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*Original Article*

# Protective effects of ginsenoside Rb2 on myocardial ischemia *in vivo* and *in vitro*

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**Abstract:** Ginsenoside Rb2 is one of the major active components of *Panax ginseng*. There is little research about cardioprotective effect of ginsenoside Rb2. The aim of this study was to explore the cardioprotective effects of ginsenoside Rb2 on animal model of myocardial ischemia/reperfusion (MI/R) injury *in vivo* and $H_2O_2$-induced stress in H9c2 cells *in vitro*. Pretreatment with ginsenoside Rb2 (10 and 20 mg/kg) could significantly improve cardiac function, remarkably decrease infarct size from 29.84% to 17.34%, decrease the activities of CK-MB, LDH, and AST in serum. Meanwhile, ginsenoside Rb2 (10 and 20 mg/kg) significantly increased the activities of SOD, CAT and GSH-PX, and decreased the level of MDA in tissue ($P < 0.05$). Histopathological results demonstrated the protective effect of ginsenoside Rb2. Furthermore, the anti-apoptotic effect of ginsenoside Rb2 was confirmed by the results of TUNEL staining. Western blot analysis demonstrated that ginsenoside Rb2 up-regulated levels of Bcl-2, Procaspase-3, -9 expression and down-regulated level of Bax expression. *In vitro*, pretreatment with ginsenoside Rb2 (1, 3 and 10 μg/mL) prior to $H_2O_2$ exposure could increase cell viability of H9c2 cells. Pretreatment with ginsenoside Rb2 (1, 3 and 10 μg/mL) also increased the activity of SOD, and decreased the level of LDH in the cultured supernatant and the MDA level in H9c2 cells. These results indicated that ginsenoside Rb2 had cardioprotective effects both *in vivo* and *in vitro*. The mechanisms involved may be attributed to the scavenging of oxidative stress products, increasing the concentration of antioxidant defense enzymes and inhibiting cardiomyocyte apoptosis.

**Keywords:** Ginsenoside Rb2, myocardial ischemia reperfusion injury, antioxidant, apoptosis, cardioprotective

## Introduction

Myocardial infarction following ischemia is a major cause of mortality worldwide. Although early reperfusion is necessary for myocardial salvage, reperfusion itself exacerbates myocardial injury [1]. Myocardial ischemia reperfusion (MI/R) injury can result in arrhythmia, cardiomyocyte apoptosis, heart hypofunction and other disorders [2, 3]. Of the many theories regarding the development of MI/R injury, the enhanced oxidative stress during the acute reperfusion phase, including the generation of superoxide anion, hydrogen peroxide, and hydroxyl radical, is the most likely one [4]. MI/R leads to oxidative stress, which subsequently leads to reactive oxygen species (ROS) production, an increase in malondialdehyde (MDA) levels and subsequent cytotoxic injury [4, 5]. Oxidative stress and the accelerated ROS production induced by MI/R play key roles in the progression of ischemic heart disease and cardiomyocyte apoptosis [5, 6]. A number of studies have suggested that cardiomyocyte apoptosis induces a spectrum of events, including cardiac remodeling, a larger infarct size and severe heart failure [7, 8]. Pharmacological preconditioning is a cardioprotective mechanism induced by drugs or chemicals that can trigger the release of some endogenous protective substances. Recently, increased attention has been focused on traditional Chinese herbal treatments due to their unique decrease of oxidative stress efficacy and limited adverse reactions [9, 10].

*Panax ginseng* C. A. Mayer (ginseng) is listed as a medicinal herb in the first class article of

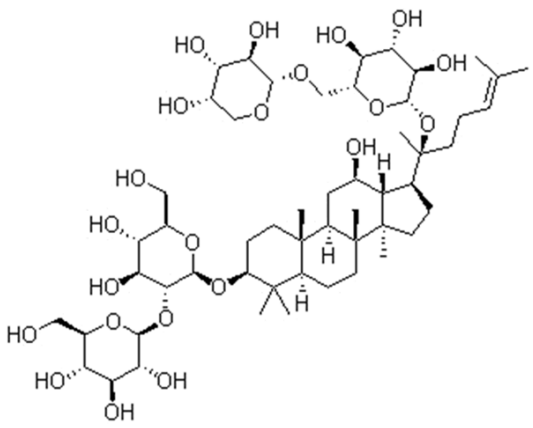

**Figure 1.** The chemical structure of ginsenoside Rb2.

Shennong Bencaojing, a typical Chinese herbal dictionary. Reports show that ginseng has a range of pharmacological and therapeutic uses [11-14]. The ginseng root consists of ginsenosides, polysaccharides, peptide, polyacetylenic alcohols and fatty acids [15]. Ginsenosides are the major active components of ginseng, and they appear to be responsible for the principle pharmacological activities of ginseng, including vasorelaxation, anti-neoplastic, anti-diabetic, anti-inflammation, and anti-oxidant effects [16-19]. Ginsenoside Rb2 is one of the major active components of *Panax ginseng* [20] (**Figure 1**). It has been reported that ginsenoside Rb2 inhibited angiogenesis and improved wound healing by enhancing epidermal cell proliferation [21, 22]. In addition, ginsenoside Rb2 might be a valuable component in the development of drugs to lower lipid levels; it was shown to be capable of lowering triacylglycerol levels in 3T3-L1 adipocytes cultured under high energy conditions by stimulating the expression of sterol regulated element binding protein and leptin mRNA [23]. To date, limited studies have been carried out to investigate cardioprotective effect of ginsenoside Rb2. The aim of this study was to explore the effects of ginsenoside Rb2 on animal model of MI/R injury *in vivo* and against $H_2O_2$-induced stress in H9c2 cells *in vitro* and its mechanism.

## Materials and methods

### *Chemicals and reagents*

Ginsenoside Rb2 was provided by Prof. Yanping Chen. The purity of ginsenoside Rb2 used in experiments was > 98% detected by HPLC. Lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). MTT and all other reagents were purchased from Sigma-Adrich Co. (St. Louis, MO, USA). TdT-mediated dUTP Nick End Labeling (TUNEL) Apoptosis Detection Kit was purchased from Roche Diagnostics (Basel, Switzerland). Antibodies against Procaspase-3, -9, Bcl-2 and Bax were purchased from Cell Signal Technology (Beverly, MA, USA). Antibody against β-actin was obtained from Tianjing Jingmai.

### *Animal's treatment*

The experimental protocol used in this study was reviewed and approved by the Animal Care and Use Committee of Jilin University and in accordance with the National Institutes of Health guidelines for the use of experimental animals. Male and female Sprague-Dawley rats weighing 230 g to 250 g (12 to 14 wk old) were provided by the Experimental Animal Center of Jilin University. All animals were allowed free access to food and water and maintained at 22-24°C under a cycle of 12 h:12 h light-dark.

Those rats were randomly assigned to experimental groups: (1) Sham group, rats were administrated intragastrically with $ddH_2O$ at a dose of 10 mL/kg; (2) MI/R group, rats were administrated intragastrically with $ddH_2O$ at a dose of 10 mL/kg; (3) Ginsenoside Rb2 10 mg/kg group, rats were administrated intragastrically with ginsenoside Rb2 at a dose of 10 mg/kg; (4) Ginsenoside Rb2 20 mg/kg group, rats were administrated intragastrically with ginsenoside Rb2 at a dose of 20 mg/kg; (5) Diltiazem group, rats were administrated intragastrically with Diltiazem at a dose of 20 mg/kg. These rats were administrated once a day for 3 days continually. One hour after the third day administration, rats were then anesthetized for the following surgery.

### *Experimental model of MI/R injury in vivo*

Those Sprague-Dawley rats were anesthetized by 3% pentobarbital sodium. Myocardial ischemia was produced by exteriorizing the heart through a left thoracic incision and placing a 6-0 silk suture and making a slipknot around the left anterior descending coronary artery.

After 30 min of ischemia, the slipknot was released, and the myocardium was reperfused for 6 h (for analysis of myocardial apoptosis and for quantification of myocardial infarct size) and 72 h (for cardiac function determination). Rats of sham group underwent the same surgical procedures except that the suture passed under the left coronary artery was left untied. Before and during the surgery, animals received different treatments.

*Echocardiography*

Two-dimensional and M-mode echocardiographic measurements were conducted in a standard setting using a 10S scan head (GE Vivid I, GE Healthcare, USA) 72 h after MI/R operation. After a short-axis two-dimensional image of the left ventricle was obtained at the level of the papillary muscles, two-dimensional guided M-mode images were acquired at a sweep speed of 100 mm/s and stored digitally. The left ventricular (LV) internal dimension at diastole (LVIDd), LV internal dimension at systole (LVIDs) were measured, allowing calculation of LV fractional shortening (FS) and LVEF (calculated using Simpson's rule). All of the parameters were measured over 3 consecutive cardiaccycles and performed by one experienced echocardiographer blinded to the treatment.

*Determination of infarct size*

At the end of 6 h reperfusion, the hearts were removed quickly. The determination of infarct size was performed according to the method with minor modification [24]. The tissues of ventricles were cut into 5 transverse slices and the slices were incubated in 0.5% nitrotetrazolium blue chloride (NBT) for 20 min. Myocardial infarction was distinguished by the different color tone (pale for ischemic myocardium and dark red for non-ischemic myocardium). The ischemic myocardium was cut and weighted. The infarct size as a percent of the ventricular mass was calculated as: weight of ischemia zone/total weight of ventricular ×100%. Myocardial infarct size is expressed as percentage of region of ventricle.

*Determination of the serum enzymes*

Blood samples were drawn at 6 h after reperfusion. The collected blood samples were clotted for 2 h at room temperature, and centrifuged at 2500 rpm for 15 min. The supernatant serums were separated and stored at -80°C. The activities of Lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB) and aspartate aminotransferase (AST) were measured using diagnostic kits according to the manufacturer's instructions.

*Biochemical analysis*

The tissues of ventricles were excised and homogenized with ice-cold buffer in homogenizer. The homogenate was centrifuged and the supernatant was taken to measure the activities of CAT, SOD and GSH-PX and the content of MDA by using diagnostic kits according to the manufacturer's instructions.

*Histopathological examination*

For light microscopic evaluation, tissue sections from the left ventricles were fixed in phosphate buffered 10% formalin solution. The specimens embedded with paraffin were cut into 5 μm thick sections and stained with hematoxylin-eosin [25]. The sections were examined by an experienced observer who was blind to the treatment under light microscope and then photomicrographs were taken.

*Determination of myocardial apoptosis*

To measure the DNA nicks that reflect the extent of myocardial apoptosis, TdT-mediated dUTP Nick End Labeling (TUNEL) was performed [26]. The heart slices were fixed in 10% formalin solution, embedded in paraffin, and sectioned on slides at 5-μm thickness. TUNEL staining with the paraffin sections was performed according to the kit manufacturer's instructions. The sections were examined using light microscopy, and then photomicrographs were taken.

*Western blot analysis*

Western blot was performed for detection of Procaspase-3, -9, Bax and Bcl-2 proteins. Rats were sacrificed after 6 h of reperfusion and heart tissue was taken out for protein extraction. The protein concentration was determined using the BCA protein assay kit. Then the protein extraction was loaded onto 12% polyacrylamide-SDS gel. After electrophoresis, the gel was blotted onto a PVDF membrane, blocked with 5% (w/v) non-fat milk for 1 h. The transferred membrane was incubated with appropri-

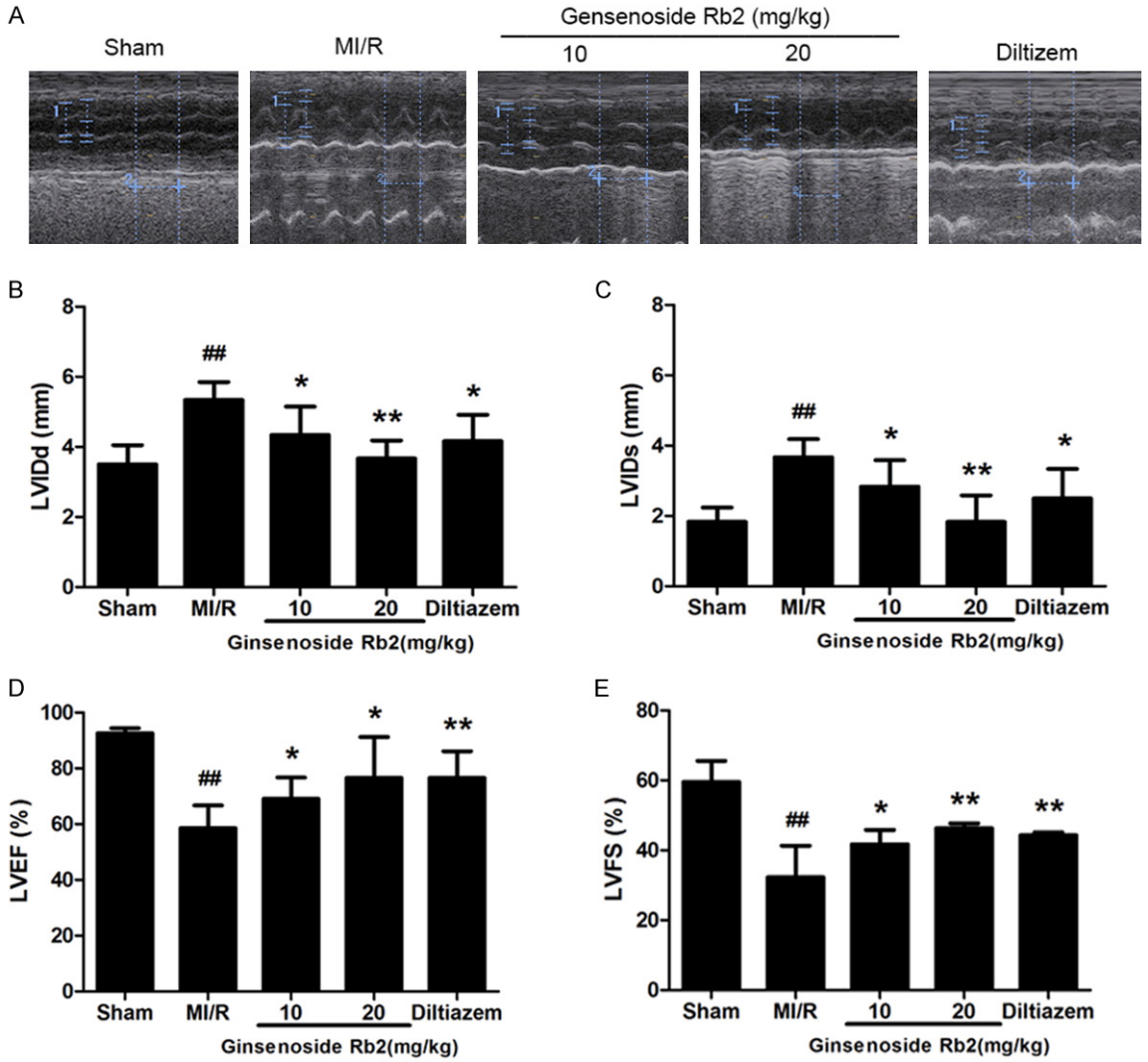


**Figure 2.** Effects of ginsenoside Rb2 on cardiac function. A. Representative M-mode images by echocardiography. Cardiac function was assessed by echocardiography at 72 h after MI/R operation. B. Left ventricular internal dimension at diastole (LVIDd). C. Left ventricular internal dimension at systole (LVIDs). D. Left ventricular ejection fraction (LVEF). E. Left ventricular fractional shortening (LVFS). Data are expressed mean ± S.D for each group. ##$P$ < 0.01 vs. Sham group; *$P$ < 0.05, **$P$ < 0.01 vs. MI/R group.

ate primary antibodies at 4°C overnight. Primary antibody binding was detected with secondary antibody conjugated to HRP, and visualized using ECL chemiluminescence. β-actin was used as the internal loading control. The results were expressed by grayscale value analyzed in NIH Image J software (National Institutes of Health, Bethesda, Maryland, USA).

*In vitro study*

Rat embryonic cardiomyoblast derived H9c2 cells were obtained from Shanghai Institute of Cell Biology, Chinese Academic of Science (Shanghai, China). H9c2 cells were cultured in DMEM supplemented with 10% heat-inactivated (56°C, 30 min) fetal bovine serum under standard cultured conditions (37°C, 95% humidified air and 5% $CO_2$). Cells were nearly 80% confluent and then treated with ginsenoside Rb2 (1, 3 and 10 μg/mL) for 4 h followed by treatment of 150 μM $H_2O_2$ for 6 h. Cell viability was measured by MTT assay as described previously [27]. After exposure of $H_2O_2$, the MTT solution (5 mg/mL) was added for 4 h. The formed formazan blue crystals remaining in the cells were dissolved in 150 μL DMSO for 10

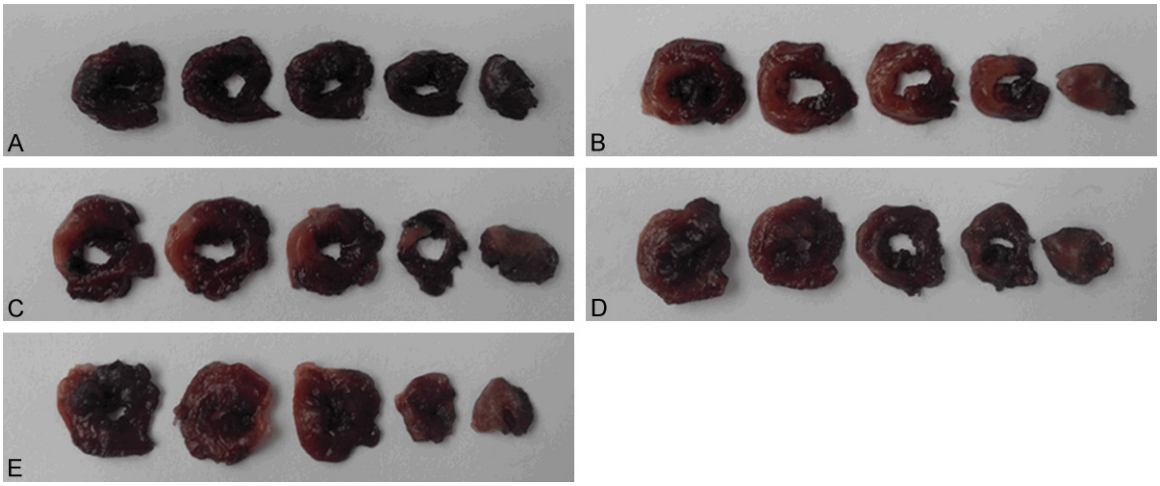


**Figure 3.** The representative heart morphological photographs of ginsenoside Rb2 on infarct zone in rats stained by NBT. The tissues of ventricles were excised and cut into transverse slice and incubated for 20 min in 0.5% nitrotetrazolium blue chloride (NBT) at 37°C. Pale color means ischemic myocardium and dark red color means non-ischemic myocardium. A. Sham group; B. MI/R group; C. Ginsenoside Rb2 10 mg/kg; D. Ginsenoside Rb2 20 mg/kg; E. Diltiazem 20 mg/kg.

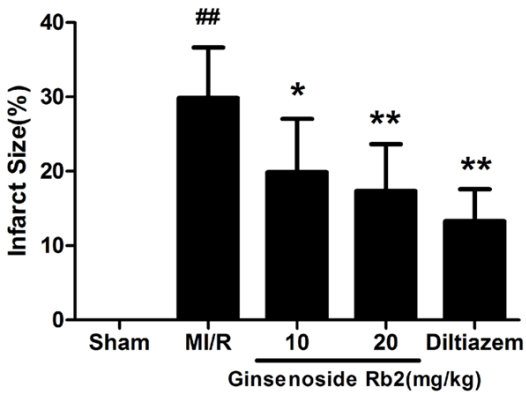


**Figure 4.** Effects of ginsenoside Rb2 on myocardial infarct size. The tissues of ventricles were excised and cut into transverse slice and incubated for 20 min in 0.5% NBT at 37°C. Pale color means ischemic myocardium and dark red color means non-ischemic myocardium. The ischemic myocardium was cut and weighted. The infarct size as a percent of the ventricular mass was calculated as: weight of ischemia zone/total weight of ventricular ×100%. Myocardial infarct size is expressed as percentage of region of ventricle. Data are expressed mean ± S.D for each group. ##$P < 0.01$ vs. Sham group; *$P < 0.05$, **$P < 0.01$ vs. MI/R group.

min. The absorbance at 570 nm was measured using spectrophotometer. The level of LDH in the cultured supernatant, the activity of SOD, GSH-PX and the content of MDA in H9c2 cells were measured using diagnostic kits according to manufacturer's instruction.

*Statistical analysis*

All data were reported as mean ± S.D. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistical significant.

## Results

*Effect of ginsenoside Rb2 on cardiac function*

After 72 h of reperfusion, echocardiographic results showed that compared with sham group, the cardiac function in MI/R rats was remarkably deteriorated, as evidenced by increased LVIDd, LVIDs and decreased LVFS, LVEF ($P < 0.05$, or $P < 0.01$). Meanwhile, we observed that pretreatment with ginsenoside Rb2 significantly ameliorated myocardial dysfunction and remodeling, which was verified by increased LVEF, LVFS and decreased LVIDd, LVIDs compared with model group ($P < 0.05$, or $P < 0.01$) (**Figure 2**). Our results indicated that ginsenoside Rb2 improved cardiac function.

*Effect of ginsenoside Rb2 on myocardial infarct size*

Based on the examination of NBT staining, a typical myocardial ischemic zone was observed in MI/R group, pale for ischemic myocardium and dark red for non-ischemic myocardium

**Table 1.** Effect of ginsenoside Rb2 on the levels of creatine-MB (CK-MB), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in serum

| Group | Infarct size (%) | CK-MB (U/L) | AST (U/L) | LDH (U/L) |
|---|---|---|---|---|
| Sham | 0.00±0.00 | 70.1±25.0 | 278.1±60.3 | 456.2±99.0 |
| MI/R | 29.84±6.83## | 314.5±139.7## | 1268.6±276.4## | 1336.1±217.0## |
| Ginsenoside Rb2 | | | | |
| 10 mg/kg | 19.88±7.18* | 193.8±65.1* | 704.9±171.2** | 1041.7±365.7* |
| 20 mg/kg | 17.34±6.30** | 182.2±57.5* | 909.1±284.0* | 1052.3±357.4* |
| Diltiazem | 13.29±4.28** | 188.5±54.4* | 944.7±290.7* | 1002.4±273.5** |

Data presented are the mean ± SD. ##$P < 0.01$ vs. Sham group; *$P < 0.05$, **$P < 0.01$ vs. MI/R group.

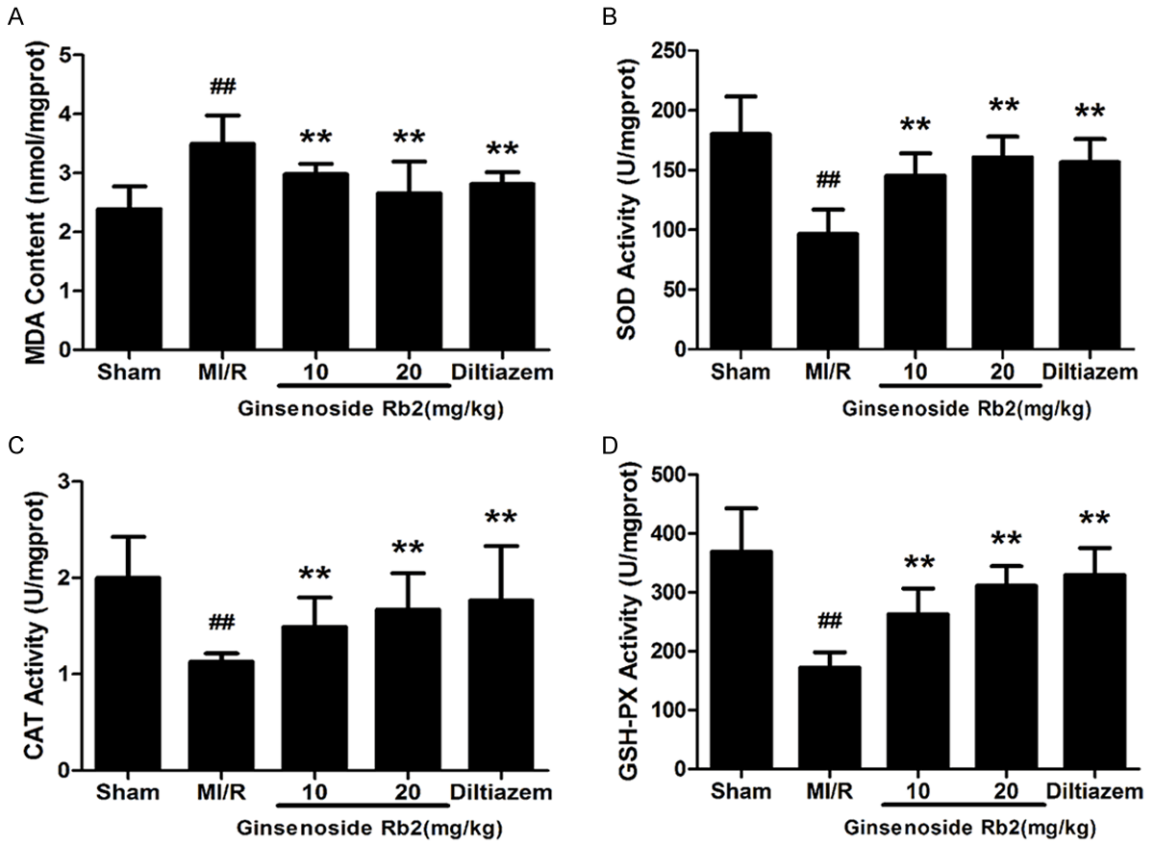


**Figure 5.** Effect of ginsenoside Rb2 on oxidative parameters in heart homogenate. A. MDA, B. SOD, C. CAT, D. GSH-PX. The tissues of ventricles were excised and homogenized with ice-cold buffer in homogenizer. The homogenate was centrifuged and the supernatant was taken to measure the content of MDA and the activities of SOD, CAT, GSH-PX by using diagnostic kits. Data are expressed mean ± S.D for each group. ##$P < 0.01$ vs. Sham group; *$P < 0.05$, **$P < 0.01$ vs. MI/R group.

(**Figure 3**). The infarct size presented as a ration of the weight of ischemic zone over ventricular mass being 29.84±6.83% in the MI/R rats. Pretreatment with ginsenoside Rb2 (10 and 20 mg/kg) resulted in dose-dependent reduction in the infarct size, 19.88±7.18 and 17.34±6.30%, respectively. There were significant differences between the MI/R rats and rats treated with ginsenoside Rb2 (10 and 20 mg/kg) ($P < 0.05$, or $P < 0.01$) (**Figure 4**).

*Effect of ginsenoside Rb2 on the activities of CK-MB, AST and LDH in serum*

CK-MB, AST and LDH release from the cardiomyocytes, which reflects cellular injury or tissue necrosis and membrane permeability. Compared with the sham group, the activities of all myocardial enzymes CK-MB, AST and LDH were increased in MI/R group. Pretreatment with ginsenoside Rb2 (10 and 20 mg/kg) inhib-

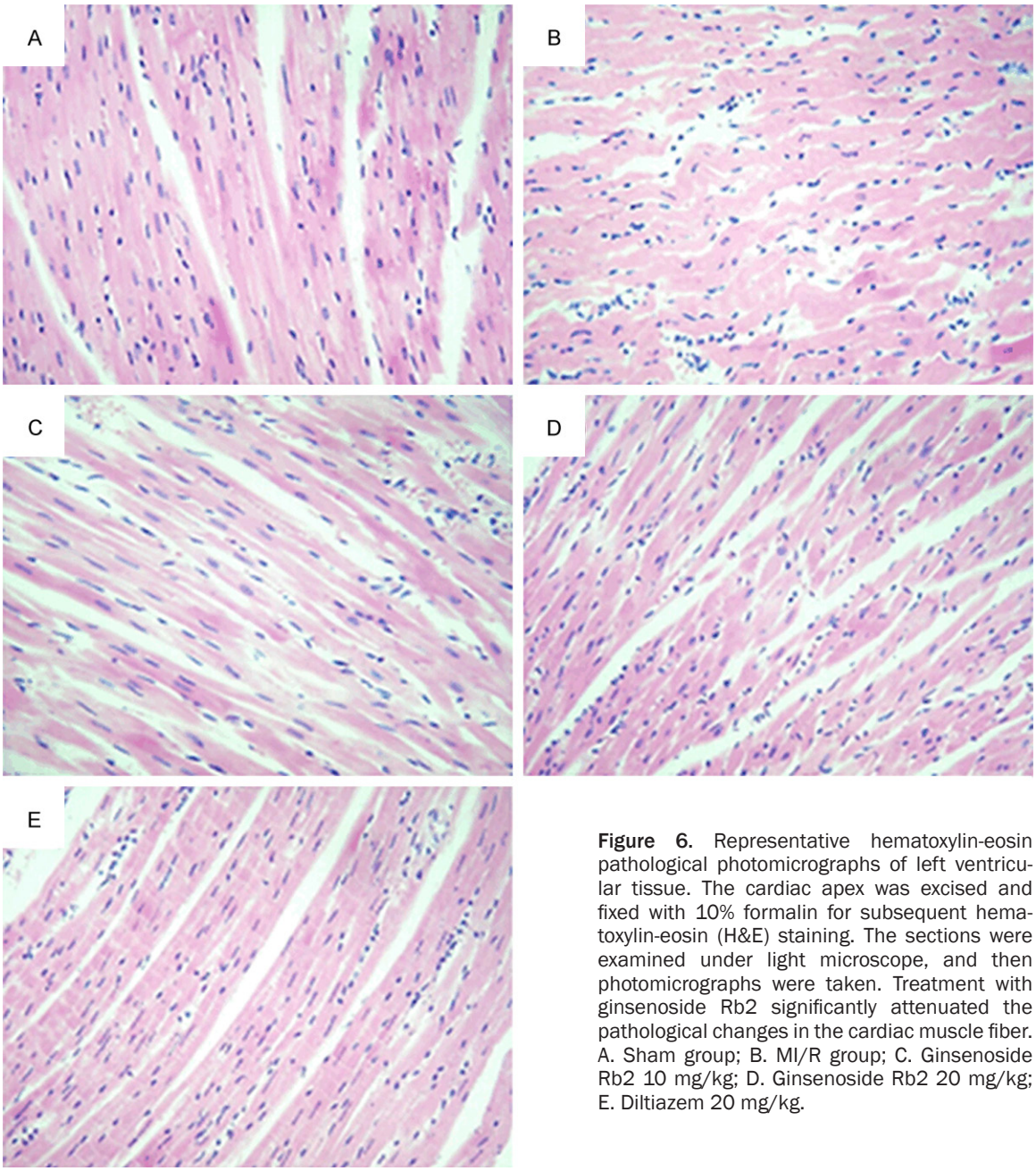


**Figure 6.** Representative hematoxylin-eosin pathological photomicrographs of left ventricular tissue. The cardiac apex was excised and fixed with 10% formalin for subsequent hematoxylin-eosin (H&E) staining. The sections were examined under light microscope, and then photomicrographs were taken. Treatment with ginsenoside Rb2 significantly attenuated the pathological changes in the cardiac muscle fiber. A. Sham group; B. MI/R group; C. Ginsenoside Rb2 10 mg/kg; D. Ginsenoside Rb2 20 mg/kg; E. Diltiazem 20 mg/kg.

ited the increase of CK-MB, AST and LDH compared with MI/R group ($P < 0.05$, or $P < 0.01$) (**Table 1**).

*Effect of ginsenoside Rb2 on the content of MDA and the activities of SOD, CAT, GSH-PX in tissue*

As shown in **Figure 5**, the content of MDA, an index of lipid peroxidation, was significantly increased, while the activities of antioxidant enzymes SOD, CAT and GSH-PX were decreased significantly in MI/R group compared with sham group. Pretreatment with ginsenoside Rb2 (10 and 20 mg/kg) could decrease the level of MDA (2.98±0.18, 2.65±0.54 nmol/mg prot, **Figure 5A**), but increase the activity of SOD (145.30±18.76, 160.49±17.49 U/mg prot, **Figure 5B**). In addition, ginsenoside Rb2 also significantly increased the activity of CAT (1.49±0.31, 1.67±0.38 U/mgprot, **Figure 5C**), and that of GSH-PX (262.77±43.73, 310.90±33.66 U/mgprot, **Figure 5D**).

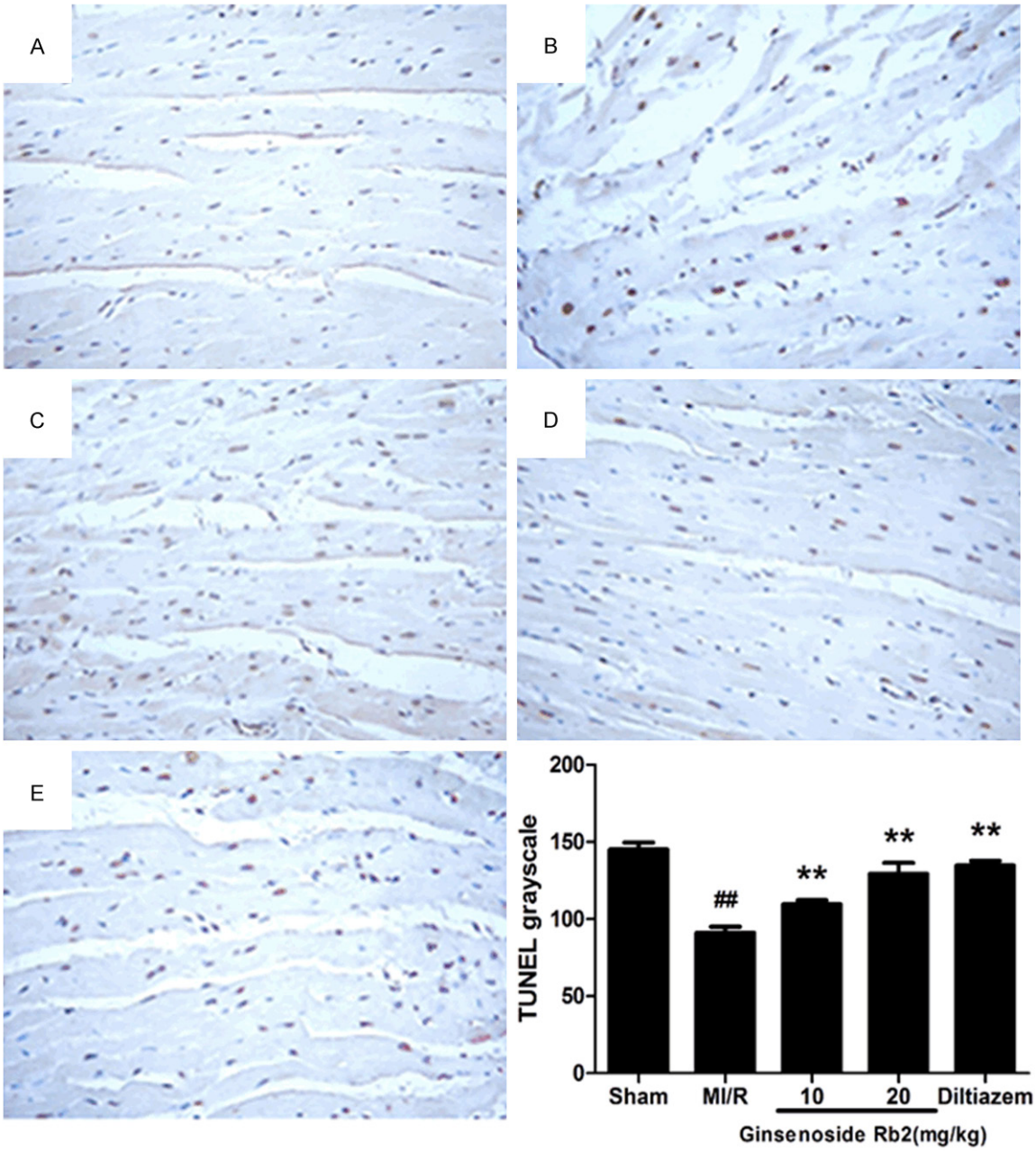


**Figure 7.** Effect of ginsenoside Rb2 against cardiomyocyte apoptosis induced MI/R injury. Representative TUNEL staining photomicrographs of myocardium tissue. A. Sham group; B. MI/R group; C. Ginsenoside Rb2 10 mg/kg; D. Ginsenoside Rb2 20 mg/kg; E. Diltiazem 20 mg/kg; Quantitative analysis of TUNEL staining. Data presented are the mean ± S.D for each group. ##$P < 0.01$ vs. Sham group; **$P < 0.01$ vs. MI/R group.

*Histopathological examination of cardiac tissues*

As shown in **Figure 6**, the heart tissues in MI/R group showed myocardial cell loss, widespread myocardial structure disorder, myocardium fragment, hyperemia and leukocyte infiltration. Pretreatment with ginsenoside Rb2 (10 and 20 mg/kg) significantly attenuated the pathophysiological changes in the cardiac muscle fiber.

*Effect of ginsenoside Rb2 against cardiomyocyte apoptosis induced MI/R injury*

As shown in **Figure 7**, apoptotic cell numbers increased significantly in MI/R group compared

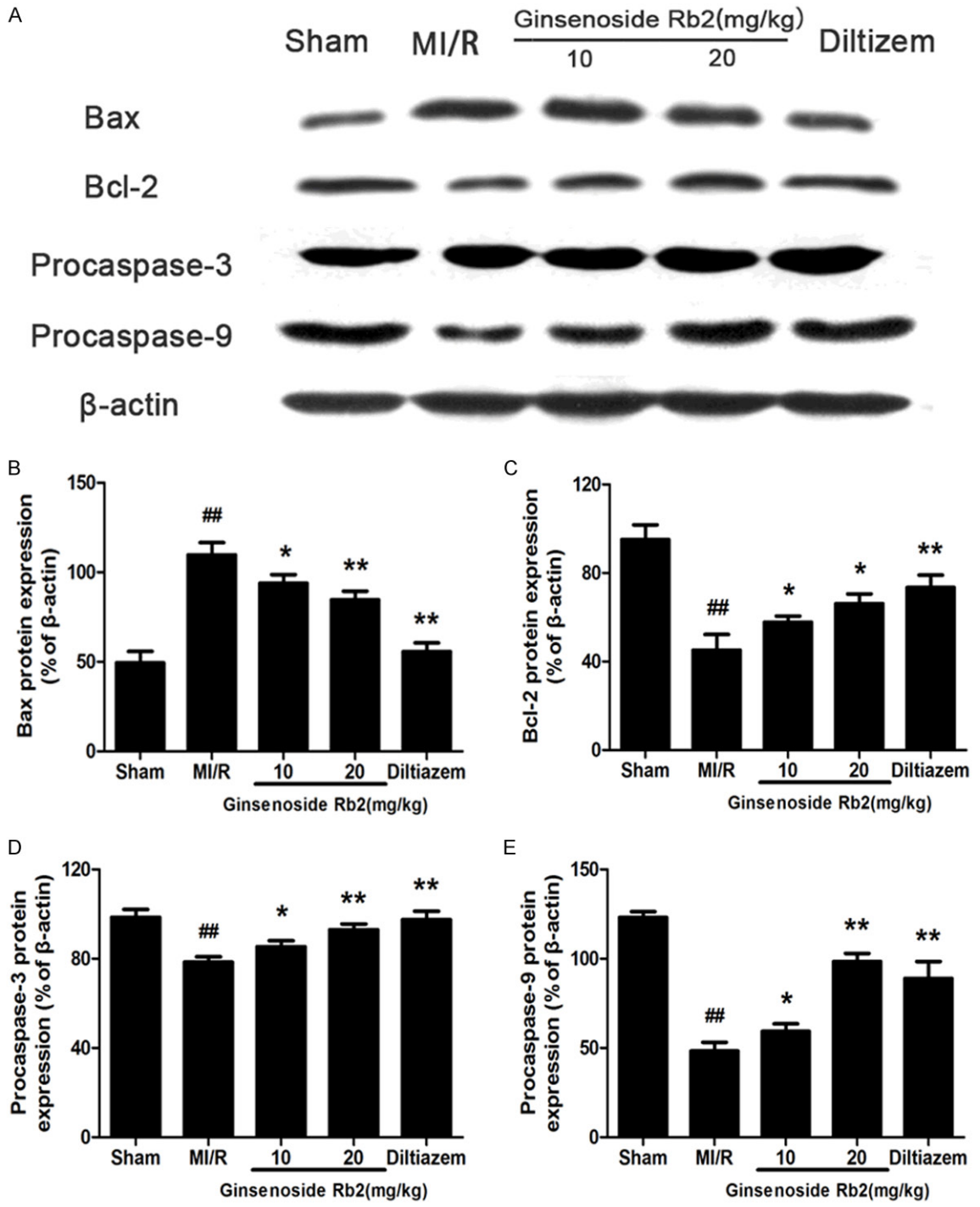


**Figure 8.** Effects of ginsenoside Rb2 on protein levels of apoptotic-related molecules in MI/R rats. A. Representative western blots. B. Quantitative analyses for Bax. C. Quantitative analyses for Bcl-2. D. Quantitative analyses for Procaspases-3. E. Quantitative analyses for Procaspases-9. Data are expressed mean ± S.D for each group. ##$P$ < 0.01 vs. Sham group; *$P$ < 0.05, **$P$ < 0.01 vs. MI/R group.

with sham group. Pretreatment with ginsenoside Rb2 (10 and 20 mg/kg) significantly decreased apoptotic cells compared with MI/R group ($P$ < 0.01).

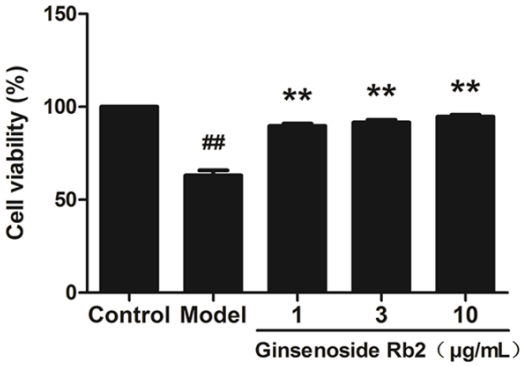


**Figure 9**. Effects of ginsenoside Rb2 on cell viability. H9c2 cells were pretreatment with different concentrations of ginsenoside Rb2 (1, 3, 10 μg/mL) for 4 h followed by treatment of 150 μM $H_2O_2$ for 6 h. Model group only treated with 150 μM $H_2O_2$ for 6 h. Cell viability was measured by MTT assay. Data are expressed mean ± S.D for each group. ##*P* < 0.01 vs. control group; ***P** < 0.01 vs. model group.

*Effects of ginsenoside Rb2 on expression of Bcl-2, Bax, and Procaspase-3, -9*

As shown in **Figure 8**, pretreatment with ginsenoside Rb2 restored protein levels of the anti-apoptotic molecule Bcl-2, Procaspase-3, -9 and reduced expression of the apoptotic molecule Bax compared with MI/R group (*P* < 0.01).

*Effect of ginsenoside Rb2 against $H_2O_2$ induced injury in H9c2 cells*

Treatment with $H_2O_2$ (150 μM, 6 h) significantly decreased the viability of H9c2 cells compared with the control group. Pretreatment with ginsenoside Rb2 (1, 3, 10 μg/mL) for 4 h increased cell viability compared with the model group, demonstrating a dose-dependent manner (*P* < 0.01, **Figure 9**). The results showed that ginsenoside Rb2 (1, 3, 10 μg/mL) protected H9c2 cells from oxidative damage.

In addition, the activity of SOD and GSH-PX in H9c2 cells were decreased while the activity of LDH in the culture supernatant and the MDA level in H9c2 cells were increased compared with the control group. Pretreatment with ginsenoside Rb2 significantly increased the activity of SOD and GSH-PX but decreased the level of LDH and MDA compared with the model group (**Figure 10**).

## Discussion

In the present study, we evaluated the cardioprotective effects of ginsenoside Rb2 on animal model of MI/R injury *in vivo* and against $H_2O_2$-induced stress in H9c2 cells *in vitro*. We found that pretreatment with ginsenoside Rb2 protected cardiomyocytes against oxidative stress induced by MI/R and $H_2O_2$ by decreasing the levels of cardiac injury makers, reducing infarct size, improving cardiac function, inhibiting cell apoptosis and increasing cell viability.

Infarct size is used for evaluation of left ventricular function. It is also an important parameter for evaluating the effectiveness of cardiovascular drugs in the treatment of ischemic heart disease [28]. We found that ginsenoside Rb2 pretreatment significantly reduced the infarct size in rats with MI/R-induced injury; this result is in line with previous reports [29, 30].

To evaluate the effect of ginsenoside Rb2 on cardial dysfunction, echocardiography parameters were incorporated into the present experiment design. As previously reported [31-33], we also found that MI/R impairs cardiac function in rats. More importantly, we found that ginsenoside Rb2 pretreatment significantly ameliorated myocardial dysfunction and remodeling, which was verified by increased LVEF, LVFS and decreased LVIDd, LVIDs.

Myocardial cell contains marker enzymes such as CK-MB, AST and LDH. Myocardial ischemia induces cell membrane to permeate or rupture, which results in the leakage of CK-MB, AST and LDH into blood. Hence, CK-MB, AST and LDH activities in serum reflect the alterations of membrane integrity and the degree of myocardial cell injury [34]. In the present study, the activities of CK-MB, AST and LDH were increased in MI/R rats. Pretreatment with ginsenoside Rb2 significantly lower those marker enzymes.

MI/R injury is characterized by oxidative stress and by changes in the antioxidant enzymes in the heart. There is increased production of oxygen free radicals during MI/R injury, which causes unsaturated fat to undergo lipid peroxidation, thereby aggravating myocardial damage [35]. MDA, CAT, SOD and GSH-PX levels are classical indices used to evaluate tissue per oxidative injury [36, 37]. MDA, which is a lipid peroxidation end product, has been used to assess oxygen free radical-mediated injury of MI/R [38]. CAT, SOD and GSH-PX, by inhibiting $O_2$ and $H_2O_2$ interaction, constitute the first line of cellular defense against oxidative injury [39]. In the present study, we demonstrated that

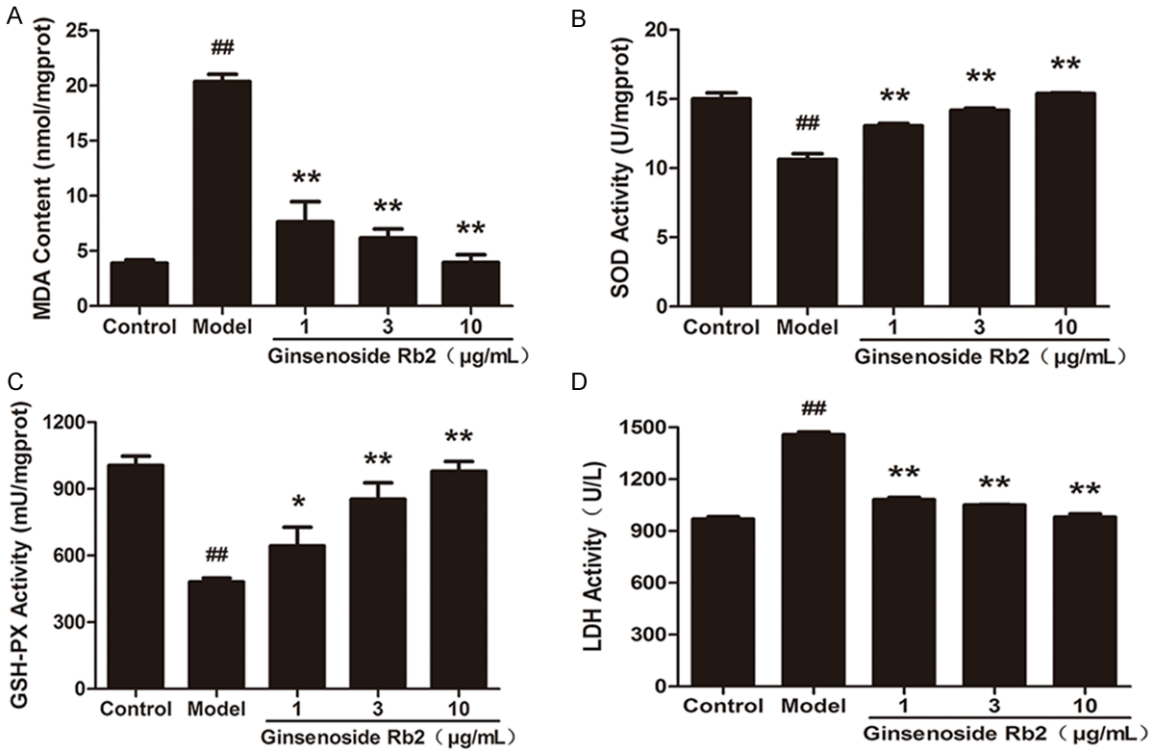


**Figure 10.** Effects of ginsenoside Rb2 on the content of MDA, the activities of SOD, GSH-PX in H9c2 cells and the level of LDH in the cultured supernatant. A. MDA, B. SOD, C. GSH-PX, D. LDH. H9c2 cells were pretreatment with different concentrations of ginsenoside Rb2 (1, 3, 10 μg/mL) for 4 h followed by treatment of 150 μM $H_2O_2$ for 6 h. Model group only treated with 150 μM $H_2O_2$ for 6 h. Data are expressed mean ± S.D for each group. ##$P$ < 0.01 vs. control group; *$P$ < 0.05, **$P$ < 0.01 vs. model group.

exposure to MI/R *in vivo* and $H_2O_2$ *in vitro* significantly increased the levels of oxidative stress products, decreased the concentrations of antioxidant defense enzymes and aggravated myocardial injury; these results are in line with previous reports [31]. In this study, pretreatment with ginsenoside Rb2 had enhanced activities of antioxidant defense enzymes (CAT, SOD and GSH-PX), but lower MDA production in comparison with the rats with MI/R induced injury. Our results suggested that ginsenoside Rb2 regulates the levels of endogenous antioxidant enzymes and oxidative stress products in rats with MI/R-induced injury and $H_2O_2$-treated H9c2 cells.

A number of studies have confirmed that cardiomyocyte apoptosis is one of the most common pathophysiological processes in injury induced by oxidative stress and MI/R [5]. Furthermore, the anti-apoptotic effects of ginsenoside Rb2 were confirmed by the results of TUNEL staining. These results strongly indicated that ginsenoside Rb2 inhibits cell apoptosis.

Apoptotic process regulatory proteins, including Bcl-2 and Bax, are located in the mitochondrial membrane. Imbalance of these regulatory proteins plays a key role on apoptosis [40]. The caspase proteases are also believed to play a critical role in mediating apoptosis. Two different caspase pathways (extrinsic and intrinsic pathways) are involved in mediating the response. Both extrinsic and intrinsic pathways lead to activating executioner Caspase (Caspase-3). Caspase-3 induces cell shrinkage, nuclear condensation and DNA fragmentation [41]. Overexpression of Bax and downregulation Bcl-2 expression might be related to the pathogenesis of apoptosis in old myocardial infarction [42]. Our data indicated that ginsenoside Rb2 could attenuate the apoptosis induced by MI/R injury through restored protein levels of Bcl-2, Procaspase-3, -9 and reduced expression of Bax. Therefore, the underlying

mechanism of protective effects of ginsenoside Rb2 was associated with its anti-apoptosis potency.

In summary, the prominent finding of this study was that ginsenoside Rb2 exerts significant cardioprotective effects against oxidative stress induced by MI/R and $H_2O_2$. The mechanisms involved may be attributed to the scavenging of oxidative stress products, increasing the concentration of antioxidant defense enzymes and inhibiting cardiomyocyte apoptosis.

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## Disclosure of conflict of interest

None.

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