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BIOMEDICAL SCIENCES

Anti-inflammatory and Anti-endoplasmic reticulum stress Effects of catalpol Against myocardial ischemia-reperfusion injury in streptozotocin-induced diabetic rats

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Abstract: The current study was designed to investigate the effects and the mechanism of catalpol on myocardial ischemia-reperfusion (MI/R) injury in a diabetic rat model. Male Sprague-Dawley rats were divided into DM + sham, DM +I/R, and DM +I/R + C groups and diabetes was induced using single injections of streptozotocin (STZ; 70 mg/kg; i.p). After confirming the induction of diabetes, rats were administered physiological saline and catalpol (10 mg/kg; i.p.) daily for 28 days. Subsequently, rats were subjected to left anterior descending (LAD) coronary artery occlusion for 30 min followed by reperfusion for 2 h. Haemodynamic parameters were recorded throughout surgery, and following sacrifice, hearts were isolated for biochemical, histopathological, and molecular analyses. Catalpol treatment significantly ameliorated MI/R injury by improving cardiac function, normalizing myocardial enzyme activities and markers of oxidative stress, and by maintaining myocardial architecture. Furthermore, expression levels of the inflammatory cytokines TNF-α and IL-6 were decreased in biochemical and immunohistochemical studies. Additionally, the cardioprotective effects of catalpol were partly related to reductions in myocardial endoplasmic reticulum stress (ERS). In conclusion, catalpol exerts cardioprotective effects in diabetic rats by attenuating inflammation and inhibiting ERS.

Key words: catalpol, ischemia-reperfusion, inflammation, endoplasmic reticulum stress.

INTRODUCTION

Diabetes mellitus (DM) is known to increase the risk of cardiovascular complications and seriously affects human health. Accordingly, the incidence of cardiovascular disease was shown to be greater in diabetics than in the non diabetic population (Brown et al. 2006), and cardiovascular disease (CVD) reportedly caused 68% of deaths among a cohort of diabetic patients (Leon & Maddox 2015). Hence, identification of new approaches for protecting against diabetic heart diseases and alleviating MI/R insults is a major priority. Accumulating evidence indicates that inflammation and endoplasmic reticulum stress (ERS) play important roles in the MI/R related injury (Gao et al. 2017, Zhang et al. 2017). Therefore, strategies that inhibit inflammation and attenuate ERS may have therapeutic potential.

Rehmanniae glutinosa L. is widely used traditional Chinese medicine, and catalpol is the the main bioactive component in the roots of *Rehmannia glutinosa* (Zhang et al. 2008). A growing body of evidence has shown that catalpol has multiple biological effects, and its anti-inflammatory, anti-diabetic and anti-ERS activities are increasingly considered as central to the protective effects of catalpol

(Gaston & Limbach 2014, Xiong et al. 2017). Our previous study has proved that catalpol has cardioprotective effects in rats (Bi et al. 2018). However, in diabetic models, the effects of catalpol following MI/R injury has still not been determined and the mechanism of catalpol's cardioprotection on MI/R has not been clearly elucidated.

Based on these studies, we investigated the protective effects of catalpol following MI/R injury in diabetic rats and determined whether these benefits were associated with the inhibition of inflammation and attenuation of ERS.

MATERIALS AND METHODS

Drug preparation

Catalpol (purity >98%, molecular formula: C15H22O10, molecular weight: 362.33) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Catalpol was dissolved in physiological saline for treatment and its concentration was 2mg/ml.

Chemicals and reagents

Lactate dehydrogenase (AST) and creatine kinase-MB (CK-MB), cardiac troponin I (cTnI), catalase (CAT), and glutathione (GSH) test kits were obtained from Nanjing Jiancheng Bioengineering Institute. Thiobarbituric acid-reactive substance (TBARS) assay kit was provided by BioAssay Systems (CA, USA). Primary antibody against C/EBP homologous protein (CHOP) was obtained from Abcam PLC (Cambridge, UK). Primary antibodies against interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), glucose regulated protein 78 (GRP78), PKR-like ER kinase (PERK), P-PERK, eukaryotic initiation factor-2α (eIF2α), P-eIF2a, Caspase-12 and nicotinamide adenine dinucleotide phosphate (NAPDH) were procured from Santa Cruz Biotechnology (CA, USA).

Animals

Adult male Sprague Dawley (200±10 g) rats were purchased from the Animal Lab Center of China Medical University, Shenyang, China. All animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, revised 1985). The study procedures were approved by the ethics committee of the China Medical University (Shenyang, China).

Induction of diabetes

After 1 week of adaptation to the laboratory environment, male Sprague Dawley rats were intraperitoneally (i.p.) injected with streptozotocin (STZ) at a dose of 70 mg/kg (Suchal et al. 2017) and diabetes was induced after an overnight fast. Seventy-two h later, blood sugar levels were measured using a glucose monitoring system and rats with blood glucose concentrations of ≥ 16.7 mmol/L were considered diabetic and were used in following experiments.

Induction of MI/R injury model

After administration of final doses of catalpol or physiological saline, myocardial I/R procedures were performed as described previously (Zheng et al. 2017). Briefly, rats were anesthetized with i.p. injections of pentobarbital (50 mg/kg) in the supine position, and surface leads were placed subcutaneously for electrocardiogram (ECG) recordings. Left lateral thoracotomy was then performed to expose the heart and identify coronary artery branches. Arteries were then occluded by snaring with a small tube, through which a ligature was passed. Successful myocardial ischemia was confirmed by changes in ECG measurements and elevated ST segments.

After 30 min of ischemia, ligatures were released and myocardial tissues were reperfused for 2 h. Rats in the sham group received the same surgical procedures without ligation.

Preliminary dose experiments

To select an appropriate dose of catalpol that protects against cardiac I/R injury, 32 diabetic rats were randomly divided into four groups to receive i.p. injections of saline- (Group 1) or 5-, 10-, or 20-mg/kg catalpol (Groups 2, 3, and 4, respectively) (Cai et al. 2014, Huang et al. 2013). After 4 weeks of pretreatments with saline or catalpol followed by induction of myocardial I/R injury, rats that received 10-mg/kg catalpol had optimal serum CK-MB and cTnI levels and myocardial histological changes.

Experimental design and protocol

Following identification of the optimal dose of catalpol, thirty diabetic rats were randomly distributed into the following three groups:

Group 1, Diabetes + sham (n = 10); diabetic rats received i.p. injections with equal volumes of physiological saline (i.p) for 28 days. On the 28th day, a thread was passed beneath the left anterior descending (LAD) coronary artery but was not occluded.

Group 2, Diabetes +I/R (n = 10); diabetic rats received i.p. injections of equal volumes of physiological saline (i.p) for 28 days. On the 28th day, LAD coronary arteries were ligated for 30 min and reperfusion was performed for 2 h.

Group 3, Diabetes +I/R + C (n = 10); diabetic rats received i.p. injections of catalpol at 10 mg/kg/day for 28 days. On the 28th day, LAD coronary arteries were ligated for 30 min and reperfusion was performed for 2 h.

Determinations of cardiac function

During the myocardial I/R period, mean arterial blood pressures (MAP) and heart rates (HR) were continuously monitored and recorded using a computerized non-invasive tail-cuff system (Visitech BP-2000 Blood Pressure Analysis System™). Simultaneously, a catheter filled with heparin saline (500 U/mL) was inserted into the left ventricle via the right common carotid artery, and a BL-420E monitor system was used to measure left ventricular end-systolic pressures (LVESP), left ventricular end-diastolic pressures (LVEDP), and rates of maximum positive and negative left ventricular pressure development (± LVdp/dtmax).

Body weights, water intake, and food consumption were monitored and recorded weekly throughout the experimental period. Plasma samples were collected weekly from tail veins after fasting overnight, and were separated using a centrifuge. Plasma glucose levels were then measured using an Accu-check Advantage glucometer and plasma insulin levels were determined using commercial diagnostic kits. After measurements of hemodynamic parameters, rats were sacrificed and blood samples and hearts were collected and stored in liquid nitrogen for biochemical and western blotting analyses, and separate heart specimens were fixed in 10% buffer formalin and embedded in paraffin for histopathology and immunohistochemistry analyses.

Analyses of biochemistry and inflammatory factors

Blood samples were collected from abdominal aortas and were centrifuged at 3000 rpm for 10 min at 4° C. AST, CK-MB, and cTnI levels were then determined in the resulting serum samples using respective kits according to the manufacturer's instructions. IL-6 and TNF-α levels in serum and myocardial tissues were measured using ELISA kits.

Measurements of myocardial lipid peroxidation and antioxidant levels

Heart tissues were taken from liquid nitrogen storage, and 20% (w/v) homogenates were prepared in 0.1-M phosphate buffer (pH 7.4). Homogenates were centrifuged at 5000 rpm for 10 min at 4°C and supernatants were collected. Lipid peroxidation levels were estimated according to thiobarbituric acid reactive substances (TBARS) using a reagent kit (BioAssay Systems, CA, USA), and activities of the antioxidant enzymes catalase (CAT) and glutathione (GSH) were determined using respective kits.

Histopathology

To assess myocardial histopathological changes, parts of paraffin sections were stained with hematoxylin and eosin (H&E) and were examined using a light microscope (Olympus, Tokyo, Japan). Photomicrographs were taken using a digital camera at a magnification of 400 ×. The damage quantification from ten areas corresponding to the myocardial tissue was graded using a five-score system: apoptosis, tubular cell necrosis, hemorrhage, cytoplasmic vacuole formation, and tubular dilatation based on a five-score system (5, histopathological changes=75-100%; 4, =50-75%; 3, =25-50%; 2, = 10-25%; and 1, <10%). The mean score for each parameter was calculated and subjected to statistical analysis (Hu et al. 2018).

Immunohistochemistry

Heart tissue sections of 5 μm in thickness were embedded in paraffin and were placed on slides for immunohistochemical analyses. Slides were then deparaffinized and were passed through a graded series of ethanol solutions and were then rehydrated. Antigen retrieval was performed on slides by heating in citrate buffer (10 mM; pH 6.0) for 10 min in a microwave oven. Subsequently, endogenous peroxidase activity was quenched using 3% hydrogen peroxide in methanol for 30 min. After rinsing with PBS, sections were incubated with primary antibodies against TNF-α (1:200, Santa Cruz) and IL-6 (1:200, Santa Cruz) overnight at 4°C. Bound primary antibodies were then detected by incubating sections with appropriate secondary antibodies for 30 min at room temperature. Colorimetric reactions were then initiated with the addition of DAB, and images were captured using a light microscope at a magnification of 400 ×. TNF-α and IL-6 expression levels were quantified using Image-ProPlus Systems.

WESTERN BLOTTING

Heart samples were homogenized in RIPA buffer containing 50-mM Tris (pH 7.4), 1% Triton C-100, and 150-mM NaCl, and freshly added phenylmethylsulfonyl fluoride (PMSF; 0.1%) protease inhibitor (Sigma-Aldrich). Final supernatants were obtained by centrifugation at 12,000 × g for 10 min at 4°C and protein concentrations were determined using a standard BCA assay kit. Proteins (40 μg) were then electrophoresed on 10%–12% sodium dodecyl sulfate polyacrylamide gels and were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Membranes were then blocked in Tris-buffered saline containing Tween-20 (TBST; 20-mM Tris, 137-mM NaCl, and 0.1% Tween-20) and 5% non-fat milk at room temperature for 1 h. PVDF membranes were then incubated with primary antibodies against GRP78 (1:500, Santa Cruz), PERK (1:1000, Santa Cruz), P-PERK (1:1000, Santa Cruz), eIF2a (1:1000, Santa Cruz), P-eIF2a (1:1000, Santa Cruz), CHOP (1:500; Abcam), Caspase-12 (1:500, Santa Cruz) and NAPDH (1:4000, Santa Cruz) overnight at 4°C. After washing in TBST, membranes were

subjected to appropriate secondary antibodies for 1 h at 37° C, and bands from heart samples were visualized using enhanced chemiluminescence (ECL) detection kits and were quantified using image J software.

Statistical analysis

Data analyses were performed using SPSS 13.0. Data are presented as means ± standard deviations (SD) and differences among groups were identified using one-way analysis of variance (ANOVA) whereas differences between groups were identified using Bonferroni post hoc tests. Differences with P values of < 0.05 were considered significant.

RESULTS

Mortality rates

During the course of this study, 2 of 30 rats (6.67%) died, including one rat from the DM +I/R group and one from the DM +I/R + C group. The cause of death in all cases was bleeding during improper carotid artery cannulation or ligation of LAD coronary arteries.

General observations

As shown in Figure 1, diabetic rats in DM + sham and DM+I/R groups presented significantly increased water intake, food consumption, and decreased body weight, compared with those of the DM +I/R + C group, especially in the third and fourth weeks (P < 0.01).

Effects of catalpol on plasma glucose and insulin levels

Rats of DM + sham and DM +I/R groups had significantly higher blood glucose concentrations and lower insulin levels than rats in the DM +I/R + C group (P < 0.01). Pretreatments of diabetic rats with catalpol significantly reversed hyperglycemia (P < 0.01) and increased insulin levels (P < 0.01; Figure 2).

Effects of catalpol on hemodynamic parameters and cardiac function

To examine the effects of catalpol on cardiac function during I/R-induced injury in diabetic rats, the hemodynamic parameters MAP and HR and left ventricular function indicators ±LVdP/dt, LVSP, and LVEDP were assessed (Figure 3 and Figure 4). Compared with rats of the DM + sham group, I/R injury led to hemodynamic impairments, with significant reductions in HR and MAP at all time points (P < 0.01). I/R injury also resulted in declines in ventricular contraction (+LVdP/dtmax and LVSP) and relaxation (-LVdP/dtmax) following increases in pre-load (LVEDP) throughout the I/R period, in comparison with rats of the DM + sham group (P < 0.01). However, four-week pretreatments with catalpol

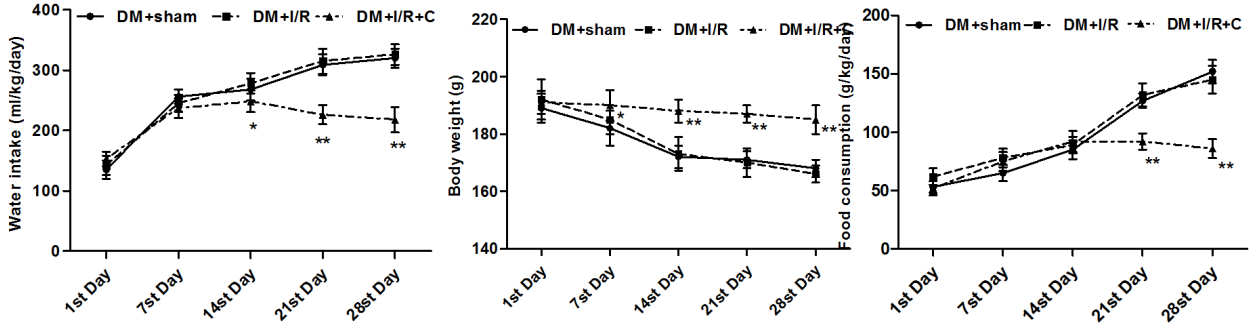

Figure 1. Efects of catalpol on water intake, body weight, and food consumption in diabetic rats. *P < 0.05 versus the DM+I/R group; **P < 0.01 versus the DM+I/R group.

significantly alleviated the detrimental effects of I/R injury, as indicated by improvements in hemodynamic parameters and preservation of ventricular functions compared with those in rats of the DM +I/R group.

Effects of catalpol on myocardial enzyme activities, oxidative stress, and inflammatory cytokine expression

AST, CK-MB, and cTnI activities in heart tissues (Table I) were significantly greater in rats of the DM +I/R group than in rats of the DM + sham group ($P < 0.01$). However, catalpol pretreatments significantly increased enzyme activities in comparison with those in DM +I/R rats ($P < 0.01$).

As shown in Figure 5, MI/R injury led to significant lipid peroxidation in the DM + sham group, with higher levels of the lipid peroxidation end product TBARS than in rats of the DM + sham group ($P < 0.01$). Catalpol pre-treated I/R animals showed significant reductions in TBARS levels when compared with DM + I/R rats ($P < 0.01$), and determinations of GSH and CAT activities showed resorative effects of catalpol treatment.

Because inflammatory responses play critical roles in I/R injury, we assessed changes in TNF-α and IL-6 levels in serum and myocardial samples (Figure 6). Compared with levels in the DM + sham group, significant increases TNF-α expression levels were observed in both serum and myocardium samples form rats of the DM +I/R group ($P < 0.01$ and $P < 0.01$, respectively). I/R-mediated increases in TNF-α levels in these samples were markedly decreased by catalpol treatments ($P < 0.01$ and $P < 0.01$, respectively). Similarly, catalpol pretreatment remarkably reduced IL-6 expression in myocardium and serum samples ($P < 0.01$ and $P < 0.01$, respectively). Finally, immunohistochemical analyses showed similar changes in protein expression levels of TNF-α and IL-6 in heart tissues (Figure 7).

Histopathological evaluation

As shown in Figure 8, orderly myofibrillar structures with striations, clear nuclear staining, and slight inflammatory responses were observed in DM + sham rats. In contrast, MI/R injury led to marked myocardial necrosis, edema, and infiltration of inflammatory cells. Cardiac tissue sections from rats that received catalpol pretreatments showed relatively limited necrosis, edema, and inflammation compared with those of the DM +I/R group, as indicated by the mean injury score.

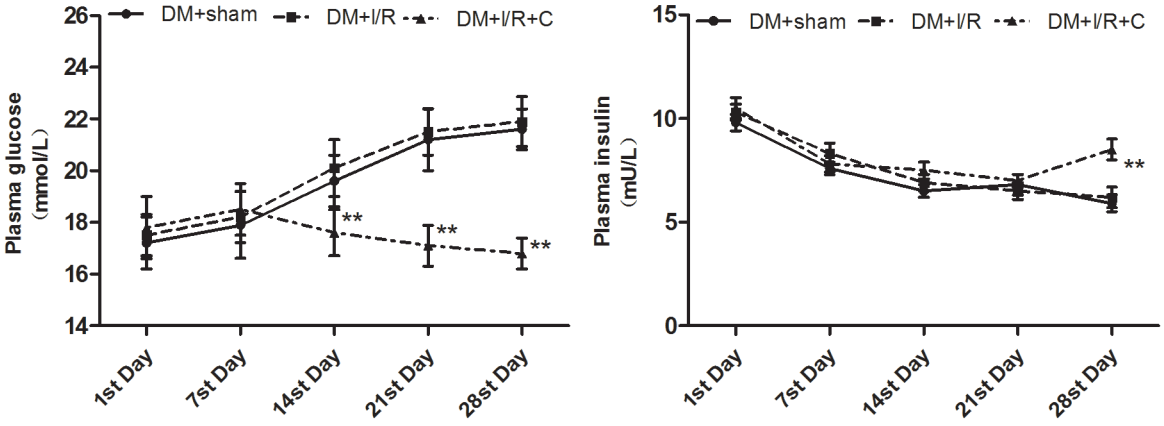

Figure 2. Effects of catalpol on plasma glucose and insulin levels. *P < 0.05 versus the DM+I/R group; **P < 0.01 versus the DM+I/R group.

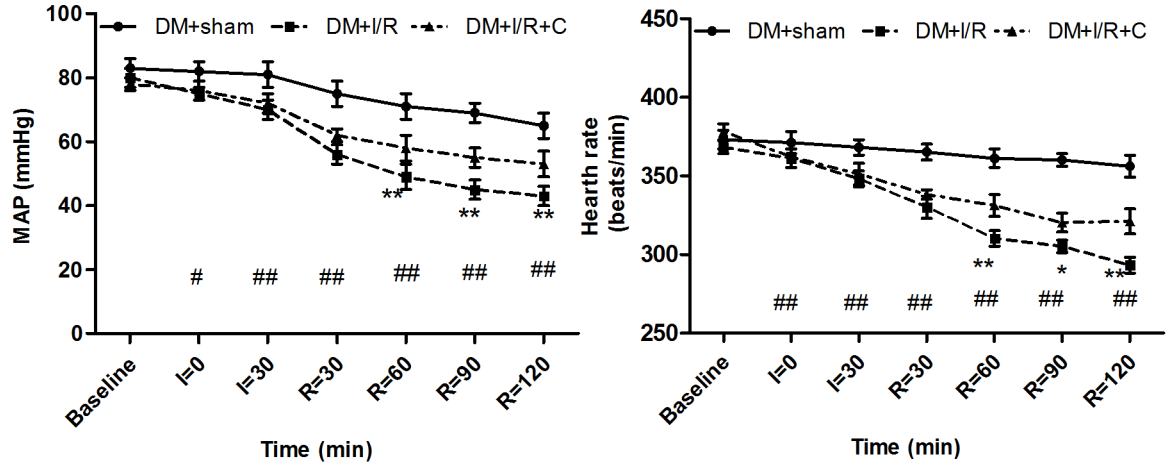

Figure 3. Effects of catalpol on hemodynamic parameters. #P < 0.05 versus the DM+sham group. ##P < 0.01 versus the DM+sham group; *P < 0.05 versus the DM+I/R group; **P < 0.01 versus the DM+I/R group.

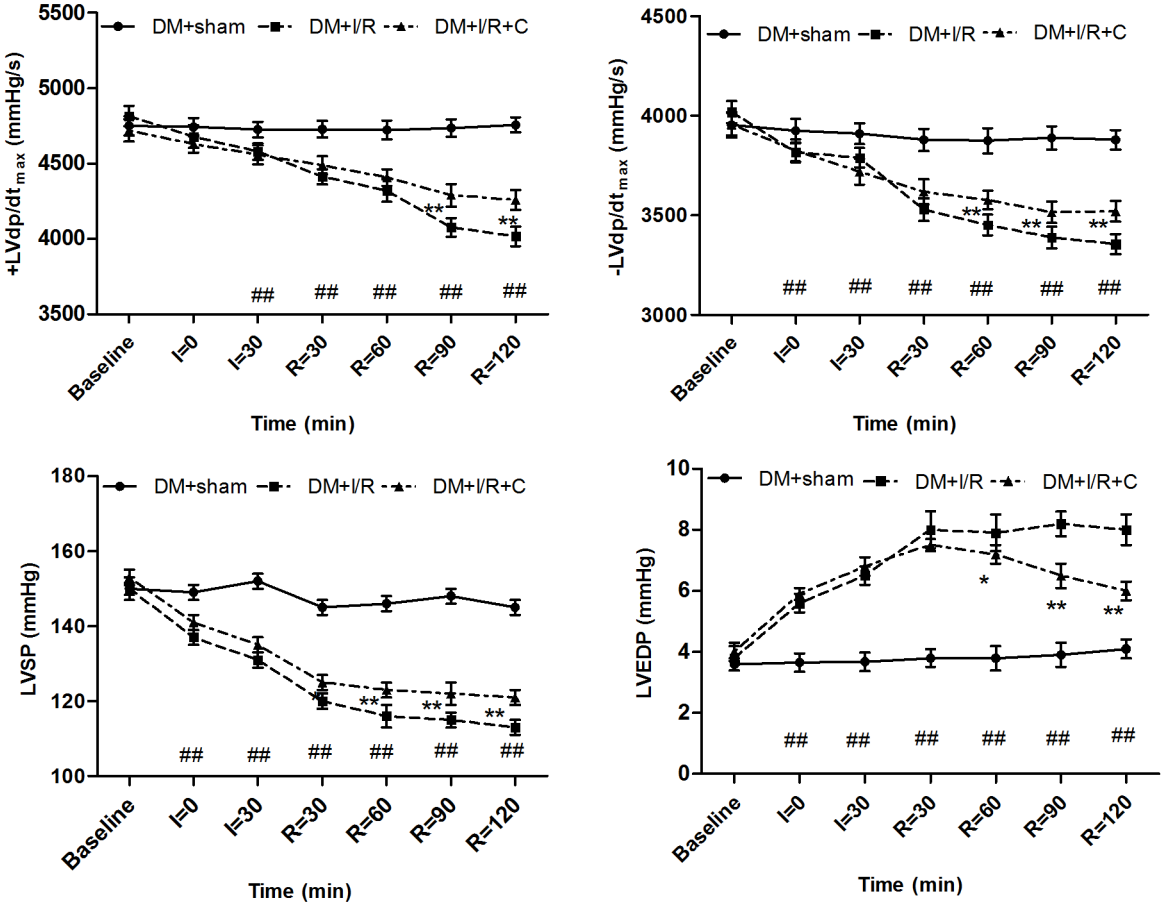

Figure 4. Effects of catalpol on cardiac function. ##P < 0.01 versus the DM+sham group; *P < 0.05 versus the DM+I/R group; **P < 0.01 versus the DM+I/R group.

Table I. Assay of cardiac marker enzymes.

Group	AST(U/L)	CK-MB(U/L)	cTnI(ng/mL)
DM+sham	161.90±18.55	205.70±15.49	1.37±0.17
DM+I/R	379.25±28.89$^{\#\#}$	457.13±20.28$^{\#\#}$	3.49±0.26$^{\#\#}$
DM+I/R+C	207.25±18.10$^{\#\#**}$	319.54±22.10$^{\#\#**}$	2.36±0.92$^{\#\#**}$

Data are presented as means ± standard deviations (SD). $^{\#\#}$P < 0.01 versus the DM+sham group; **P < 0.01 versus the DM+I/R group.

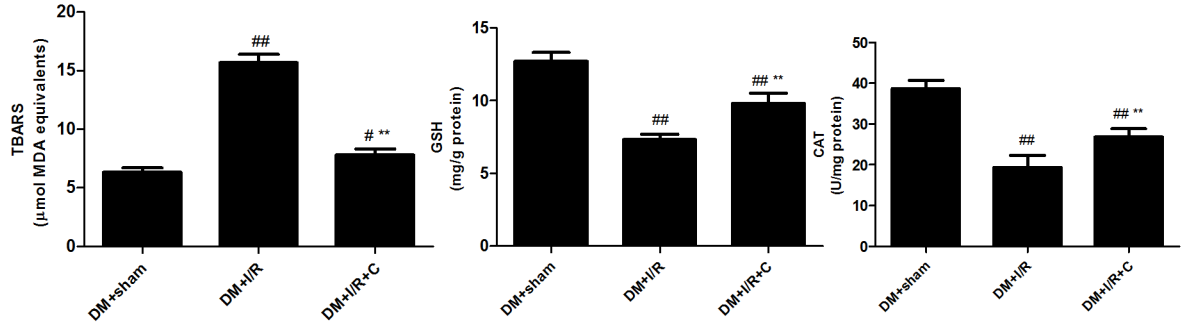

Figure 5. Effects of catalpol on myocardial oxidative stress. $^{\#}$P < 0.05 versus the DM+sham group. $^{\#\#}$P < 0.01 versus the DM+sham group; **P < 0.01 versus the DM+I/R group.

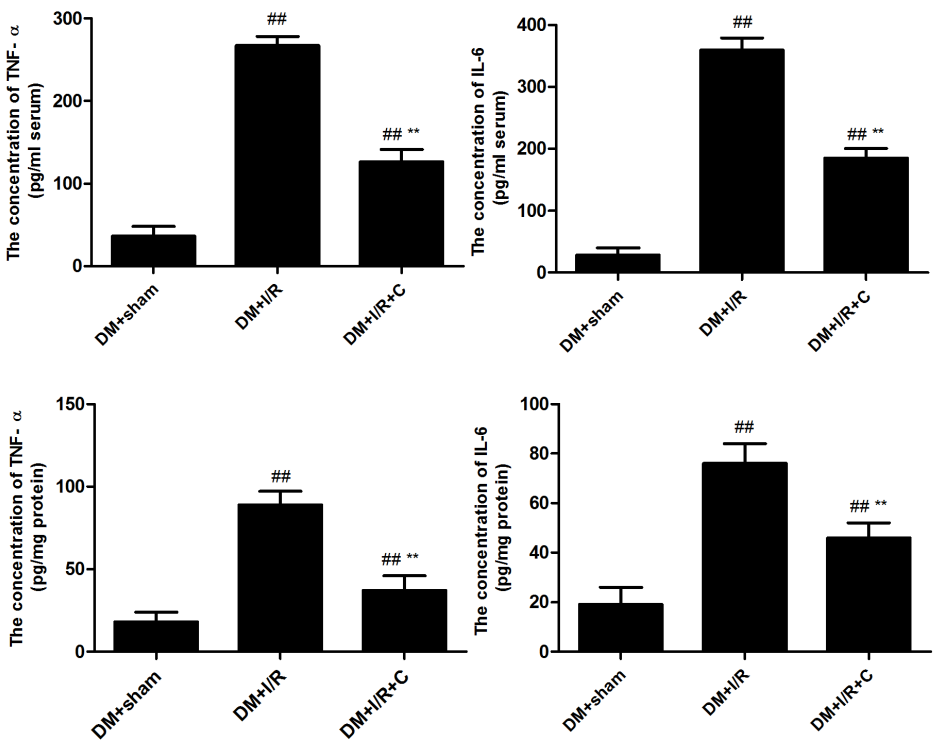

Figure 6. Effects of catalpol on serum and myocardial levels of inflammatory cytokines (TNF-α and IL-6). $^{\#\#}$P < 0.01 versus the DM+sham group; **P < 0.01 versus the DM+I/R group.

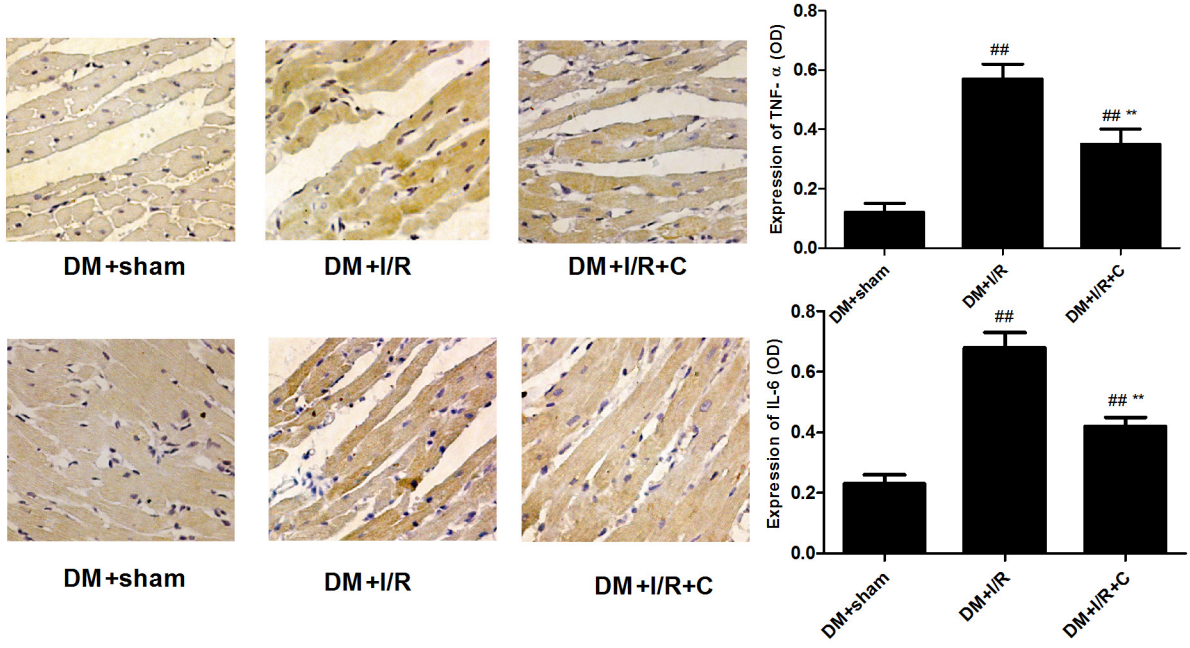

Figure 7. Effects of catalpol on protein expression of inflammatory cytokines (TNF-α and IL-6) (400 ×). $^{\#\#}P < 0.01$ versus the DM+sham group; $^{**}P < 0.01$ versus the DM+I/R group.

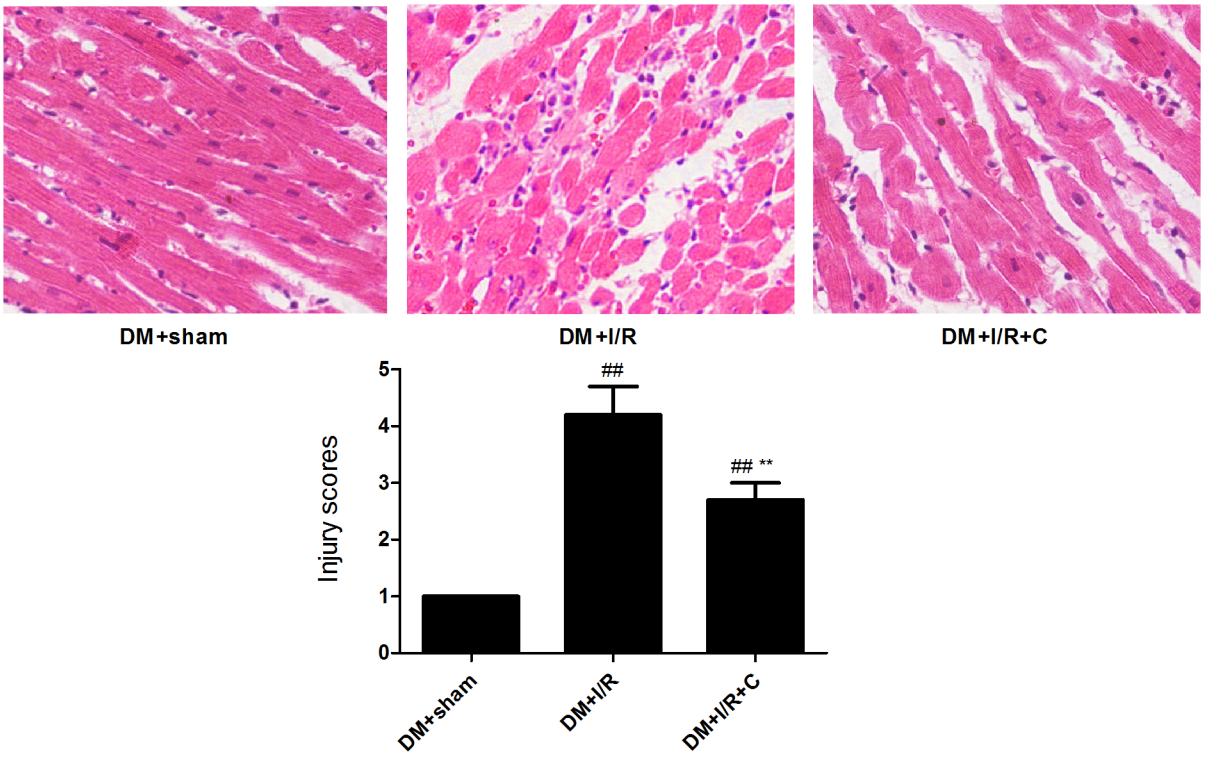

Figure 8. Effects of catalpol on myocardial histopathology in diabetic rats (400 ×).

Effects of catalpol on ERS

Numerous studies have shown that excessive ERS aggravates MI/R injury and ultimately causes apoptosis in cardiomyocytes. To examine the effects of catalpol on ERS following MI/R injury in diabetic rats, we determined relative expression levels of these proteins (Figure 9) and showed that MI/R injury significantly increases the expression of ERS related proteins, such as Grp78, P-PERK, P-eIF-α, CHOP, and caspase-12 ($P < 0.01$, $P < 0.01$, $P < 0.01$, $P < 0.01$, and $P < 0.01$), indicating increased ERS. These changes were significantly suppressed by catalpol. Compared with the DM +I/R group, catalpol pretreatment significantly decreased Grp78, P-PERK, P-eIF-α, CHOP, and caspase-12 ($P < 0.01$, $P < 0.05$, $P < 0.01$, $P < 0.05$, and $P < 0.01$) protein expression, suggesting that catalpol inhibits I/R-initiated ERS in diabetic rats.

DISCUSSION

Diabetes is an independent risk factor for cardiovascular disease, and severely affects human health. Numerous laboratory and epidemiological studies show that diabetic hearts are more vulnerable to ischemic injury and have decreased protective capacities (Di Filippo et al. 2005, Marfella et al. 2002, Miki et al. 2012, Ravingerova et al. 2003). Despite improvements in treatments, ischemic heart disease, especially in cases of DM, remains among the most serious health problems in many countries. Accumulating evidence strongly demonstrates that traditional Chinese medicine has great advantages in the treatment of many cardiovascular diseases. Thus, it seems reasonable to seek novel natural drugs to protect diabetic hearts.

The present investigation reveals that catalpol treatment protects heart tissues against acute ischemia-reperfusion injury in diabetic rats. This is evident from the following supporting data: 1. improvements in cardiac function; 2. reduced leakage of myocardial injury marker enzymes; 3. restoration of antioxidant status; and 4. reversal of histopathological changes. Furthermore, we explored the effect of catalpol treatment on inflammation and endoplasmic reticulum stress.

As one of the most commonly used traditional Chinese medicines, *R. glutinosa* L. possesses a wide range of biological and pharmacological activities, such as protective effects against diabetic disorders and heart disease, and neuroprotective effects. Catalpol is the main chemical component of root isolates from *R. glutinosa* L. plants, and possesses diverse biological activities including anti-tumor (Liu et al. 2017), anti-hyperglycemia (Liu et al. 2016, Shieh et al. 2011), antioxidant (Cai et al. 2016, Hu et al. 2016), anti-inflammatory (Zhang et al. 2013, Zhu et al. 2017), anti-ERS (Xiong et al. 2017), and antiapoptosis properties (Hu et al. 2016).

It is widely accepted that MI/R injury leads to oxidative stress, as reflected by disruptions of the balance between oxidants and antioxidants and increases in lipid peroxidation. Because lipids are the main constituents of biological membranes, peroxidation reactions can lead to cell damage and death. In the present study, increased lipid peroxidation products were indicated by elevated TBARS, and were likely responsible for the observed membrane damage, which leads to the release of cardiac enzymes such as AST, CK-MB, and cTnI from intracellular compartments into the blood (Li et al. 2012, Zhang et al. 2017). In agreement, activities of the antioxidant enzymes GSH and CAT, which are endogenous free radical scavengers, were increased by catalpol pretreatments, and thus by bolstering endogenous antioxidant defense systems, catalpol prevented the release of AST,

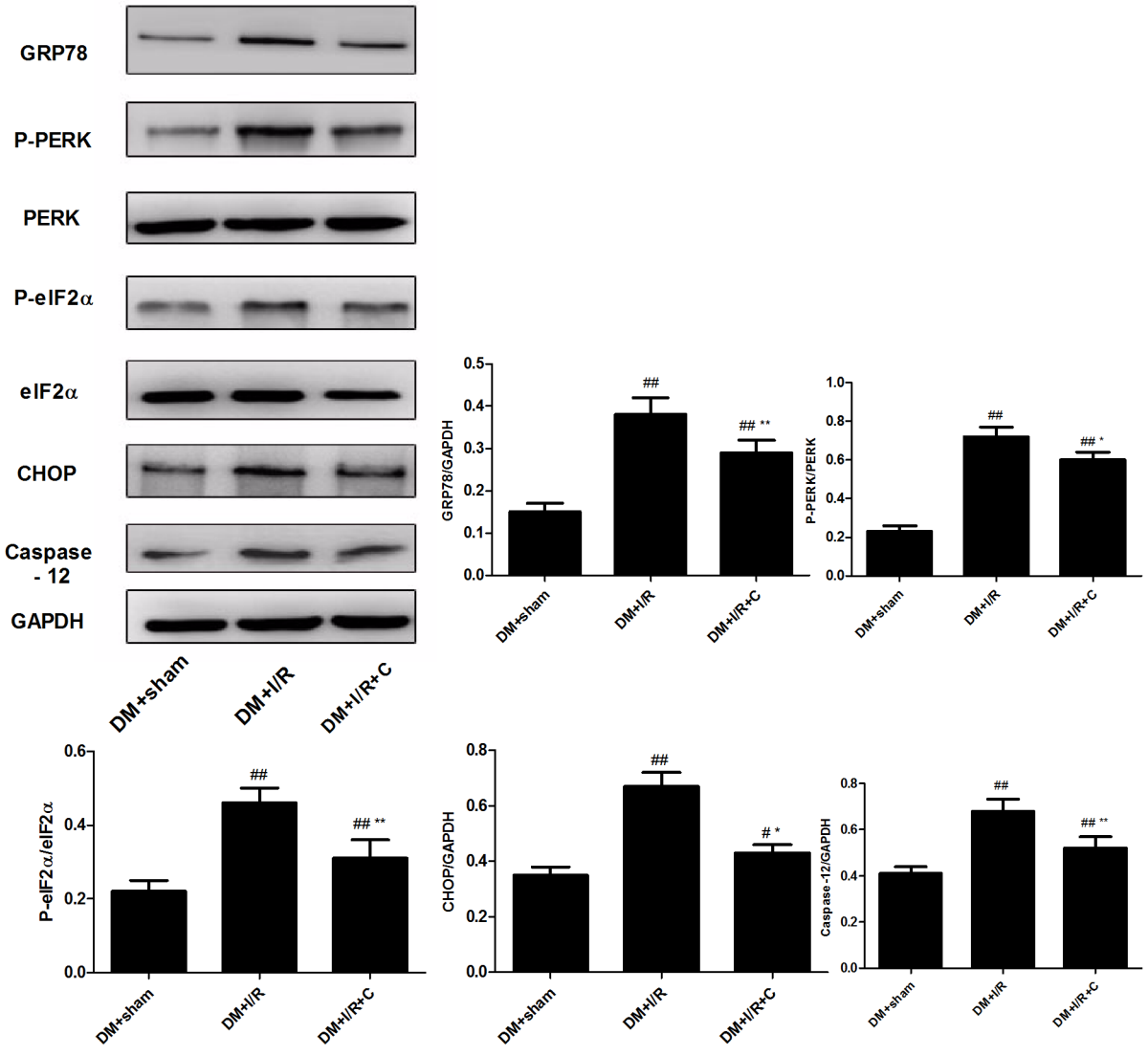

Figure 9. Effects of catalpol on expression of ERS-associated protein in diabetic myocardial tissues. $^{\#}P < 0.05$ versus the DM+sham group. $^{\#\#}P < 0.01$ versus the DM+sham group; $^{*}P < 0.05$ versus the DM+I/R group; $^{**}P < 0.01$ versus the DM+I/R group.

CK-MB, and cTnI into extracellular fluids, and preserved membrane integrity. These data show that catalpol modulates the production of lipid peroxides and enhances antioxidant defense systems in myocardial tissues, thereby providing protection from MI/R injury. In a recent supporting study, MI/R injury significantly influenced heart function, as indicated by decreases in MAP and inotropic and lusitropic states (±LVdP/dtmax), and increased ventricular remodeling (LVEDP) (Agrawal et al. 2014). We also showed that catalpol treatment prevents these changes, and our histopathological results showed that the marked increases in myocardial-fiber necrosis and inflammatory cell infiltration in the DM +I/R group were significantly ameliorated by catalpol, leading to protection of cardiac tissue structure

integrity. These results further confirmed that catalpol is cardioprotective in diabetic rats.

Increasing evidence shows that inflammation plays important roles in many cardiovascular diseases, especially in the pathogenesis of MI/R injury (Li et al. 2011, Timmers et al. 2012). During MI/R injury, myocardial cells secrete various inflammatory cytokines, such as TNF-a and IL-6, and these likely contribute to neutrophil infiltration into the myocardium. In the present study, we found that diabetic-IR rats have increased levels of inflammatory markers such as TNF-α and IL-6. These molecular changes were further supported by inflammatory and histopathological tissue changes that were ameliorated by treatments with catalpol following diabetic myocardial I/R injury.

The endoplasmic reticulum (ER) is essential for multiple cellular functions, including protein synthesis and transportation, biosynthesis of lipids, and calcium homeostasis (Hotamisligil 2010). Hence, exposures to pathological or physiological stimuli that disturb ER homeostasis cause ERS. Initially, ER stress is an adaptive mechanism that contributes to the recovery of normal function. However, severe or sustained ER stress initiates the unfolded protein response (UPR), which then induces oxidative stress, inflammation, and apoptosis. Previous studies show that during ERS-initiated apoptosis, CHOP is a key pro-apoptotic transcription factor that acts downstream of the PERK-eIF2α pathway (Marwarha et al. 2012, Song et al. 2015). Accordingly, PERK activation induces the expression of CHOP and ultimately promotes apoptosis. Caspase-12 is a member of the interleukin-1b converting enzyme (ICE) subfamily of caspases, and has activities that are specifically related to ERS–associated apoptosis following release from the ER upon initiation of ERS (Bravo et al. 2012, Qi et al. 2007). Accumulating evidence reveals that ERS is closely involved in the pathogenesis of various cardiovascular diseases, such as heart failure, and MI/R injury (Guo et al. 2017, Misaka et al. 2018). Hence, inhibition of ERS may be an effective therapeutic strategy for MI/R injury. To confirm the effect of catalpol on ERS during MI/R in diabetic rats, we investigated the expression of proteins with established roles in ERS. In these experiments, catalpol suppressed ERS markers, such as GRP78, PERK, eIF2a, CHOP, and caspase-12, after MI/R injury. Thus, the cardioprotective effects of catalpol reflect reduced activation of ERS.

In conclusion, our results suggested catalpol exerts cardioprotective effects in diabetic rats. The protective effect is associated with attenuation inflammation and inhibition ERS. However, a more comprehensive understanding of the cardioprotective mechanisms of catalpol will be achieved in further intensive *in vivo* and *in vitro* investigations.

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Fangjie Bi performed the biological assays; Guangxin Chen and Pan Wang performed the western blotting; Yujia Xu analyzed results and performed major manuscript corrections. All authors had corrected and approved the final manuscript version.