

The protective effect of glycyrrhizin on hepatic ischemia-reperfusion injury in rats and possible related signal pathway

Xiaoni Kou^{1,2}, Jiang Zhu³, Xinke Xie², Mingxia Hao², Yingren Zhao^{1*}

¹ Department of Infectious Diseases, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710000, Shaanxi Province, China

² Department of Hepatopathy, Affiliated hospital of Shaanxi University of Chinese Medicine, Xianyang 712000, Shaanxi Province, China

³ Department of Galactophore, Shaanxi Provincial Tumor Hospital, Xi'an 710061, Shaanxi Province, China

ARTICLE INFO

Article type:
Original article

Article history:
Received: Oct 30, 2019
Accepted: Jun 30, 2020

Keywords:
Apoptosis
Glycyrrhizic acid
Inflammation
Liver
Oxidative stress
Reperfusion injury

ABSTRACT

Objective(s): To investigate the protective effect of glycyrrhizin (GL) on hepatic ischemia-reperfusion injury (HIRI).

Materials and Methods: Forty SD rats were randomly divided into sham group, HIRI group, GL 100 mg/kg group, and GL 200 mg/kg group. The pathological alterations of liver tissue in each group were observed. The levels of alanine transaminase (ALT), aspartate aminotransferase (AST), endothelin-1 (ET-1), nitric oxide (NO), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) were detected. Western blot was used to detect the expression levels of cytoplasmic protein caspase-3, Bax, Bcl-2, heme oxygenase-1 (HO-1), nuclear factor erythroid 2-related factor 2 (Nrf2), and nuclear protein Nrf2.

Results: Compared with the HIRI group, the levels of AST, ALT, ET-1, TNF- α , IL-1 β , and IL-6 in GL groups were lower, serum NO content was higher, MDA content was lower, SOD and GSH-Px activities were significantly increased, apoptosis index was lower ($P < 0.05$), which was more obvious in high-dose GL (200 mg/kg) group. The LC3-II/LC3-I ratio and Beclin-1 protein expression levels in the GL group were significantly lower than the HIRI group, but the expression levels of cytoplasmic protein HO-1 and nuclear protein Nrf2 were significantly higher than those of the HIRI group, which was more obvious in the high-dose GL group ($P < 0.05$).

Conclusion: GL has a protective effect on the liver of HIRI rats, and its mechanism may be related to activation of the Nrf2/HO-1 signaling pathway, inhibition of oxidative stress, inflammation, autophagy, and apoptosis.

► Please cite this article as:

Kou X, Zhu J, Xie X, Hao M, Zhao Y. The protective effect of glycyrrhizin on hepatic ischemia-reperfusion injury in rats and possible related signal pathway. Iran J Basic Med Sci 2020; 23:1232-1238. doi: 10.22038/ijbms.2020.44101.10334

Introduction

Hepatic ischemia-reperfusion injury (HIRI) widely exists in clinical procedures such as liver resection, organ transplantation, trauma, and shock. HIRI can cause liver function damage and liver failure, which directly affects the operative successful rate and prognosis of patients (1). Current studies suggest that HIRI often leads to cell injury in the hepatic sinus, which induces the accumulation of reactive oxygen radicals, releases pro-inflammatory cytokines, causes hepatic cell necrosis, and also causes apoptosis and autophagy of hepatic cells, leading to abnormal liver function (2, 3). Therefore, the prevention and treatment of HIRI have always been an important research field in liver surgery. Various Chinese traditional medicines have been reported that play an important role in these kinds of ischemia-reperfusion injury. Glycyrrhizin (GL), whose chemical formula is $C_{42}H_{62}O_{16}$ and known as glycyrrhizic acid, is one of the active constituents extracted from the traditional Chinese medicine *Glycyrrhiza uralensis*. GL has many pharmacological effects, such as anti-inflammatory, anti-oxidative, anti-apoptotic, and immune regulation, and has been reported as an inhibitor of High-mobility group protein-1 (HMGB1) in many pathological conditions. There is evidence that GL has a protective

effect on ischemia-reperfusion injury of various organs (4-6). Meanwhile, current studies have shown that GL could attenuate liver injury by inhibiting TNF- α -induced hepatocyte apoptosis and oxidative stress (7, 8) and attenuate tissue damage and Kupffer cells pyroptosis during HIRI (9). So, it could be speculated that GL may have some positive effect on HIRI, however, this opinion has not yet been verified and the mechanism is not clear. This study aimed to explore the effect of GL on HIRI and the related mechanism preliminarily.

Materials and Methods

Animals

Male Sprague-Dawley rats (8~10 weeks old, 220~250 g body weight, Experimental Animal Center of Zhengzhou University) were kept under constant temperature (20~22 °C) and humidity (40~50%) with a 12 hr light/dark cycle, and fed a standard diet and tap water.

Drugs and reagents

GL (Minophagen Pharmaceutical Co., Ltd.). Endothelin-1 (ET-1) kit, nitric oxide (NO) kit, malondialdehyde (MDA) kit, SOD kit, GSH-Px kit, tumor necrosis factor- α (TNF- α) kit, interleukin-1 β (IL-1 β)

*Corresponding author: Yingren Zhao. Department of Infectious Diseases, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710000, Shaanxi Province, China. Tel/ Fax: +86-13509187086; Email: yingren_grk@163.com

kit, and interleukin-6 (IL-6) kit were purchased from Beijing solarbio science & technology Co., Ltd. Caspase-3 antibody, Bax antibody, LC3 antibody Beclin-1 antibody, HO-1 antibody, Nrf2 antibody, PCNA antibody, and β -actin were purchased from Abcam, UK.

Groups

40 rats were randomly divided into sham group, HIRI group, GL 100 mg/kg group, and GL 200 mg/kg group, with 10 rats in each group. All animal experiments were approved by the Ethics Committee of Shaanxi University of Chinese Medicine Affiliated Hospital.

HIRI modeling and GL intervention

Rats were fasted for 12 hr before the operation, and rats in GL groups were injected GL 100 mg/kg and 200 mg/kg 1 hr before modeling, while sham group and HIRI group were injected with equal volume normal saline.

After being anesthetized by 1% pentobarbital sodium (50 mg/kg) intraperitoneal injection, the rats were fixed on the 37 °C thermostatic operating plate. The liver was exposed through a longitudinal abdominal midline incision. The portal vein and hepatic artery were clamped with a non-invasive vascular clamp to make the middle and left liver lobes ischemic (about 70%). After 90 min, the clamp was released to restore liver blood flow for reperfusion, and the abdomen was closed by suture. The success criteria of hepatic ischemia were gray liver surface color and soft liver texture, and the success criteria of reperfusion were restored ruddy liver surface color. On the sham group was conducted the same operations as the HIRI group except clamping hepatic arteries and veins. After 6 hr of reperfusion, all rats were executed by drawing-out heart blood and liver tissue was collected under aseptic condition and frozen at -80 °C for later use.

Pathological examination of the liver

A part of liver tissues was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin (HE). The morphology of liver tissues was observed under an ordinary optical microscope. The degree of liver injury was evaluated according to the SUZUKI grading standard(10). The SUZUKI grading standard contains 5 grades (0~4) according to tissue congestion, cell ballooning, and necrosis area. No tissue congestion, ballooning, and necrosis were recorded as grade 0, small tissue congestion, ballooning, and necrosis were recorded as grade 1, mild tissue congestion, ballooning, and necrosis were recorded as grade 2, moderate tissue congestion, ballooning, and necrosis were recorded as grade 3, severe tissue congestion, ballooning, and necrosis over 60% of the area were grade 4. The grades among all groups were compared.

Determination of liver function and liver injury indexes

The supernatant was obtained after centrifugation. The serum levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) were detected by the automatic biochemical analyzer, and the levels of plasma ET-1 and serum NO were detected using the ELISA kit.

Determination of inflammatory cytokines

Serum levels of inflammatory cytokines TNF- α , IL-1 β , and IL-6 were detected according to the operating instructions of the ELISA kit.

Detection of oxidative stress indicators

The liver tissues of each group were made into homogenates. The content of MDA was determined by the thiobarbituric acid method, the activity of SOD was measured by the xanthine oxidase method, and GSH-Px activity was determined by the dithionitrotoluidine method, which was carried out in strict accordance with the operating instructions of the kit.

Observation of apoptosis by TUNEL staining

The frozen liver tissue was cut into 6 μ m sections on the slicing machine. After overnight fixing with 4% paraformaldehyde, the liver tissue was stained with a TUNEL cell apoptosis detection kit, which was carried out in strict accordance with the operating instructions of the kit. Apoptosis was observed under the fluorescence microscope. Apoptotic cells were brown, normal cells were blue-purple, apoptosis index (AI) = apoptotic cells / total cells * 100%.

Western blot

The nuclear proteins and cytoplasmic proteins were extracted from liver tissues by nuclear proteins and cytoplasmic proteins extraction kits, respectively, strictly according to the instructions of the kits. Cytoplasmic proteins were used to detect the expression of Caspase-3, Bax, Bcl-2, LC3, Beclin-1, Nrf2, and HO-1 proteins, and nuclear proteins were used to detect the expression of Nrf2 proteins. Protein concentration was determined by the BCA method. 40 μ g protein samples were separated by polyacrylamide gel electrophoresis and were transferred to polyvinylidene fluoride membranes (PVDF membrane). Blocking at room temperature with skimmed milk powder at room temperature for 2 hr, and then were incubated with Bax, Bcl-2, Caspase-3, LC3, Beclin-1, HO-1, Nrf2, PCNA and β -actin (1:1000) antibodies at 4 °C overnight. Subsequently, the membranes were incubated with the corresponding secondary antibody IgG (1:2000) for 1 hr at room temperature. After chemiluminescence development, image J was used to calculate the ratio of the target band and internal reference band gray value, that is, the relative expression of the target protein.

Statistical analysis

All data were analyzed using SPSS 19.0 and presented as mean \pm standard deviation ($\bar{x} \pm s$). Data between multiple groups were calculated with one-way ANOVA, pairwise comparison was performed by the LSD test. Results between the two groups were analyzed with an independent sample *t*-test. Statistical significance was accepted for *P*-value < 0.05.

Results

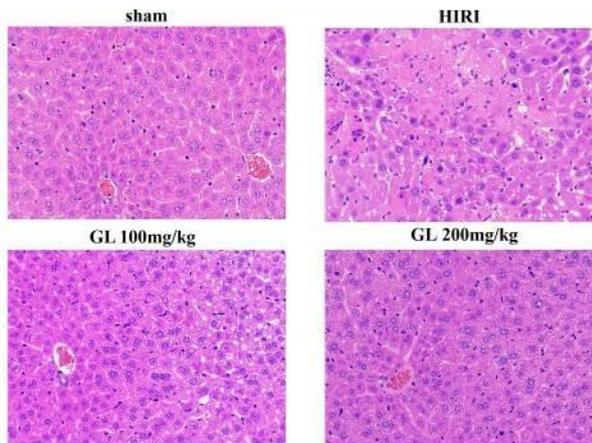
Effects of GL on pathological findings in the liver

HE staining showed that, compared with the sham group, liver cells in the HIRI group were patchy necrosis, liver cells around the central vein were moderate

Table 1. The levels of liver injury indexes in each group of rats

Group	AST (U/l)	ALT (U/l)	NO (μmol/l)	ET-1 (ng/l)
Sham	122.20±8.28	79.87±8.86	109.11±6.71	52.11±4.79
HIRI	543.76±16.62*	348.19±10.74*	45.27±4.03*	158.22±7.00*
GL 100 mg/kg	349.74±12.24*#	258.04±22.23*#	64.34±5.47*#	126.99±6.52*#
GL 200 mg/kg	243.92±11.03*#	139.67±7.13*#	82.74±6.59*#	97.30±5.72*#

Compared with Sham group, * $P<0.05$; compared with HIRI group, # $P<0.05$ AST: Aspartate aminotransferase; ALT: Alanine transaminase; ET-1: Endothelin-1; NO: Nitric oxide; HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

**Figure 1.** Pathological alterations of liver tissue in each group (magnification, ×200)

HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

and severe edema, obvious hepatic sinus block, and inflammatory cell infiltration. Compared with the HIRI group, liver cells in the GL groups showed focal or spot-like necrosis, mild edema of liver cells around the central vein, hepatic sinus block, and attenuated inflammatory cell infiltration (Figure 1). The SUZUKI score in the HIRI group was 3.60 ± 0.49 , and those in GL 100 mg/kg and 200 mg/kg groups were 2.50 ± 0.67 and 1.90 ± 0.70 , respectively, which were both lower than that of the HIRI group (both $P<0.05$).

Effects of GL on liver function and liver injury indexes

Compared with the sham group, the levels of AST, ALT, and ET-1 in the HIRI group and GL group were significantly increased, while the serum level of NO was significantly reduced ($P<0.05$). Compared with the HIRI group, the levels of AST, ALT, and ET-1 in the GL group were significantly reduced, while the level of NO was significantly increased, and the changes in the GL 200

Table 2. The levels of inflammatory cytokines in each group of rats

Group	TNF-α (ng/l)	IL-1β (ng/l)	IL-6 (ng/l)
Sham	42.59±4.66	63.41±4.85	34.38±2.53
HIRI	183.42±7.14*	234.03±10.36*	134.05±5.21*
GL 100 mg/kg	156.11±6.02*#	187.94±9.53*#	104.57±6.77*#
GL 200 mg/kg	113.71±5.34*#	141.19±6.51*#	64.01±2.88*#

Compared with Sham group, * $P<0.05$; compared with HIRI group, # $P<0.05$

TNF-α: Tumor necrosis factor-α, IL-1β: Interleukin-1β, IL-6: Interleukin-6; HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

Table 3. The levels of oxidative stress indicators in each group of rats

Group	MDA (nmol/ml)	SOD (U/ml)	GSH-Px (U/ml)
Sham	5.75±0.31	122.46±7.81	185.99±7.17
HIRI	19.64±1.95*	56.87±4.05*	121.21±5.60*
GL 100 mg/kg	14.01±1.43*#	76.78±4.38*#	143.27±5.26*#
GL 200 mg/kg	9.63±0.57*#	104.18±5.24*#	170.09±6.87*#

Compared with Sham group, * $P<0.05$; compared with HIRI group, # $P<0.05$ SOD: Superoxide dismutase; MDA: Malondialdehyde, GSH-Px: Glutathione peroxidase; HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

mg/kg group were more obvious ($P<0.05$, Table 1).

Inhibition of GL on inflammation

Compared with the sham group, the serum levels of TNF-α, IL-1β, and IL-6 in the HIRI group and GL group were significantly increased ($P<0.05$, Table 2). Compared with the HIRI group, the levels of TNF-α, IL-1β, and IL-6 in the GL group were significantly decreased, and the changes in GL 200 mg/kg group were more obvious ($P<0.05$, Table 2).

Inhibition of GL on the oxidative stress response

Compared with the sham group, MDA content in the HIRI group was significantly increased, while the activities of antioxidant enzymes SOD and GSH-Px were significantly decreased ($P<0.05$, Table 3). Compared with the HIRI group, MDA content in the GL group was significantly decreased, the activities of antioxidant enzymes SOD and GSH-Px were significantly increased, and the changes in GL200 mg/kg group were more obvious ($P<0.05$, Table 3).

GL improved apoptosis of hepatocytes in HIRI rats

TUNEL staining showed that only a few apoptotic positive cells were observed in the Sham group, and a

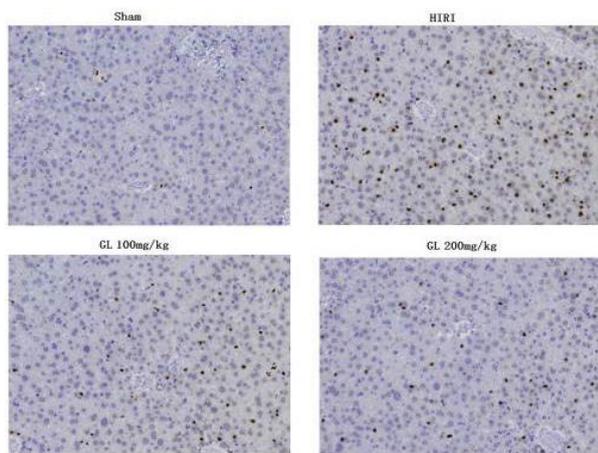


Figure 2. Apoptosis of hepatocytes in each group of rats (magnification, ×200)
HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

large number of positive cells with yellow staining were observed in the HIRI group. The positive cells in the GL group were significantly less than those in the HIRI group (Figure 2). There were statistically significant differences in AI between each group ($P < 0.05$, Table 4).

Western blot showed that the expression levels of Caspase-3 and Bax protein in the HIRI group were significantly higher than the sham group, while the expression levels of Bcl-2 protein were significantly lower than the sham group ($P < 0.05$). Meanwhile, the expression levels of Caspase-3 and Bax protein in the GL group were significantly lower than the HIRI group, while the expression levels of Bcl-2 protein were significantly higher than the HIRI group, and the changes were more obvious in the GL 200 mg/kg group ($P < 0.05$, Figure 3).

Effect of GL on the expression of autophagy-related proteins LC3 and Beclin-1

Western blot showed that the LC3-II/LC3-I ratio and Beclin-1 protein expression levels in the liver tissues of the HIRI group were significantly higher than the sham group ($P < 0.05$). Meanwhile, the LC3-II/LC3-I ratio and Beclin-1 protein expression levels in the liver tissue of the GL group were significantly lower than the HIRI group ($P < 0.05$), and the changes were more obvious in the GL 200 mg/kg group ($P < 0.05$, Figure 4)

Effect of GL on the Nrf2/HO-1 signal pathway

The molecular mechanism of GL protecting the

Table 4. The apoptosis rate of liver tissue in each group of rats

Group	n	AI (%)
Sham	10	3.89±0.60
HIRI	10	27.66±4.76*
GL100 mg/kg	10	20.57±4.09*#
GL200 mg/kg	10	15.43±3.66*#

Compared with Sham group, * $P < 0.05$; compared with HIRI group, # $P < 0.05$
HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

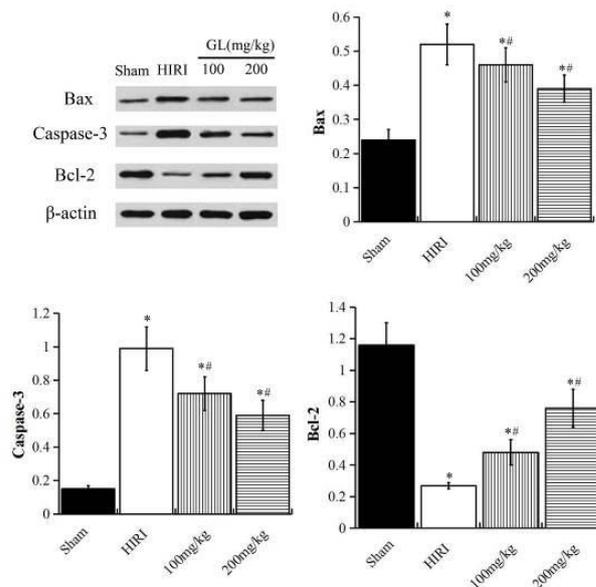


Figure 3. Effects of GL on the expression of apoptosis-related proteins. Compared with the Sham group, * $P < 0.05$; compared with the HIRI group, # $P < 0.05$
HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

liver was explored by detecting the expression levels of Nrf2/HO-1 pathway-related proteins. There was no statistically significant difference in the expression of cytoplasmic protein Nrf2 between the sham group, HIRI group, and GL group ($P > 0.05$). Compared with the sham group, the expression levels of cytoplasmic protein HO-1 and nuclear protein Nrf2 were significantly increased ($P < 0.05$). Compared with the HIRI group, the expression levels of cytoplasmic protein HO-1 and nuclear protein Nrf2 were significantly increased, and the changes were more obvious in the GL200 mg/kg group ($P < 0.05$, Figure 5).

Discussion

HIRI is an important cause of liver dysfunction after liver surgery, liver failure, and primary liver transplantation without function. Current studies have found that the mechanism of HIRI may be related to various factors, such as oxygen-free radical production, inflammatory reaction, calcium overload,

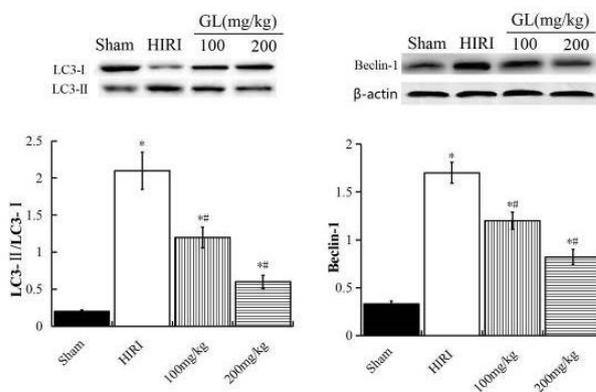


Figure 4. Effects of GL on the expression of autophagy-related proteins. Compared with the Sham group, * $P < 0.05$; compared with the HIRI group, # $P < 0.05$
HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

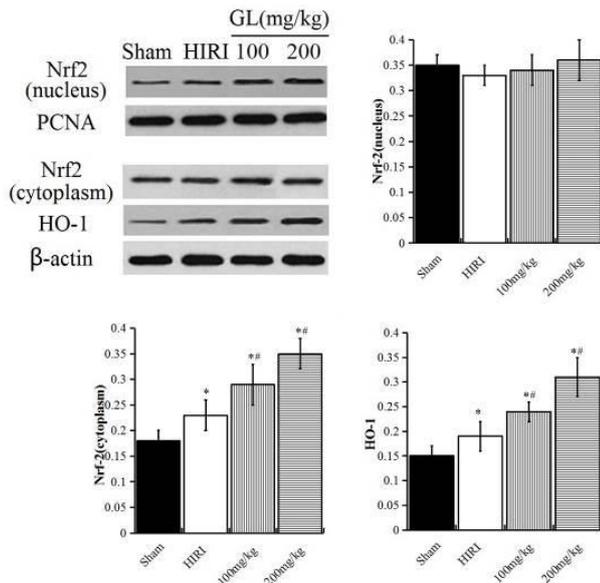


Figure 5. The effect of GL on the expression of Nrf2 and HO-1 protein. A: Nuclear protein, B: Cytoplasmic protein. Compared with the Sham group, * $P < 0.05$; ; compared with the HIRI group, # $P < 0.05$ HIRI: Hepatic ischemia-reperfusion injury; GL: Glycyrrhizin

and cell apoptosis (11, 12). Under normal physiological conditions, inflammatory and anti-inflammatory reactions, oxidation, and anti-oxidation systems are in a dynamic equilibrium state. After ischemia-reperfusion, neutrophils infiltrate the inflammatory injury site, which can promote the release of inflammatory cytokines and stimulate inflammatory cascade reaction (13). At the same time, a large number of reactive oxygen species (ROS) are produced in liver tissues, which react with lipids to produce a large amount of lipid peroxides (MDA) and reduce the activities of antioxidant enzymes such as SOD and GSH-Px, resulting in an imbalance of tissue oxidation and antioxidant system, eventually leading to tissue damage and necrosis. The results of this study suggested that HIRI could promote inflammatory response and apoptosis, weaken the antioxidant ability, and cause liver tissue damage and necrosis, which is consistent with the present study (14-16). Meanwhile, autophagy levels will increase in HIRI (17, 18). At present, many autophagy associated genes (ATG) involved in autophagy have been identified such as LC3 and Beclin-1, which is the earliest positive regulator of autophagy. LC3-II is a specific marker of the autophagic membrane, which is located in pre-autophagosome and autophagosome. By detecting the change of the LC3-II/LC3-I ratio, the intensity of autophagic activity can be determined (3). The results of this study showed that the LC3-II/LC3-I ratio and Beclin-1 protein were highly expressed in HIRI tissue, suggesting that the autophagy level in HIRI tissue was increased.

GL is the main physiological active substance extracted from the root of *G. uralensis*. It has many biological activities, such as anti-oxidant, anti-inflammatory, and immune enhancer. Studies have shown that GL can inhibit the release of pro-inflammatory factors, reduce MDA content, improve the activity of antioxidant enzymes, maintain the balance of free radicals/oxidants and antioxidants in the intracellular environment, inhibit

apoptosis, and alleviate ischemia-reperfusion injury (4, 19, 20). Yan T *et al.* showed that GL could weaken liver injury by inhibiting hepatocyte apoptosis, which could be used against acetaminophen (APAP) overdose (8). Sil *et al.* indicated that GL treatment could improve the hepatocellular damage in metabolic syndrome rats by inhibiting oxidative stress, inflammation, and cell apoptosis (21). The same effect of GL was observed in hepatic injury induced by LPS/D-galactosamine (22). The results of this study showed that GL could significantly reduce the necrosis of liver cells, reduce hepatic sinus block, decrease inflammatory cell infiltration, reduce the inflammatory reaction and oxidative stress induced by HIRI, improve antioxidant capacity, alleviate HIRI, and protect liver tissue. Besides, GL could inhibit liver cell apoptosis, which was consistent with the existing research results.

Autophagy is a pathway to maintain homeostasis by transporting damaged aging or denatured proteins and organelles to lysosomes for digestion and clearance (17). However, excessive autophagy can lead to excessive degradation of normal proteins and organelles and damaged cells (18). The present study showed that some natural products such as resveratrol, berberine, and curcumin can trigger autophagy via canonical (Beclin-1-dependent) and non-canonical (Beclin-1-independent) pathways. A study showed that coptisine treatment increased cell survival, inhibited apoptosis, and reduced the protein level of LC3-II, cleaved Caspase-3, Beclin1, and Sirt1, suggesting that coptisine may protect cardiomyocyte damage by H/R through suppressing autophagy (23). Meanwhile, results of a study show that pretreatment with glycyrrhizin significantly reduced 3-NP-mediated activation of autophagy marker LC3-II (24). The results of this study showed that GL could up-regulate the expression of Beclin-1 and LC3-II/LC3-I ratio in HIRI tissue, suggesting that GL may inhibit the formation and maturation of autophagosomes by inhibiting the conversion of LC3-I to LC3-II and the synthesis of Beclin-1, thus alleviating HIRI in rats.

Nrf2 is a leucine zipper transcription factor in the alkaline region. It is also a regulator of many physiological processes, such as anti-oxidant, anti-inflammatory, and anti-apoptotic (25). The signaling pathway composed of Nrf2, cytoplasmic protein Keap1, and antioxidant response sequence element (ARE) has the effect of antioxidant stress damage in many tissues and organs. In normal physiological conditions, Nrf2 binds to Keap1, which makes Nrf2 in an inhibitory state. When oxidative stress occurs, Nrf2 is activated, and the activated Nrf2 is released from Keap1 and transferred to the nucleus, which combines with ARE in the nucleus, up-regulates the expression of downstream phase II detoxifying enzymes and antioxidant genes, and enhances the activity of antioxidant enzymes, to realize the effect of antioxidant stress (26, 27). HO-1 is a small molecular heat shock protein, which can catalyze the degradation of heme to produce biliverdin, CO and Fe^{2+} , which together form an endogenous protective system against oxidative stress injury, weaken oxidative stress and inflammatory response, inhibit the expression of pro-apoptotic proteins, to exert its anti-apoptotic effect (28). Activation of the Nrf2 signaling pathway can

induce the up-regulation of HO-1 expression, inhibit the formation and release of inflammatory mediators, exert anti-inflammatory, antioxidant and anti-apoptotic effects, protect cells (29). The results of this study showed that GL could activate Nrf2, promote the nuclear transfer of Nrf2 protein, up-regulate the expression of HO-1, inhibit oxidative stress response, and protect liver tissue.

Conclusion

GL has an obvious protective effect on HIRI, and its mechanism may be related to activation of the Nrf2/HO-1 signaling pathway, inhibition of oxidative stress, inflammation, autophagy, and apoptosis.

Acknowledgment

This study was supported by Shaanxi Province Social Development key project (Grant NO. 2018SF-295).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

- Chaves JC, Neto FS, Ikejiri AT, Bertolotto PR, Teruya R, Santos Simoes R, *et al*. Period of hyperbaric oxygen delivery leads to different degrees of hepatic ischemia/reperfusion injury in rats. *Transplant Proc* 2016; 48:516-520.
- Tao X, Wan X, Xu Y, Xu L, Qi Y, Yin L, *et al*. Dioscin attenuates hepatic ischemia-reperfusion injury in rats through inhibition of oxidative-nitrative stress, inflammation, and apoptosis. *Transplantation* 2014; 98:604-611.
- Xue L, Wu Z, Ji XP, Gao XQ, Guo YH. Effect and mechanism of salvianolic acid B on the myocardial ischemia-reperfusion injury in rats. *Asian Pac J Trop Med* 2014; 7:280-284.
- Cai X, Wang X, Li J, Chen S. Protective effect of glycyrrhizin on myocardial ischemia/reperfusion injury-induced oxidative stress, inducible nitric oxide synthase and inflammatory reactions through high-mobility group box 1 and mitogen-activated protein kinase expression. *Exp Ther Med* 2017; 14:1219-1226.
- Gong G, Xiang L, Yuan L, Hu L, Wu W, Cai L, *et al*. Protective effect of glycyrrhizin, a direct HMGB1 inhibitor, on focal cerebral ischemia/reperfusion-induced inflammation, oxidative stress, and apoptosis in rats. *PLoS One* 2014; 9:e89450.
- Ni B, Cao Z, Liu Y. Glycyrrhizin protects spinal cord and reduces inflammation in spinal cord ischemia-reperfusion injury. *Int J Neurosci* 2013; 123:745-751.
- Wang Y, Chen Q, Shi C, Jiao F, Gong Z. Mechanism of glycyrrhizin on ferroptosis during acute liver failure by inhibiting oxidative stress. *Mol Med Rep* 2019; 20:4081-4090.
- Yan T, Wang H, Zhao M, Yagai T, Chai Y, Krausz KW, *et al*. Glycyrrhizin protects against acetaminophen-induced acute liver injury via alleviating tumor necrosis factor alpha-mediated apoptosis. *Drug Metab Dispos* 2016; 44:720-731.
- Hua S, Ma M, Fei X, Zhang Y, Gong F, Fang M. Glycyrrhizin attenuates hepatic ischemia-reperfusion injury by suppressing HMGB1-dependent GSDMD-mediated kupffer cells pyroptosis. *Int Immunopharmacol* 2019; 68:145-155.
- Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. *Transplantation* 1993; 55:1265-1272.
- Zhang Q, Lai Y, Deng J, Wang M, Wang Z, Wang M, *et al*. Vagus nerve stimulation attenuates hepatic ischemia/reperfusion injury via the Nrf2/HO-1 Pathway. *Oxid Med Cell Longev* 2019; 2019:9549506.
- Zhao Y, Cai H, Zhou P, Lin S, Pan Y, Liang X. Protective effect of ulinastatin on hepatic ischemia reperfusion injury through autophagy activation in Chang liver cells. *J Cell Biochem* 2019; 120:14960-14970.
- Sehitoglu I, Tumkaya L, Bedir R, Kalkan Y, Cure MC, Yucel AF, *et al*. Zoledronic acid aggravates kidney damage during ischemia reperfusion injury in rat. *J Environ Pathol Toxicol Oncol* 2015; 34:53-61.
- Ye Z, Chen O, Zhang R, Nakao A, Fan D, Zhang T, *et al*. Methane attenuates hepatic ischemia/reperfusion injury in rats through antiapoptotic, anti-inflammatory, and antioxidative actions. *Shock* 2015; 44:181-187.
- Tao YE, Wen Z, Song Y, Wang H. Paeoniflorin attenuates hepatic ischemia/reperfusion injury via anti-oxidative, anti-inflammatory and anti-apoptotic pathways. *Exp Ther Med* 2016; 11:263-268.
- Zhang CB, Tang YC, Xu XJ, Guo SX, Wang HZ. Hydrogen gas inhalation protects against liver ischemia/reperfusion injury by activating the NF-kappa B signaling pathway. *Exp Ther Med* 2015; 9:2114-2120.
- Deng J, Feng J, Liu T, Lu X, Wang W, Liu N, *et al*. Beraprost sodium preconditioning prevents inflammation, apoptosis, and autophagy during hepatic ischemia-reperfusion injury in mice via the P38 and JNK pathways. *Drug Des Devel Ther* 2018; 12:4067-4082.
- Cursio R, Colosetti P, Gugenheim J. Autophagy and liver ischemia-reperfusion injury. *Biomed Res Int* 2015; 2015:417590.
- Ye S, Zhu Y, Ming Y, She X, Liu H, Ye Q. Glycyrrhizin protects mice against renal ischemia-reperfusion injury through inhibition of apoptosis and inflammation by downregulating p38 mitogen-activated protein kinase signaling. *Exp Ther Med* 2014; 7:1247-1252.
- Fei L, Jifeng F, Tiantian W, Yi H, Linghui P. Glycyrrhizin ameliorate ischemia reperfusion lung injury through down-regulate TLR2 signaling cascade in alveolar macrophages. *Front Pharmacol* 2017; 8:389.
- Sil R, Ray D, Chakraborti AS. Glycyrrhizin ameliorates metabolic syndrome-induced liver damage in experimental rat model. *Mol Cell Biochem* 2015; 409:177-189.
- Ikeda T, Abe K, Kuroda N, Kida Y, Inoue H, Wake K, *et al*. The inhibition of apoptosis by glycyrrhizin in hepatic injury induced by injection of lipopolysaccharide / D-galactosamine in mice. *Arch Histol Cytol* 2008; 71:163-178.
- Wang Y, Wang Q, Zhang L, Ke Z, Zhao Y, Wang D, *et al*. Coptisine protects cardiomyocyte against hypoxia/reoxygenation-induced damage via inhibition of autophagy. *Biochem Biophys Res Commun* 2017; 490:231-238.
- Qi L, Sun X, Li FE, Zhu BS, Braun FK, Liu ZQ, *et al*. HMGB1 promotes mitochondrial dysfunction-triggered striatal neurodegeneration via autophagy and apoptosis activation. *PLoS One* 2015; 10:e0142901.
- Hu T, Wei G, Xi M, Yan J, Wu X, Wang Y, *et al*. Synergistic cardioprotective effects of Danshensu and hydroxysafflor yellow A against myocardial ischemia-reperfusion injury are mediated through the Akt/Nrf2/HO-1 pathway. *Int J Mol Med* 2016; 38:83-94.
- Chai J, Luo L, Hou F, Fan X, Yu J, Ma W, *et al*. Agmatine reduces lipopolysaccharide-mediated oxidant response via activating PI3K/Akt pathway and up-regulating Nrf2 and HO-1 expression in macrophages. *PLoS One* 2016; 11:e0163634.
- Gu L, Ye P, Li H, Wang Y, Xu Y, Tian Q, *et al*. Lunasin attenuates oxidant-induced endothelial injury and inhibits atherosclerotic plaque progression in ApoE(-/-) mice by up-regulating heme oxygenase-1 via PI3K/Akt/Nrf2/ARE pathway. *FASEB J* 2019; 33:4836-4850.
- He M, Pan H, Chang RC, So KF, Brecha NC, Pu M. Activation of the Nrf2/HO-1 anti-oxidant pathway contributes to the protective effects of Lycium barbarum polysaccharides in the rodent retina after ischemia-reperfusion-induced damage. *PLoS One* 2014; 9:e84800.

29. Kim YM, Kim HJ, Chang KC. Glycyrrhizin reduces HMGB1 secretion in lipopolysaccharide-activated RAW 264.7 cells and endotoxemic mice by p38/Nrf2-dependent induction of HO-1. *Int Immunopharmacol* 2015; 26:112-118.