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ORIGINAL ARTICLE

Protective effect of glycyrrhizin on coronary microembolization-induced myocardial dysfunction in rats

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Abstract

Coronary microembolization (CME)-induced inflammation and cardiomyocyte apoptosis are two key factors contributing to CME-induced myocardial dysfunction. High-mobility group box-1 (HMGB1) plays essential role in progression of CME-induced injury and inhibition of HMGB1 has been shown to be protective. In present study, the potential effects of glycyrrhizin, a HMGB1 inhibitor, on CME-induced myocardial dysfunction are evaluated. Using a rat model of CME, we administrated glycyrrhizin in rats prior to CME induction. The level of HMGB1, TNF-α, iNOS, IL-6, IL-1β, cleaved caspase-3, Bax, and Bcl-2 were measured. The serum level of cardiac troponin I, creatine kinase, was detected. The cardiac function and cardiomyocyte apoptosis were evaluated. The activation of TLR4/NF-κB signaling pathway was analyzed. Glycyrrhizin prevented CME-induced production of HMGB1, TNF-α, iNOS, IL-6, and IL-1β. Glycyrrhizin inhibited CME-induced cardiomyocyte apoptosis and the expression of cleaved caspase-3 and Bax, while enhanced the expression of Bcl-2. Glycyrrhizin decreased cardiac troponin I and creatine kinase levels and improved cardiac function. Glycyrrhizin prevented the activation of HMGB1/TLR4/NF-κB signaling pathway. Glycyrrhizin ameliorated myocardial dysfunction in CME rats by preventing inflammation and apoptosis of cardiomyocytes.

KEYWORDS
apoptosis, coronary microembolization, glycyrrhizin, HMGB1, inflammation

1 | INTRODUCTION

Coronary microembolization (CME), a frequent complication of acute myocardial infarction with an occurrence rate of 15%–20%, is critical for dysfunctional heart.[1] CME has been shown to cause myocardial perfusion–contraction mismatch, micro-infarction, arrhythmias, and results in fatal consequences.[2]

Local myocardial inflammation induced by CME has been shown to be essential in cardiac dysfunction.[3] Inflammatory cells infiltration and release of inflammatory factors in the area of CME-induced myocardial microinfarction have been identified. Extensively activated nuclear factor kappa-B (NF-κB) signaling pathway results in excessive production of inflammatory cytokines including tumor necrosis factor alpha (TNF-α) and interleukin 1beta (IL-1β) while blocking NF-κB activity alleviates inflammatory response and improves cardiac function.[4] Neutralization of TNF-α by antibodies has been shown to attenuate CME-induced progressive cardiac dysfunction.[5] Cardiomyocyte apoptosis is another key

Abbreviations: CME, Coronary microembolization; HMGB1, High-mobility group box-1; NF-κB, nuclear factor kappa-B; TNF-α, tumor necrosis factor alpha.

cause of CME-induced dysfunction.[6] TNF-α has been shown to contribute to cardiomyocyte apoptosis and suppression of cardiomyocyte apoptosis improves cardiac function in CME.[7] Therefore, targeting inflammation and apoptosis could be effective therapy to treat CME-induced injury.

High-mobility group box-1 (HMGB1) is DNA binding protein which could function to regulate transcription when it is in nucleus. HMGB1 could be secreted out of the cells like a pro-inflammatory cytokine after various stimulations and exerts pro-inflammatory effects.[8] HMGB1 plays important roles in multiple diseases including CME. Inhibition of HMGB1 by siRNA has been shown to reduce CME-induced myocardial injury and ameliorate cardiac function,[3] indicating HMGB1 could be a potential target for CME treatment. Glycyrrhizin is a HMGB1 inhibitor which blocks HMGB1 function by directly binding.[9] Glycyrrhizin has been described to inhibit inflammation, oxidative stress, and apoptosis in ischemia by targeting HMGB1.[10] Until now, the potential effects of glycyrrhizin on CME-induced injury are not described yet. In current study, the effects of glycyrrhizin on CME-induced myocardial dysfunction are investigated.

2 | MATERIALS AND METHODS

2.1 | CME rat model

Male Sprague Dawley rats with weight of 250–300 g were purchased from Charles River and kept in a room with controlled temperature and 12/12 light/dark cycle (a total number of 126 rats were used for the whole experiments). The CME rat model was established following protocol described previously.[3] Briefly, rats were anesthetized by intraperitoneally injecting with 30 mg/kg pentobarbital.[3] When performing the thoracotomy, the ascending aorta was separated and clamped for 10 s. Totally, 3000 microembolism spheres (diameter 42 μm; Biosphere Medical Inc) were suspended in 0.1 mL of saline and then directly injected to the left ventricle. For sham group, rats were injected with saline only. When heartbeat was stable, the chest was sealed. After the surgery, both sham group rats and rats injected with spheres were injected with 800 000 U of penicillin.

The glycyrrhizin was purchased from Sigma. For glycyrrhizin treatment, rats were intraperitoneally injected with 2, 4, or 10 mg/kg glycyrrhizin once per day for consecutive 7 days prior to Coronary microembolization. The doses of glycyrrhizin used in present study followed the previous study.[10] Animal studies were in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, NIH). The study was approved by the ethics commitment of Cangzhou Central Hospital of Tianjin Medical University.

2.2 | Enzyme-linked immunosorbent assay (ELISA)

Serum level of HMGB1 was detected using commercial rat HMGB1 ELISA kit (LSBio) following manufacture's protocols. Serum level of creatine kinase was measured using commercial rat creatine kinase ELISA kit (Nanjing Jianchen Bioengineering Institute). Serum lactate dehydrogenase (LDH) activities were determined using the LDH assay kit (Jiancheng). The concentrations of TNF-α, inducible nitric oxide synthase (iNOS), IL-1β and IL-6 in myocardial tissues were measured using commercial ELISA kits (Abcam) following manufacturer's instructions.

2.3 | Real-time polymerase chain reaction (RT-PCR)

Total RNA from myocardial tissues was extracted using NucleoSpin® RNA Plus kit (Takara). Then, cDNA was synthesis by using PrimeScript™ II First-Strand cDNA Synthesis Kit (Takara). The real-time PCR was performed using TB Green® Advantage® qPCR Premix (Takara). The primers used for real-time PCR were as follows: *HMGB1* Forward: 5'-CTGATGCAGCTTA TACGAAG-3', Reverse 5'-TCAGGTAAGGAGCAGAACAT-3'; *Bax* Forward: 5'-AGA CACCTGACCTTGGA-3', Reverse: 5'-TTGAAGTTGCCATCAGCAA ACA-3'; *Bcl-2* Forward: 5'-AGACACCTGACCTT GGA-3', Reverse: 5'-TTGAAGTTGCCATCAGCAAACA-3'; *IL-6* Forward: 5'-GGAT ACC ACCCACAACAG AC-3', Reverse: 5-TTGCCGAGTAGACCTCA TAG-3'; *iNOS* Forward: 5'-GCATCCCAAGTACGAGTGGT-3', Reverse: 5'-GAAGGCGTAG CTGAACAAGG-3'; *TNF-α* Forward:5'-CCCCTTTATCGTCTACTCCTC-3', Reverse: 5'-GCTGGTAGTTTAGC TCCGTTT-3'; *IL-1β* Forward: 5'-TCATTGTGGCTGTGGAGAAG-3', Reverse: 5'-CTATGTCCCGACCATTGCTG-3'; *GAPDH* Forward: 5'-CCATCACTGCCACTCAGAAGA-3', and Reverse: 5'-CATGAGGTC CAC CACCCTGT- 3'. The mRNA expression of each gene was normalized to *GAPDH* using the $2^{-\Delta\Delta Ct}$ method and then normalized to sham group.

2.4 | Western blot

Total proteins from myocardial tissues were extracted using radioimmunoprecipitation lysis buffer (Sigma) and 25 μg proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis. After transfer and blockage in 5% nonfat milk, membranes were incubated with primary antibodies including anti-HMGB1 (ab18256, 1:1000, Abcam), anti-GAPDH (ab9484, 1:2000, Abcam), anti-cleaved Caspase-3 (ab49822, 1:1000, Abcam), anti-Bax (ab32503, 1:1000, Abcam), anti-Bcl2 (ab194583, 1:2000, Abcam), anti-TLR4 (ab217274, 1:1000, Abcam), anti-phospho-p65 (ab76302, 1:1000, Abcam), and anti-p65 (ab32536, 1:1000, Abcam) for overnight at 4°C. Next day, corresponding HRP-conjugated secondary antibodies were used for detecting immune-reactive bands. ImageJ was used for quantitation analysis.

2.5 | TUNEL assay

The hearts were harvested immediately after induction of cardiac arrest and then fixed in 4% paraformaldehyde. After sectioning, the

slides were subjected to the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) by using a commercial TUNEL assay kit (Roche, Shanghai, China) following manufacturer's instructions. Apoptotic nuclei were stained and showed yellow-brown (TUNEL positive). A total of 20 areas from each slice were randomly selected and observed. The number of apoptotic cardiomyocytes and total cardiomyocytes were counted.

2.6 | Measurement of serum cardiac Troponin I

Twelve hours after CME, rat blood was collected and the serum cardiac troponin I (cTnI) level was measured using electrochemistry method following manufacturer's protocol (Roche Diagnostics).

2.7 | Detection of cardiac function

Twelve hours after CME, the left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDd), left ventricular fractional shortening (LVFS), and cardiac output (CO) were analyzed using an ultrasound instrument from Philip Technologies.

2.8 | Statistical analysis

Data were analyzed by statistical product and service solutions (SPSS 16.0, SPSS Inc) and expressed as means ± standard deviation (SD). The statistical difference was determined using one-way or two-way ANOVA with *post hoc* test. The difference was considered as significant when p value is less than .05. The sample size/number of experiments was set before data. All the data have been repeated three times to confirm the results.

3 | RESULTS

3.1 | Coronary microembolization promoted HMGB1 expression in rats

First, we evaluated the expression of HMGB1 after the induction of CME. As shown in Figure 1A, the serum level of HMGB1 in rats from sham group did not change after CME induction. In contrast, serum level of HMGB1 in rats with CME was significantly higher than that in sham rats at 6, 12, 24, and 48 h post-CME. In addition, the serum level of HMGB1 in rats with CME increased from 6 h to 24 h post-CME while slightly decreased at 48 h post-CME. Similarly, we detected significant higher myocardial mRNA level of HMGB1 in CME rats than that in sham rats (Figure 1B). Correspondingly, the protein level of myocardial HMGB1 in rats with CME was significantly increased after CME when compared to that in sham rats (Figure 1C and D).

3.2 | Glycyrrhizin ameliorated cardiac function after coronary microembolization induction

To investigate the potential effects of glycyrrhizin on CME-induced cardiac dysfunction, we pretreated rats with different concentration of glycyrrhizin prior to CME induction and then monitored

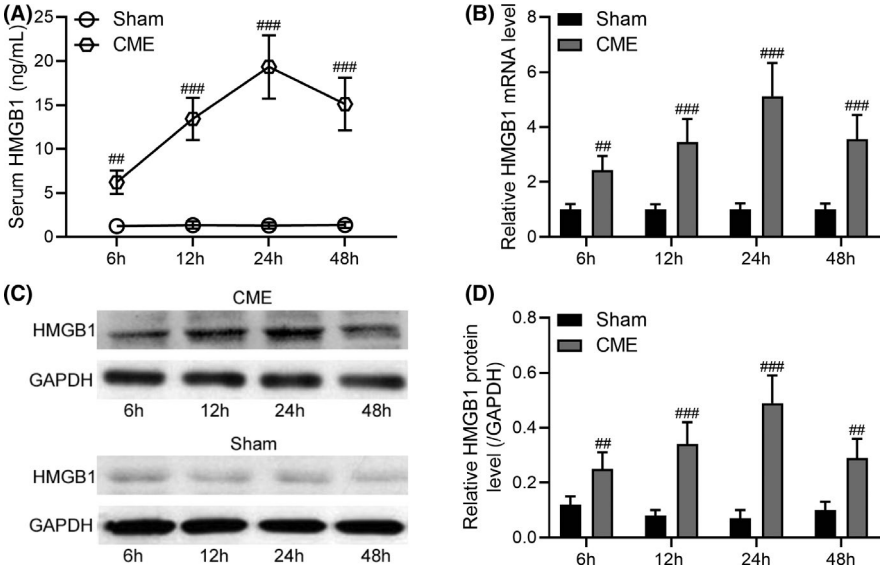

FIGURE 1 Coronary microembolization induced the expression of HMGB1 in Rats. (A) Serum HMGB1 concentrations were determined by ELISA after the surgery of coronary microembolization. (B) Myocardial HMGB1 mRNA expressions were measured by Real-time PCR after the surgery of coronary microembolization. (C) Myocardial HMGB1 protein expressions were measured by Western blotting after the surgery of coronary microembolization and the relative expressions were normalized to GAPDH. (D) Data are presented as mean ± SD. $N = 10$ for each group. $^{\#\#}p < .01$, $^{\#\#\#}p < .001$ compared to sham. Two-way ANOVA followed Sidak's multiple comparisons test

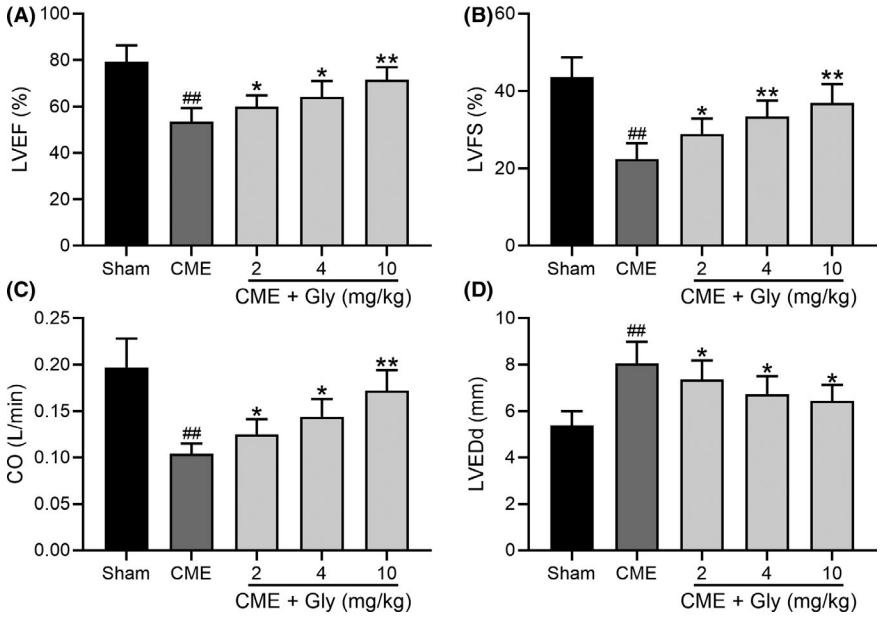

FIGURE 2 Glycyrrhizin pretreatment improved cardiac function after coronary microembolization induction. (A) Left ventricular ejection fraction (LVEF); (B) left ventricular fractional shortening (LVFS); (C) cardiac output (CO); and (D) left ventricular end-diastolic diameter (LVEDd) were detected. Data are presented as mean ± SD. $N = 10$ for each group. $^{\#\#}p < .01$ compared to sham, $^{*}p < .05$, $^{**}p < .01$ compared to CME. One-way ANOVA followed Dunn's multiple comparisons test

the cardiac function. Rats with CME had significantly decreased LVEF (Figure 2A), LVFS (Figure 2B), and CO (Figure 2C), while had significantly increased LVEDd (Figure 2D) when compared to sham rats. In contrast, CME rats pretreated with glycyrrhizin had significantly increased LVEF (Figure 2A), LVFS (Figure 2B), and CO (Figure 2C) but had decreased LVEDd (Figure 2D) when compared to non-treated CME rats, indicating glycyrrhizin improved cardiac function. The glycyrrhizin enhanced cardiac function in a dose-dependent manner. CME rats treated with highest dose of glycyrrhizin (10 mg/kg) had the highest values of LVEF (Figure 2A), LVFS (Figure 2B), and CO (Figure 2C), while had the lowest value of LVEDd (Figure 2D), indicating this dose had the best protective effects.

3.3 | Glycyrrhizin pretreatment prevented CME-induced production of serum cardiac troponin I, creatine kinase, and LDH

We continued to investigate the effects of glycyrrhizin on serum cardiac troponin I and creatine kinase, two markers of cardiac injury.[11] As shown in Figure 3A, CME rats had significantly increased serum cardiac troponin when compared to sham rats. Glycyrrhizin pretreatment significantly decreased serum cardiac troponin level in a dose-dependent manner. CME rats pretreated with 10 mg/kg glycyrrhizin had the lowest serum cardiac troponin level. CME also promoted the serum level of creatine kinase (Figure 3B) while glycyrrhizin pretreatment significantly decreased serum level of creatine kinase in a dose-dependent manner. In addition, we detected significantly increased LDH in serum from CME rats. In contrast, CME rats treated with glycyrrhizin had significantly decreased serum level of LDH (Figure S1). CME rats treated with 10 mg/kg glycyrrhizin had the lowest level of cardiac troponin, creatine kinase, and LDH, indicating the best protective effects of this dose. Therefore, this dose was chosen for next experiments.

3.4 | Glycyrrhizin pretreatment prevented CME-induced myocardial apoptosis

Next, we investigated the effects of glycyrrhizin on CME-induced myocardial apoptosis. The myocardial apoptosis was monitored 12 h post-CME by using TUNEL assay. As shown in Figure 4A, there was no obvious apoptotic signal detected in sham rats. In contrast, there was obvious apoptotic cardiomyocytes detected in CME rats while the apoptotic signal decreased in CME rats pretreated with glycyrrhizin. After quantitation, CME rats pretreated with glycyrrhizin had significantly decreased percentage of apoptotic cardiomyocytes when compared to non-treated CME rats (Figure 4B).

3.5 | Glycyrrhizin prevented CME-induced expression of pro-apoptotic factors and enhanced expression of anti-apoptotic factor Bcl-2

We continued to investigate the effects of glycyrrhizin on expression of apoptosis-related factors including cleaved caspase-3, bax, and bcl-2. As shown in Figure 5A, we detected significantly increased mRNA level of Bax in myocardial tissue of CME rats when

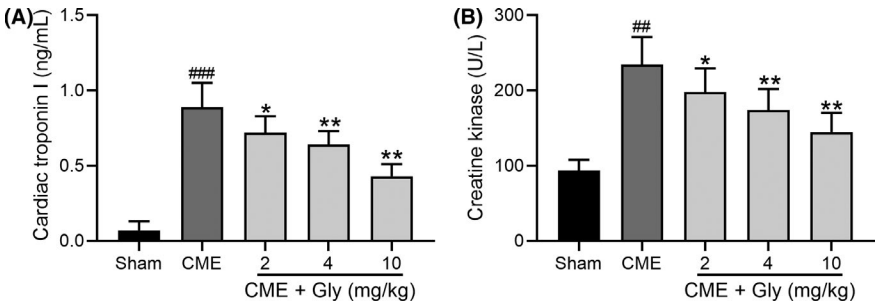

FIGURE 3 Protective effect of glycyrrhizin pretreatment on (A) serum cardiac troponin I level and (B) creatine kinase level. Data are presented as mean ± SD. $N = 10$ for each group. $^{\#\#}p < .01$, $^{\#\#\#}p < .001$ compared to sham, $^{*}p < .05$, $^{**}p < .01$ compared to CME. One-way ANOVA followed Dunn's multiple comparisons test

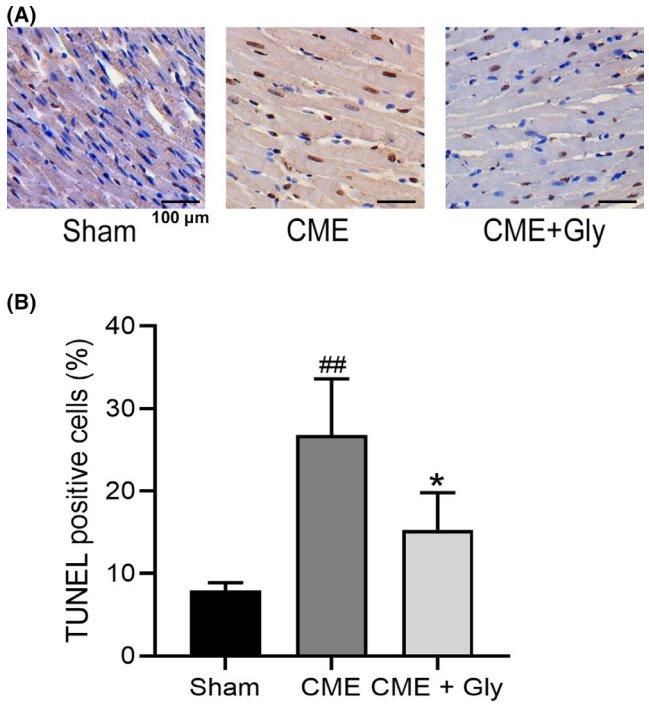

FIGURE 4 Glycyrrhizin pretreatment ameliorated myocardial apoptosis after coronary microembolization induction. (A) Representative TUNEL staining for detecting myocardial apoptosis among different groups. Magnification, ×200, Scale bar = 100 μm. (B) The percentage of TUNEL positive cells. Data are presented as mean ± SD. $N = 6$ for each group. $^{\#\#}p < .01$ compared to sham, $^{*}p < .05$ compared to CME. One-way ANOVA followed Dunn's multiple comparisons test

compared to that in myocardial tissue of sham rats. In contrast, the mRNA level of Bax in myocardial tissue was significantly decreased in CME rats pretreated with glycyrrhizin. We detected significantly decreased mRNA level of anti-apoptotic factor Bcl-2 in myocardial tissue of CME rats while glycyrrhizin pretreatment significantly increased Bcl-2 mRNA (Figure 5B). Correspondingly, we detected significantly increased protein level of cleaved caspase-3 (Figure 5C and D) and Bax (Figure 5C and E), and significantly decreased protein level of Bcl-2 (Figure 5C and F) in myocardial tissue of CME rats.

3.6 | Glycyrrhizin prevented CME-induced inflammation

Next, we investigated the effects of glycyrrhizin on inflammation after CME induction. CME rats had significantly increased TNF-α (Figure 6A), iNOS (Figure 6B), IL-1β (Figure 6C), and IL-6 (Figure 6D) in myocardial tissues when compared to sham rats. Correspondingly, the mRNA level of TNF-α (Figure 6E), iNOS (Figure 6F), IL-1β (Figure 6G), and IL-6 (Figure 6H) in myocardial tissues of CME rats was significantly higher than that in myocardial tissues of sham rats. In contrast, CME rats treated with glycyrrhizin had significantly decreased TNF-α (Figure 6A), iNOS (Figure 6B), IL-1β (Figure 6C), and IL-6 (Figure 6D), and mRNA level of TNF-α (Figure 6E), iNOS (Figure 6F), IL-1β (Figure 6G), and IL-6 (Figure 6H) in myocardial tissues when compared to non-treated CME rats.

3.7 | Glycyrrhizin inhibited the activation of HMGB1/TLR4/NF-κB pathway in CME

We continued to investigate the effects of glycyrrhizin on HMGB1 and its downstream TLR4/NF-κB signaling pathway. We detected significantly increased HMGB1 protein level (Figure 7A and C) and mRNA level (Figure 7B) in myocardial tissues of CME rats when compared to these in myocardial tissues of sham rats. Similarly, CME rats had significantly increased protein level of TLR4 (Figure 7A and D) and phosphorylated-p65 (Figure 7A and E) in myocardial tissues when compared to sham rats, indicating CME activated HMGB1/TLR4/NF–κB signaling pathway. In contrast, CME rats treated with glycyrrhizin had significantly decreased HMGB1 protein level (Figure 7A and C) and mRNA level (Figure 7B), and significantly decreased protein level of TLR4 (Figure 7A and D) and phosphorylated-p65 (Figure 7A and E) in myocardial tissues when compared to non-treated CME rats.

4 | DISCUSSION

In present study, we evaluated the effects of glycyrrhizin, a HMGB1 inhibitor, on CME-induced damage in CME rat model. We

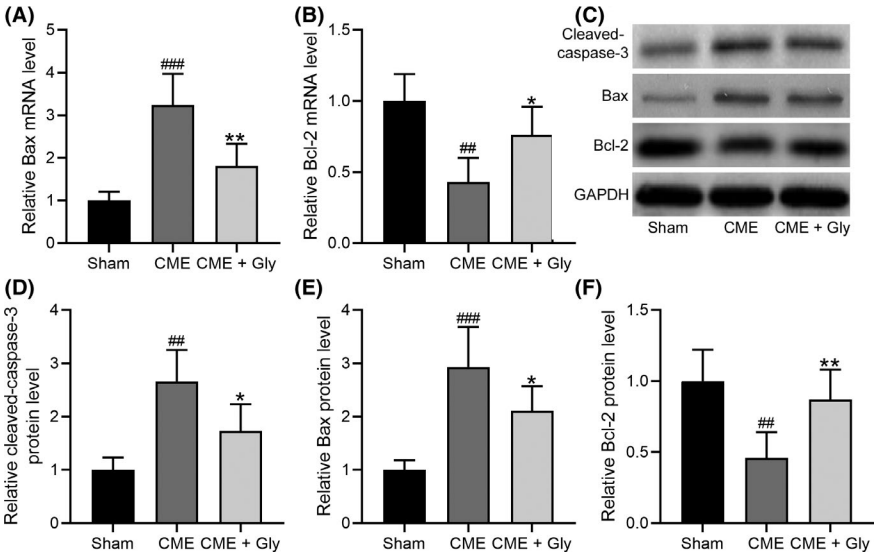

FIGURE 5 Glycyrrhizin pretreatment suppressed myocardial apoptosis after coronary microembolization induction. (A and B), The mRNA expressions of bax and bcl-2 in myocardial tissues were measured by Real-time PCR. (C), Western blot was used to detect the protein expressions of cleaved caspase-3, bax, and bcl-2 in myocardial tissues and the relative expressions were normalized to sham (D–F). Data are presented as mean ± SD. $N = 8$ for each group. $^{\#\#}p < .01$, $^{\#\#\#}p < .001$ compared to sham, $^{*}p < .05$, $^{**}p < .01$ compared to CME. One-way ANOVA followed Dunn's multiple comparisons test

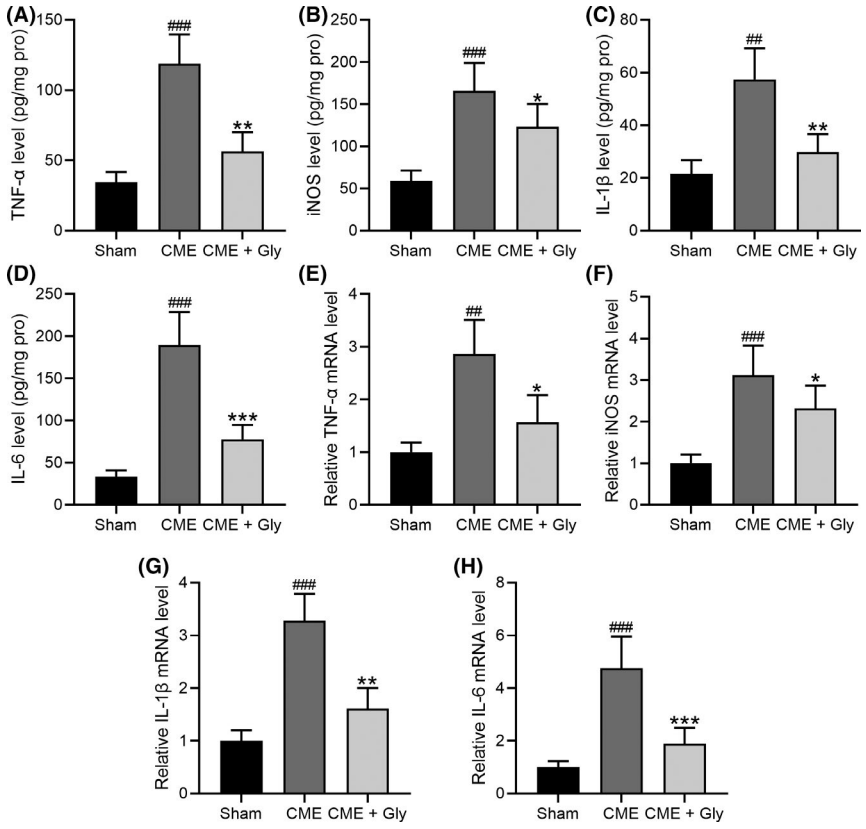

FIGURE 6 Glycyrrhizin pretreatment ameliorated myocardial inflammatory response after coronary microembolization induction. (A–D), ELISA was used to measure the concentrations of TNF-α (A), iNOS (B), IL-1β (C), and IL-6 (D) in myocardial tissues. (E–H), The mRNA expressions of TNF-α(E), iNOS (F), IL-1β(G), and IL-6 (H) in myocardial tissues were measured by Real-time PCR. The relative expressions were normalized to sham. Data are presented as mean ± SD. $N = 8$ for each group. $^{\#\#}p < .01$, $^{\#\#\#}p < .001$ compared to sham, $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ compared to CME. One-way ANOVA followed Dunn's multiple comparisons test

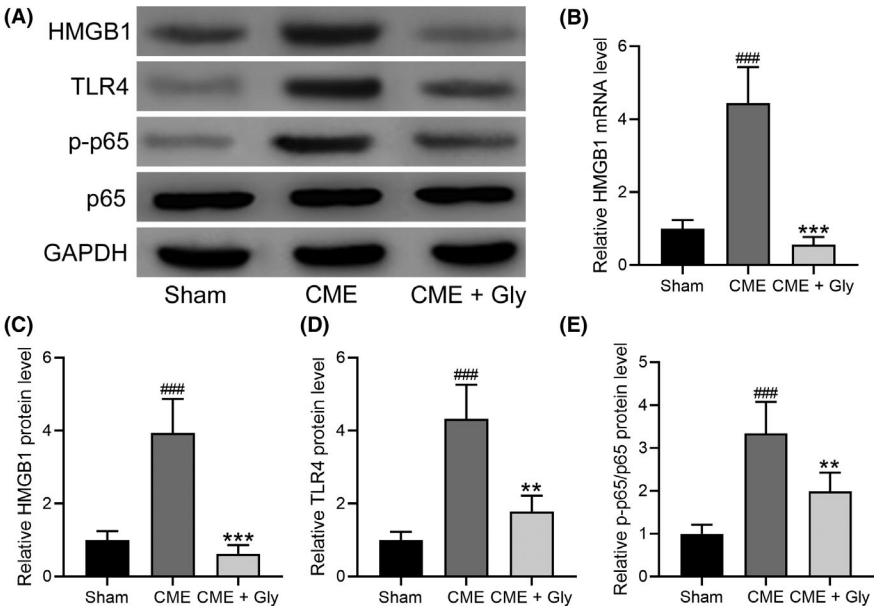

FIGURE 7 Glycyrrhizin pretreatment inhibited the activation of HMGB1/TLR4/NF-κB pathway. (A) Western blotting was used to assay the protein expressions of HMGB1, TLR4, p-p65, and p65 in myocardial tissues. GAPDH was used as loading control and the relative expressions were normalized to sham group (C–E). The mRNA expressions of HMGB1 (B) in myocardial tissues were measured by Real-time PCR. Data are presented as mean ± SD. $N = 8$ for each group. $^{###}p < .001$ compared to sham, $^{**}p < .01$ and $^{***}p < .001$ compared to CME. One-way ANOVA followed Dunn's multiple comparisons test

demonstrated that CME promoted HMGB1 expression, induced inflammatory response and cardiomyocytes apoptosis, and caused cardiac injury in rats. Glycyrrhizin inhibited the CME-induced production of inflammatory cytokines, prevented cardiomyocytes apoptosis, and ameliorated cardiac function. We further demonstrated that glycyrrhizin prevented CME-induced activation of HMGB1/TLR4/NF-κB signaling pathway.

CME occurs in acute myocardial infarction and causes no reflow or slow blood flow.[3] Inflammation including inflammatory cells infiltration and inflammatory cytokines production has been observed after CME, which leads to CME-induced myocardial injury. Significantly increased serum TNF-α was detected in clinical patients and animal with experimental CME.[7,12] TNF-α is a critical inflammatory factor which is involved in development of heart failure, atherosclerosis.[7] After binding to its receptor, TNF-α regulates multiple cellular events including NF-κB activation, oxidative stress, and apoptosis. The direct evidences that TNF-α contribute to cardiomyocyte apoptosis and resulted in cardiac dysfunction have been described. Skyschally and colleagues reported that animal treated with TNF-α neutralization antibody had attenuated progressive contractile dysfunction.[5] By using TNF-α antibody adalimumab, Chen et.al demonstrated that neutralization of TNF-α suppressed cardiomyocyte apoptosis and improved cardiac function after CME in mini pigs.[7] Apoptosis, which occurred in the beginning of ischemia, plays significant role in myocardial dysfunction. As cardiomyocytes cannot self-renew, the cardiomyocytes apoptosis could cause myocardial dysfunction. Su and colleagues described that metoprolol inhibited apoptosis and improved cardiac function in rats with CME.[13,14] Liang et al. reported that upregulation and activation of Nrf2/HO-1 signaling pathway prevented myocardial apoptosis and improved CME-induced cardiac dysfunction. Therefore, therapies targeting TNF-α and apoptosis could be used to treat CME-induced cardiac injury. In the present study, we identified that glycyrrhizin had strong protective effects against apoptosis and inhibited TNF-α expression in CME, indicating these effects contributed to the amelioration of CME by glycyrrhizin.

Troponin I is essential for the calcium-mediated regulation of cardiac muscle contraction. When there is damage to heart muscle cells, troponin is released into the blood. The presence of cardiac troponins in the serum indicates myocardial injury.[15] In CME rats, we detected significantly increased cardiac troponin I in serum. In contrast, the serum cardiac troponin was significantly decreased after glycyrrhizin treatment, suggesting glycyrrhizin prevented cardiac damage. Similar to troponin, creatine kinase is another diagnostic marker of heart failure.[16] Increased amounts of creatine kinase are released into the blood when there is muscle damage. Glycyrrhizin decreased the serum level of creatin kinase suggesting the protective effects of glycyrrhizin on cardiac damage.

The NF-κB signaling pathway has been shown to play essential role in CME-induced damage. Blockage of NF-κB using NF-κB-specific inhibitor pyrrolidine dithiocarbmate has been shown to prevent CME-induced local production of TNF-α, IL-6, and improved cardiac function.[4] Furthermore, the involvement of NF-κB pathway upstream factors TLR4/MyD88 in CME-induced inflammation has been demonstrated. Su and colleagues reported that nicorandil significantly decreased the expression and activation of TLR4/MyD88/NF-κB signaling, which reduced myocardial inflammation and injury after induction of CME in rats.[17] TLR4 is one of

the HMGB1 receptors. When extracellular HMGB1 binds to TLR4, the downstream signaling MyD88/NF-κB is activated, resulting in cytokines and chemokines expression. In the present study, we confirmed the CME-induced activation of HMGB1/TLR4/NF-κB signaling pathway in rats. The essential roles of HMGB1 in CME-induced cardiac dysfunction have been described. Using a rat CME model, Chen and colleagues described that knocking down HMGB1 expression by siRNA treatment decreased the activation of NF-κB, inhibited the expression of TNF-α and IL-1β, inhibited apoptosis, and improved cardiac function.[3] The protective effects of HMGB1 inhibition have been proven in other diseases models. Oozawa and colleges reported that neutralizing HMGB1 using antibody ameliorated ischemia-reperfusion induced heart injury in rats.[18]

Glycyrrhizin is a HMGB1 inhibitor with great anti-inflammatory activities. Gong and colleagues described that glycyrrhizin protected the ischemia-reperfusion injury by inhibiting inflammation, oxidative stress, and cell apoptosis through targeting HMGB1.[10] Thakur et al. demonstrated that glycyrrhizin inhibited TLR4/NF-κB pathway activation and reduced the inflammation in diabetic kidney disease.[19] Glycyrrhizin also blocked the binding of HMGB1 to RAGE and TLR4, suppressed the downstream MAPKs/NF-κB pathways, and protected rats against sepsis.[20] In the present study, we also demonstrated that glycyrrhizin inhibited the production of inflammatory cytokines and iNOS, prevented CME-induced cell apoptosis and injury. These results strongly suggested that glycyrrhizin could be used as a potential therapeutic drug to prevent CME-induced injury.

The results in our study are consistent with a previous report where the authors suppressed HMBG1 using siRNA treatment.[3] Glycyrrhizin is a natural compound isolated from *Glycyrrhiza glabra* (liquorice) root. Glycyrrhizin has been used for a long time to treat various diseases and has been proved to be safe.[21–23] In contrast, major challenges in most siRNA treatments remain unsolved such as efficient and target-oriented delivery of siRNA, reducing their therapeutic applications.[24] Therefore, our study shed light on the utilization of glycyrrhizin to treat CME by targeting HMGB1.

There are still several important and interesting questions that need to be further addressed. For example, besides TLR4, TLR2 is another receptor for HMGB1. TLR2 has been shown to be involved in cardiac ischemia-reperfusion-induced injury.[25,26] Whether TLR2 functions as downstream factor of HMGB1 and contributes to CME-induced inflammation and cardiac dysfunction remains unknown and need be further elucidated. In addition, whether TLR2 and/or TLR4 are/is required for glycyrrhizin-mediated protection against CME-induced injury need further determined.

5 | CONCLUSION

Glycyrrhizin prevented CME-induced inflammation and myocardial apoptosis, ameliorated cardiac function, and suppressed CME-induced activation of HMGB1/TLR4/NF-κB signaling pathway.

ETHICS STATEMENT

The study was approved by the ethics commitment of Cangzhou Central Hospital of Tianjin Medical University.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Yonggang Yuan, Bing Li, Wanzhong Peng, and Zesheng Xu performed the experiments, analyzed and interpreted the data. Zesheng Xu was the major contributors in writing the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data could be obtained upon request to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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