8 Nigericin abrogates the protective effects of corynoline on H/R injury in BRL-3A cells. **a–c**. The ALT, AST, and LDH levels in cell culture supernatants; **d–e**. MDA and SOD concentrations in cells; **f–g**. Representative ROS staining images in BRL-3A cells and quantitative analysis of ROS fluorescence intensity. Scale bar = 100 μm; **h–j**. TNF-α, IL-6, and IL-1β levels in cell culture supernatants; K-M. The relative mRNA expression levels of IL-6, TNF-α, and IL-1β in cells; The experiments were repeated at least three times. All data are presented as the mean ± standard deviation. $^{**}P < 0.01$ versus the Control group; $^{\#\#}P < 0.01$ versus the H/R group; $\$\$P < 0.01$ versus the H/R + COR group

pharmacologically reducing the inflammatory response and oxidative damage is a potential strategy for ameliorating hepatic I/R injury. Corynoline has been shown to display anti-inflammatory and antioxidant biological activities in many disorders. In this study, we provide in vivo and in vitro evidence suggesting for the first time that corynoline can alleviate hepatic I/R injury by attenuating oxidative stress and inflammatory responses. More specifically, corynoline pretreatment attenuated liver necrosis and hepatocyte apoptosis levels, reduced inflammatory cell infiltration, lowered pro-inflammatory cytokine release, and inhibited mitochondrial dysfunction and ROS production during hepatic I/R injury. Additional investigations into the mechanisms revealed that corynoline exerted its protective effect on hepatic I/R injury primarily by suppressing NLRP3 inflammasome activation and promoting the initiation of Nrf2 antioxidant signaling (Fig. 9). Thus, corynoline treatment is a promising new intervention method that could be used in clinical practice.

Hepatic ischemia triggers a series of cellular events, which are worsened by reperfusion, and can cause structural and functional damage to the liver [2, 29]. Hepatocytes are the most important cellular component of the liver and frequently undergo apoptosis and necrosis during I/R injury [29]. Previous studies have shown that corynoline treatment can attenuate LPS-induced lung injury and zearalenone-induced liver injury [21, 26]. The present study unveiled that corynoline pretreatment exhibited a notable mitigation of liver I/R injury, as demonstrated by significantly decreased serum ALT and AST levels, improved pathological impairment, reduced TUNEL-positive cell count, and suppressed pro-apoptotic protein expression levels. In addition, in vitro experiments demonstrated that corynoline treatment improved the survival rates and reduced the apoptosis levels of H/R-induced BRL-3A cells. These results indicate that corynoline could improve I/R-induced liver damage and hepatocyte apoptosis both in vivo and in vitro. Nevertheless, additional investigations are needed to explore the underlying mechanism.

The innate immune response and sterile inflammatory response play pivotal roles in hepatic I/R injury, with inflammasomes being key components of the innate immune response [4, 29]. NLRP3 inflammasome activation triggers the initiation of inflammatory responses, leading to the

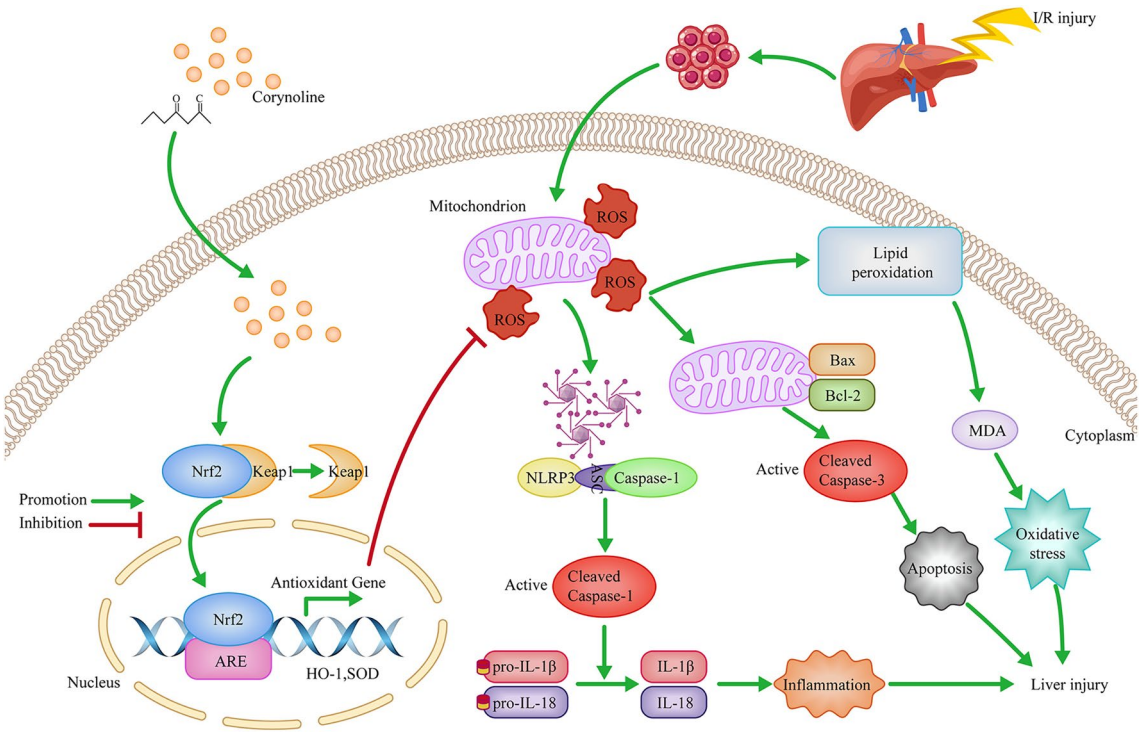

Fig. 9 Schematic diagram illustrating the protective effect of corynoline on hepatic I/R injury and its underlying mechanisms. Corynoline can attenuate hepatic I/R injury by inhibiting oxidative stress, inflammation, and apoptosis, possibly via inhibiting ROS-induced activation of the NLRP3 inflammasome by enhancing Nrf2/HO-1 signaling

maturation of IL-1β and IL-18 by cleaving pro-caspase1 into activated caspase1 [5]. These two key regulators play significant roles in propagating the inflammatory response following hepatic I/R injury by activating Kupffer cells and recruiting neutrophils. This thereby promotes the production and release of various pro-inflammatory cytokines, like IL-6 and TNF-α [4, 6]. The potential role of NLRP3 inflammasome activation in hepatic I/R injury has been investigated both in vitro and in vivo. Data have confirmed that NLRP3 inflammasome activation is crucially involved in initiating the inflammatory response cascade following reperfusion, with NLRP3 knockdown decreasing inflammatory cell infiltration and protecting the liver from I/R injury [6, 30–32]. In this study, we demonstrated that NLRP3 inflammasome activation during I/R led to exacerbated liver inflammation. This was supported by increased serum levels of IL-1β, IL-6, and TNF-α, as well as enhanced neutrophil and macrophage infiltration in the liver. However, pretreatment with corynoline effectively inhibited NLRP3 inflammasome activation and subsequently suppressed its downstream inflammatory cascade. In addition, the data obtained from BRL-3A cells induced by H/R further confirmed the inhibitory effect of corynoline treatment on NLRP3. These findings suggest that corynoline can suppress NLRP3 inflammasome activation, thereby reducing inflammatory responses. Previous research has indicated that corynoline pretreatment can effectively hinder the production of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, by inhibiting NF-κB activation [24, 25]. However, to our knowledge, this study is the first to demonstrate that corynoline exhibits a suppressive effect on NLRP3 inflammasome activation for mitigating hepatic I/R injury.

Oxidative stress is a deleterious cellular event characterized by elevated ROS production and/or inadequate antioxidant defense mechanisms [11]. ROS primarily originate from mitochondria in hepatocytes during hepatic I/R injury. After mitochondrial damage occurs, a substantial quantity of ROS and oxidation byproducts are generated, thereby exacerbating the extent of the damage [33–36]. MDA serves as a marker for lipid peroxidation and can indirectly indicate the degree of oxidative stress in tissues [37]. SOD is an antioxidant enzyme that plays a fundamental and indispensable role in fighting free radicals [38]. A previous study showed that corynoline pretreatment could attenuate ROS production and lipid peroxidation, as well as boost antioxidant defenses, in the colon tissues of DSS-treated mice [25]. Another study also demonstrated that corynoline treatment could alleviate oxidative stress injury by enhancing the activities of GSH, CAT, and SOD, while reducing MDA levels, in a CFA-induced rheumatoid arthritis rat model [19]. Consistent with these findings, our study also demonstrated that corynoline pretreatment dramatically induced

increased SOD levels and decreased MDA and ROS levels in hepatic I/R injury. Further in vitro work in BRL-3A cells indicated that H/R significantly induced ROS accumulation, MDA formation, and SOD depletion, while corynoline pretreatment effectively inhibited these H/R-stimulated effects. Mitochondrial damage is an indicator of ROS release, which is the same as that found in our experiments. TEM analysis indicated that corynoline pretreatment could improve hepatocyte mitochondrial function in I/R injury, with decreased mitochondrial swelling and a reduced disappearance of the mitochondrial ridge and cavitation formation. Collectively, these results suggested that corynoline treatment can provide significant liver protection against I/R injury by mitigating oxidative stress and suppressing the inflammatory response.

Experimental evidence suggests that oxidative stress can initiate or augment the inflammatory response [8]. ROS are reportedly involved in the pro-inflammatory response, affecting antioxidant enzyme activity and oxidative stress, leading to NLRP3 inflammasome activation [39, 40]. Because of this critical upstream signal, scavenging ROS can significantly impede NLRP3 inflammasome activation. Nrf2 serves as a key regulator for various cytoprotective genes that combat oxidative and inflammatory damage, playing a pivotal role in indirectly eliminating ROS [41]. When cells perceive oxidative stress, Nrf2 can translocate from the cytoplasm to the nucleus and transcriptionally activate genes that encode antioxidant enzymes, such as HO-1. As a result, excessive ROS and harmful metabolites are effectively eliminated within cells, thereby ensuring the maintenance of intracellular homeostasis [13]. Studies have shown that the livers of Nrf2-deficient mice exhibit enhanced oxidative stress, inflammatory responses, and tissue damage following I/R injury, while Nrf2 activation could greatly mitigate this liver damage [42]. An increasing body of recent research has revealed that Nrf2 plays a pivotal role in regulating the NLRP3 pathway [43–46]. NLRP3 inflammasome activation is initiated by ROS-induced oxidative stress, while the absence of Nrf2 impacts the expression patterns of both NLRP3 and its downstream signaling molecules. However, it should be noted that silencing NLRP3 does not have any effect on the expression levels of Nrf2 pathway components, suggesting that NLRP3 inflammasome activation could be primarily mediated through the Nrf2/HO-1 signaling cascade [46]. Importantly, previous studies have demonstrated that corynoline treatment could attenuate oxidative stress and inflammatory injury in several diseases, such as acute lung injury and liver injury, by activating Nrf2 [21, 26]. We therefore speculated that corynoline could regulate Nrf2 activation, leading to a reduction in ROS production and subsequent prevention of NLRP3 inflammasome activation during hepatic I/R injury. Our findings from this investigation indicate that corynoline treatment can significantly promote Nrf2 translocation into the nucleus, elevate HO-1 expression levels, decrease ROS levels, and hinder NLRP3 inflammasome activation in BRL-3A cells subjected to H/R and rat liver injury induced by I/R. However, when ML385 treatment was used to inhibit Nrf2 activity, the suppressive effects of corynoline on ROS generation and NLRP3 inflammasome activation were attenuated. In addition, ML385 treatment suppressed the antioxidative stress, anti-inflammatory, and anti-apoptotic activities of corynoline, as well as aggravated liver injury after I/R. These results suggest that corynoline-mediated inhibition of NLRP3 inflammasome activation possibly involves the Nrf2 signaling pathway. To further confirm the relationship between NLRP3 and Nrf2 activation by corynoline, BRL-3A cells were pretreated with nigericin, an NLRP3 activator. The data showed that nigericin pretreatment induced NLRP3 inflammasome activation, but did not affect Nrf2 or HO-2 protein expression levels. These findings indicate that activation of Nrf2 and the NLRP3 inflammasome induced by corynoline may involve a regulatory relationship, with Nrf2 potentially acting upstream of the NLRP3 inflammasome. Importantly, corynoline treatment effectively suppressed the inflammatory response, mitigated oxidative damage, and attenuated hepatocyte apoptosis in vitro. However, these beneficial effects were abrogated upon subsequent nigericin treatment. Overall, our experimental results indicate that activation of the Nrf2 signaling pathway by corynoline can effectively inhibit the ROS-induced activation of the NLRP3 inflammasome. This mechanism enables corynoline to exhibit antioxidative, anti-inflammatory, and anti-apoptotic properties, as well as provide hepatoprotective effects during hepatic I/R injury.

Conclusions

In summary, our findings demonstrate that corynoline pretreatment exerts protective effects against hepatic I/R injury by effectively inhibiting oxidative stress and inflammatory responses. These effects are primarily mediated through upregulation of Nrf2 pathway components, leading to suppressed ROS-induced activation of the NLRP3 inflammasome. Therefore, these results suggest that corynoline holds great promise as a potential therapeutic agent for both the treatment and prevention of hepatic I/R injury.

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Author contributions Peng Du and Panliang Wang are responsible for the conception and design of the research; Peng Du and Xin Ge performed the experiments, Peng Du and Wendong Wang analyzed data, and wrote the manuscript; Yue Gu prepared the figures; Wenzhi Guo edited and revised the manuscript; All authors approved the final version of the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest There are no conflict of interest in this work.

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