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Original Article

Cistanche deserticola ethanol extract attenuates left ventricular remodeling and dysfunction by reducing the inflammatory response after myocardial infarction

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Abstract: Objective: This study aimed to investigate whether *Cistanche deserticol*a extract (CDE) exerted an inhibitory effect on the cardiac inflammatory response and prevented adverse remodeling after myocardial infarction (MI) in rats. Methods: Wistar rats were randomly divided into seven groups: normal, sham, MI (LAD coronary artery ligation), 3 CDE groups (high dosage, middle dosage, and low dosage (LAD ligation and treated with CDE)), and a captopril group (LAD ligation and treated with captopril as the positive control drug). After six weeks, the effect of CDE on left ventricular (LV) remodeling was assessed by examining cardiac function and histology. Indicators of fibrosis (Masson and matrix metalloproteinases (MMPs)) and inflammation-related factors were evaluated. Results: CDE treatment significantly reduced the heart weight to body weight ratio and LV dilation and improved ejection fraction and fractional shortening in rat hearts. It also decreased myocardial hypertrophy and interstitial fibrosis in the non-infarcted myocardium and significantly decreased inflammatory cytokines (TNF-α and IL-1β) and inhibited the expression of MMP-9. However, CDE treatment produced no effect on MMP-2 and markedly diminished TLR-4 and NF-κB p65 expression in the non-infarcted area. Conclusion: CDE has the potential to improve cardiac remodeling and dysfunction following MI by modulation of myocardial inflammation, which may be attributed to mitigation of the TLR-4/NF-κB signaling pathway. CDE may be considered a potential therapeutic drug for the treatment of cardiac diseases.

Keywords: *Cistanche deserticola*, myocardial infarction, ventricular remodeling, inflammation, TLR-4/NF-κB pathway

Introduction

Acute myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide. Advances in therapy have significantly reduced early mortality during the acute phase, but the incidence of chronic heart failure (HF) resulting from ventricular remodeling (VR) is reaching epidemic proportions [1, 2]. VR after acute MI is a complicated pathological process that includes thinning of the ventricular wall, progressive expansion of the initial infarct area, dilation of the left ventricular (LV) lumen, myocardial hypertrophy, and cardiomyocyte replacement by fibrous tissue deposition in the ventricular wall [3-6]. Therefore, early inhibition of VR is increasingly becoming recognized as an effective method for postponing HF induced by MI or other cardiovascular diseases [7].

Extensive experimental evidence suggests that an intense inflammatory response after MI plays a crucial role in the pathogenesis of VR. Although a certain amount of inflammation is required for proper healing and scar formation in the damaged myocardium, a persistent activation of the innate immune system is deleterious to the injured heart and ultimately results in heart failure [8-12]. All cells within the heart can cause an inflammatory response after being triggered by activation of toll-like receptors (TLRs) and nuclear factor-kappaB (NF-κB) signaling pathways [13-15]. Several studies indicated that TLR-4 signaling increased after cardiac injury, which promoted an inflammatory cascade through the TLR-4/NF-κB pathway [16, 17]. TLR-4-deficient mice exhibited a smaller infarct size with suppression of inflammatory

reactions and less adverse remodeling following MI [18]. Although NF-κB was shown to be cardioprotective during acute hypoxia and reperfusion injury [19], prolonged activation of NF-κB appeared to be detrimental and to promote HF by eliciting signals that triggered chronic inflammation through enhanced production of cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6. This led to endoplasmic reticulum stress responses and cell death [20]. Inhibition of the TLR-4/NF-κB pathway was shown to improve left ventricular remodeling and dysfunction following MI. For example, fluvastatin, a hydroxy-methylglutaryl-CoA reductase inhibitor, improved cardiac function by inhibiting the expression of TLR-4, which reduced NF-κB activity and TNF-α expression [21, 22]. Agents that affect the molecular pathways that are elicited during post-infarct remodeling are promising therapeutic candidates.

Cistanche deserticola Y. C. Ma (Rou Cong Rong), a genus of parasitic plants that belong to the Orobanchaceae family, is classified as a tonifying agent in Oriental traditional medicine and is commonly used to treat renal disorders, body weakness, and infertility [23]. *C. deserticola* is also widely used clinically to treat cardiovascular diseases such as coronary heart disease and hypertension. *C. deserticola* contains a variety of active components, including phenylethanoid glycosides (PhGs), iridoids, lignans, alditols, oligosaccharides, and polysaccharides. The major bioactive components of the *Cistanche* species are thought to be PhGs [24]. Modern pharmacological studies have demonstrated that *C. deserticol*a and its constituents such as PhGs, echinacoside and verbascoside, possess a variety of pharmacological activities, including anti-inflammatory [25, 26], antioxidant [27], and neuroprotective activities [28]. In recent years, *C. deserticola* extracts and PhGs have also been shown to protect H9c2 cardiomyocytes from hypoxia/reoxygenation-induced apoptosis, relax rat aortic rings, and decrease myocardial ischemia/reperfusion (I/R) injury in rats [29-31].

However, there remains a lack of evidence for the role of CDE in post-infarct remodeling. The present study was performed to explore the effects of CDE on VR and the underlying mechanism in rats.

Materials and methods

Chemicals

Echinacoside (Sample Code 111670-201304, purity > 93%) and verbascoside (Sample Code 111530-201310, purity > 93%) were purchased from the National Institutes for Food and Drug Control (Beijing, China). High performance liquid chromatography (HPLC) grade methanol and methanoic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Materials

Dried succulent stem of *C. deserticola* Y. C. MA (Lot: 150761021, cultivated in Inner-Mongolia, China) was purchased from Beijing Kangmei Pharmaceutical Co., Ltd. (Beijing, China). The samples used to prepare the extracts were authenticated by Professor Cui-Ying Zhang, a specialist in *Cistanche* species at the Pharmacologic Lab of Chinese Medicine, Guang'anmen Hospital, China Academy of Chinese Medical Science.

CDE preparation

The ratio of plant: ethanol used for the extraction was 1:7 (w/w). CDE was prepared as previously described [32]. The dried roots were extracted under reflux three times with 70% ethanol. Thereafter, the extract liquor was filtered and concentrated to a relative density of 1.11-1.13 under reduced pressure at 60°C. The yield of *C. deserticola* extract was approximately 3.54%, and the PhG content was 30.4%. This concentrate was then vacuum-dried and stored at 4°C.

HPLC analysis

Two PhGs, echinacoside and verbascoside, were used as quality standards for C. deserticola, according to the Chinese Pharmacopoeia [23]. Therefore, the echinacoside and verbascoside components of CDE were analyzed using HPLC as previously described [24]. Briefly, 100 mg of CDE powder was dissolved in 10.0 mL purified water and, after filtration, was injected into the HPLC system. HPLC analysis was performed using an Agilent 1200 liquid chromatography system (Agilent Co., Santa Clara, CA, USA). The mobile phase consisted of a mixture of methanol (A) and 0.1% methanoic acid (B). A

gradient chromatography program was employed as follows: 0-17 min: 26.5% (A) and 73.5% (B); 17-20 min: 26.5-29.5% (A) and 73.5-70.5% (B); and 20-17 min: 29.5% (A) and 70.5% (B). The flow rate was held constant at 1.0 mL/min, the injection volume was 10 μL, and the column temperature was maintained at 25°C. A UV detector set at 330 nm was used to monitor the column outflow and generate chromatograms. Echinacoside and verbascoside were identified based on their retention times and absorption spectra.

Animals

Healthy adult male Wistar rats weighing 230±10 g were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All animals were housed with food and water available under standard animal room conditions (temperature 21±1°C; humidity 55%-60%) for one week before the study. Our experimental procedures complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (document 55, 2001).

Acute MI model and treatment

A total of 215 rats were used in the study. The normal group consisted of 10 rats. One hundred ninety-five rats underwent left coronary ligation to induce acute MI as described previously [33]. The rats were anesthetized intraperitoneally with 2% pentobarbital sodium solution (4.6 mg/100 g body weight). Acute MI was induced by performing a left coronary artery (LCA) ligation approximately 3 mm from its origin using a 6-0 polypropylene suture (Surgipro, New Haven, CT, USA).

Twenty-four hours after surgery, the surviving 65 rats were randomly divided into five groups as follows: MI group, CDE 50 mg/kg per day group (L), CDE 100 mg/kg per day group (M), CDE 200 mg/kg per day group (H), and captopril 30 mg/kg per day group. An additional 10 rats were assigned to the sham group and underwent the same procedure, except for the ligation of the coronary artery. Treated rats received CDE water solution by gavage daily for six weeks after acute MI. Rats in the sham group and acute MI group received equivalent doses of water.

Assessment of cardiac function

Three and six weeks after drug administration, changes in left ventricular function were evaluated by transthoracic echocardiography using an ultrasound machine (Prosound SSD-5000 SV, manufactured by Hitachi Aloka Medical, Ltd., Tokyo, Japan) equipped with a 10 MHz phased-array transducer.

Left ventricular systolic diameter (LVSd) and left ventricular diastolic diameter (LVDd) were measured concurrently. Ejection fraction (EF) and fractional shortening (FS) were calculated from M-mode recordings. All measurements were averaged over three to five consecutive cardiac cycles according to the standards of the American Society of Echocardiography. FS and EF were calculated as previously described [34].

All images were analyzed using Vevo 770 3.0.0 software from VisualSonics Inc. (Toronto, Canada).

Histological and histomorphometric assessment

Six weeks after drug administration, rats were euthanized with 1% sodium phenobarbital (40-60 mg/kg, i.p.) and the hearts were collected. To investigate myocardial fibrosis and cardiomyocyte hypertrophy, each heart was cut transversely into three pieces, and equatorial regions of the heart were routinely processed and paraffin-embedded. Sections were stained with hematoxylin and eosin and Masson's Trichrome using standard protocols for histomorphometric analysis. At least one section of the three pieces of each heart sample was examined.

Masson's trichrome staining was used to evaluate collagen deposition. The extent of cardiac fibrosis in the peri-infarct region was assessed by calculating collagen volume fraction (CVF). Quantitative assessments for myocardial fibrotic area were performed on five sections in five randomly selected fields per section, each of which were imaged at 200 × magnification by bright-field microscopy (IX71, Olympus, Tokyo, Japan).

The extent of cardiomyocyte hypertrophy was determined on hematoxylin and eosin-stained transverse sections by measurement of the cardiomyocyte cross-sectional area using optical cursors with computerized Image Pro-Plus

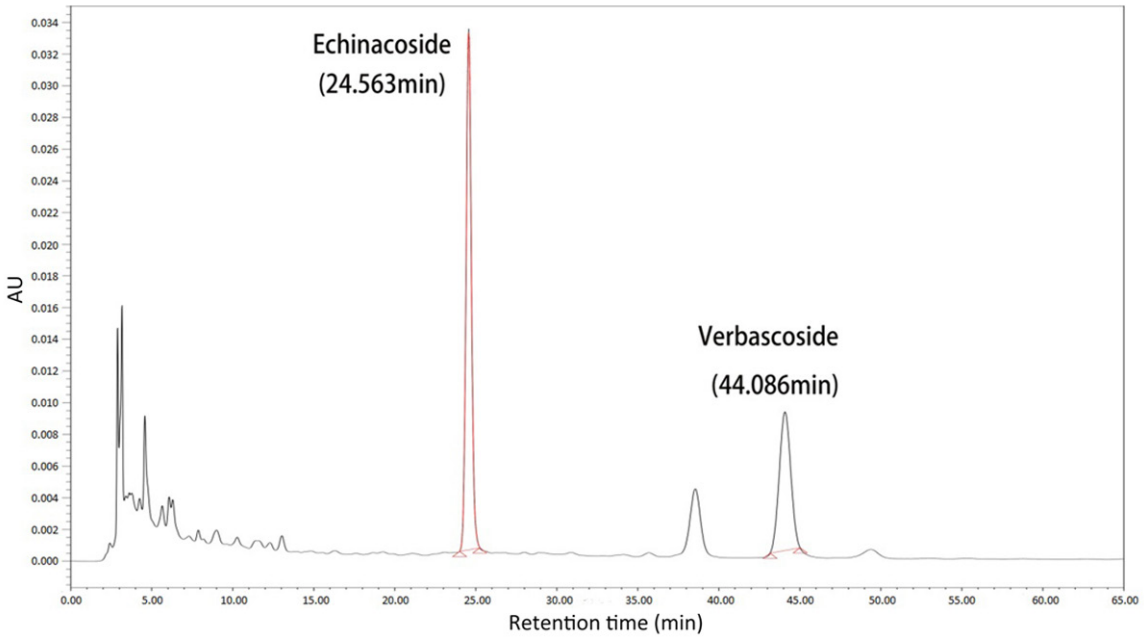

Figure 1. HPLC analysis of CDE and two phenylethanoid glycosides, echinacoside and verbascoside.

Table 1. Tissue weight of rats before and after MI and treatment with CDE ($\bar{x}\pm S$)

Group	Normal	Sham	MI	CDE (H)	CDE (M)	CDE (L)	Captopril
Initial BW	241.90±6.37	240.10±5.53	241.70±4.2	242.8±5.75	243.18±7.05	239.10±7.52	241.90±4.6
BW after 6 wk	460.00±23.84**	452.90±39.11*	423.90±26.25#	430.00±14.77	422.20±12.89#	420.00±25.36##	419.20±30.9##
HW/BW ratio	2.37±0.13**	2.37±0.06**	2.63±0.31#	2.46±0.05*	2.49±0.11	2.50±0.2	2.45±0.11*

n ≥ 10. #*P* < 0.05 and ##*P* < 0.01 vs. sham; **P* < 0.05 and ***P* < 0.01 vs. MI. Abbreviations: MI, myocardial infarction; CDE, *Cistanche deserticola* extract; BW: body weight; HW: heart weight.

6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). Cells with central nuclei as judged by the eye were chosen for measurements.

Measurement of plasma indicators by ELISA

TNF-α, IL-1β, MMP-2, and MMP-9 levels were quantified using commercial ELISA kits (Cusabio Inc., China). Each assay was performed following the related manufacturer instructions. Standards at a series of concentrations were run in parallel with the samples. The concentrations of the samples were calculated by reference to the corresponding standard curves.

Protein isolation and western blot

Equal amounts of protein were extracted from the left ventricular posterior wall (100 2 g/lane as determined by the Bradford method) and were separated using 10% sodium dodecyl sulfate-polyacrylamide gels. After electrophoresis, the proteins were electrophoretically transferred to nitrocellulose membranes blocked with 5% bovine serum albumin in Tris-buffered saline containing 0.1% Tween 20 at room temperature for 40 min. The membranes were then incubated overnight at 4°C with primary antibodies against TLR-4, NF-κB p65 (1:500 diluted), and GAPDH (1:1000 diluted). The antibody-tagged membranes were probed with a secondary antibody solution consisting of a 1:1000 dilution of horseradish peroxidase (HRP)-conjugated goat antirabbit IgG (Jackson, USA) for TLR-4, NF-κB p65, and GAPDH.

An enhanced chemiluminescent detection system was used to detect the immunoblot protein. The optical density of the bands (measured in arbitrary densitometry units) was determined using Image-Pro Plus, and the densitometry of the immunoblot was normalized against GAPDH.

Quantitative reverse transcription-PCR

Total RNA was extracted from cardiac tissues using TRIZOL reagent (Cwbio. Co. Ltd, Beijing,

Table 2. Echocardiography of MI rats after treatment with CDE for 3 and 6 weeks ($\bar{x} \pm S$)

Group	Normal	Sham	MI	CDE (H)	CDE (M)	CDE (L)	Captopr
3 weeks							
HR, beats/min	487.25±24.6	476.50±9.46	465.88±46.09	447.00±27.91	453.29±26.63	472.50±50.08	450.38±35.11
EF (%)	95.39±3.86**	92.54±4.67**	63.26±4.2##	84.74±1.5##,**	78.11±5.72##,**	77.15±4.57##,**	73.10±6.2##,**
FS (%)	67.83±8.91**	61.5±9.62**	30.06±2.68##	48.65±1.8##,**	42.13±5.28##,**	41.11±4.08##,**	37.75±5.24##,*
LVDd (mm)	0.61±0.05**	0.61±0.05**	0.75±0.04##	0.68±0.06	0.72±0.03##	0.74±0.11##	0.69±0.10#
LVSd (mm)	0.20±0.06**	0.24±0.06**	0.48±0.08##	0.36±0.02##,**	0.38±0.04##,**	0.44±0.08##	0.47±0.05##
6 weeks							
HR, beats/min	473.25±44.09	448.00±26.03	474.25±39.99	444.75±25.96	443.00±35.09	461.50±42.04	456.00±24.51
EF (%)	94.95±2.83**	94.73±1.38**	52.75±6.06##	74.61±3.85##,**	73.16±9.83##,**	71.55±3.25##,**	62.71±7.44##,**
FS (%)	66.26±7.67**	64.50±3.28**	23.78±3.436##	38.88±3.26##,**	38.31±7.996##,**	36.28±2.576##,**	30.16±5.146##,*
LVDd (mm)	0.60±0.06**	0.65±0.08**	0.78±0.12##	0.76±0.11#	0.81±0.04##	0.78±0.11##	0.81±0.08##
LVSd (mm)	0.20±0.05**	0.22±0.03**	0.60±0.11##	0.47±0.06##,**	0.49±0.13##,*	0.49±0.72##,**	0.57±0.08##

Notes: n ≥ 10. #P < 0.05 and ##P < 0.01 vs. sham. *P < 0.05 and **P < 0.01 vs. MI. Abbreviations: MI, myocardial infarction; CDE, *Cistanche deserticola* extract; HR, heart rate; EF, ejection fraction; FS, fractional shortening; LVDd, left ventricular diastolic diameter; LVSd, left ventricular systolic diameter.

China). After reverse transcription, the cDNA obtained was analyzed using quantitative reverse transcription-PCR to determine the expression of TLR-4 and NF-κB p65. mRNA levels were evaluated using Fast SYBR Green Master Mix (Cwbio. Co. Ltd) reagents with the following parameters: 95°C for 10 min followed by 45 cycles of 95°C for 15 s and 59°C for 60 s. GAPDH was used as an internal control. The primer sequences were: TLR-4, forward, 5'-ACTGGGTGAGAAACGAGCTG-3', reverse, 5'-CAGCAATGGCTACACCAGGA-3'; NF-κB p65, forward, 5'-GGAGTCCTTTCCTCTCTC-3', reverse, 5'-GGCTCAATGAGCATGCTT-3'; and GAPDH, forward, 5'-CAGTGCCAGCCTCGTCTCAT-3', reverse, 5'-AGGGGCCATCCACAGTCTTC-3'.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). All data are presented as mean ± standard deviation (SD). Statistical analysis was carried out on three or more groups using one-way analysis of variance (ANOVA) and Dunnetts' test. A value of $P < 0.05$ was considered statistically significant.

Results

Quantitative determination of the verbascoside content and echinacoside content of C. deserticola

The contents of verbascoside and echinacoside in CDE were quantified using external standard calibration curves and were found to be 4.7% and 10.9%, respectively. The HPLC chromatogram of *C. deserticola* extract is shown in **Figure 1**.

Mortality and heart weight

One rat in the captopril group died after the commencement of treatment two days post-MI induction; there was no mortality in the CDE-treated or sham groups. Six weeks after MI, BW was reduced by 6.4% and HW/BW was increased by 9.9% in the MI group ($P < 0.05$ vs. sham). In the group treated with CDE (200 mg/kg per day), BW was increased by 1.4% and HW/BW was decreased by 6.5% compared with the MI group. Treatment with captopril resulted in a decrease in HW/BW, but there was no effect on BW (**Table 1**).

Effect of CDE on cardiac function evaluated by echocardiography

Echocardiography was performed three and six weeks after CDE administration (**Table 2**). At both time points, the MI hearts were significantly dilated as evidenced by an increase in LVDd and LVSd ($P < 0.01$ vs. sham), whereas EF and FS were significantly decreased ($P < 0.01$ vs. sham). These results indicated that cardiac function was impaired. However, heart rate (HR) did not change significantly after MI ($P > 0.05$ vs. sham). CDE treatment attenuated VR by significantly decreasing LVSd and increasing EF and FS ($P < 0.05$ vs. MI). These results indicated that CDE had a protective effect on cardiac function. Captopril treatment had a similar

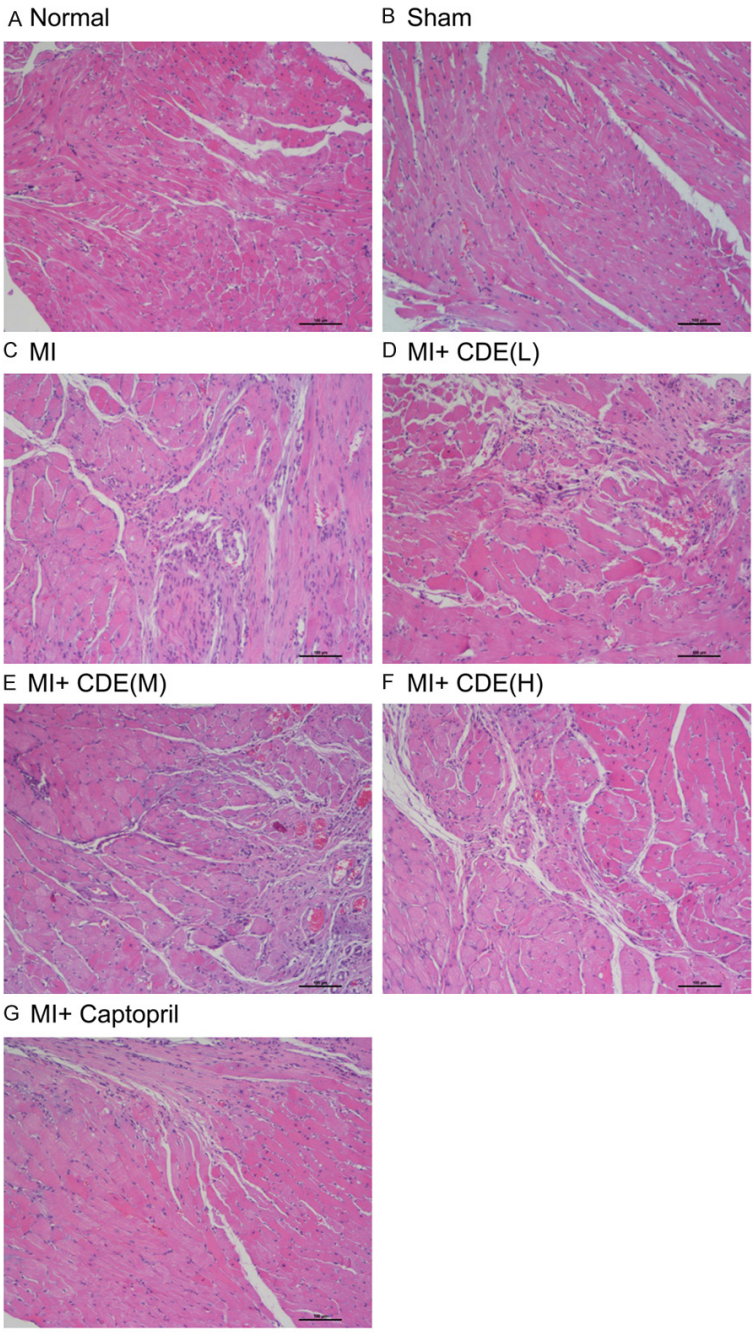

Figure 2. Effect of CDE on HE results after MI and treatment with CDE for 6 weeks. Notes: A: Cardiomyocytes from normal group. B: Cardiomyocytes from sham group. C: Cardiomyocytes from model group. D: Cardiomyocytes from CDE (200 mg/kg per day) group. E: Cardiomyocytes from CDE (100 mg/kg per day) group. F: Cardiomyocytes from CDE (50 mg/kg per day) group. G: Cardiomyocytes from captopril (30 mg/kg per day) group. n ≥ 5 in each group. Abbreviations: MI, myocardial infarction; CDE, *Cistanche deserticola* extract.

effect on these indicators ($P < 0.05$). Results from rats treated with CDE did not differ from rats in the MI group in terms of HR and LVDd ($P > 0.05$) (**Table 2**).

Hypertrophy and collagen

The HE-stained images of left ventricular tissue are shown in **Figure 2**. Cardiomyocytes in the normal group and sham group were arranged in an orderly fashion, and the nuclei were lightly stained and located in the center of muscle fibers. Thickening and lengthening of myocardial fibers was observed in the MI group, wherein the nuclei were darkly stained and displayed local tissue fibrosis. Cellular degeneration and inflammatory cell infiltration were significantly improved in the CDE groups and captopril group compared with those in the MI group.

Myocardial hypertrophy is frequently observed in ischemic HF, which reflects the existence of compensatory mechanisms in response to impaired pump function. The myocyte cross-sectional area (CSA) was measured to evaluate the extent of cardiomyocyte hypertrophy six weeks following MI. Morphometric analysis further revealed that the CDE (200 mg/kg per day) and CDE (100 mg/kg per day) groups had a smaller cardiomyocyte CSA compared to the MI group in the remote LV area. The captopril group displayed the same effect on cardiomyocyte CSA ($P < 0.01$) (**Figure 3**).

In addition to myocardial hypertrophy, interstitial fibrosis in the remote non-infarcted myocardium is commonly observed in failing hearts and contributes to functional impairment. The collagen volume fraction (CVF) of MI rats increased significantly compared with the sham group (8.36±2.56% for sham vs. 21.85±4.15% for MI, $P < 0.01$). Compared with the MI group, treatment with CDE at doses of 50 mg/kg per day, 100 mg/kg per day, and 200 mg/kg per day

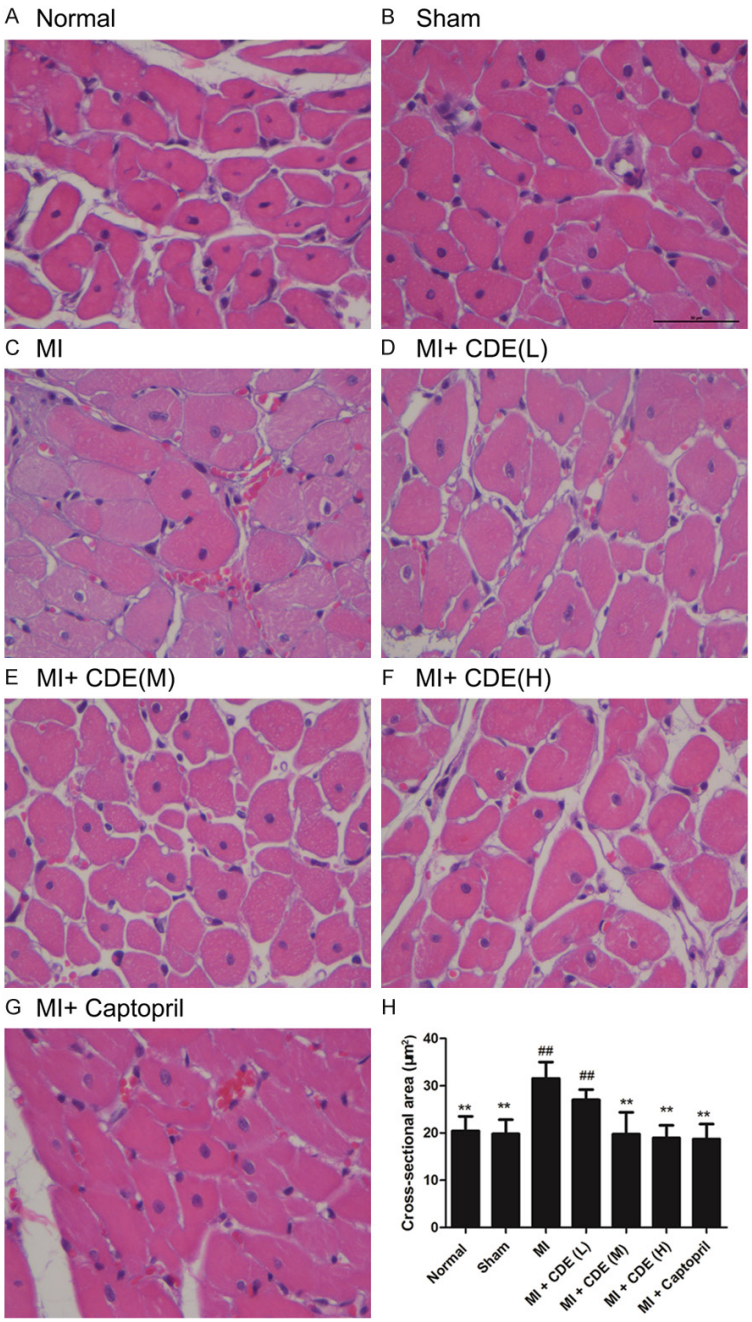

Figure 3. Effect of CDE on myocardial hypertrophy in the non-infarcted myocardium of rats after MI ($\bar{x}$±S). Notes: A-G: Representative sections of heart stained with H&E viewed at a magnification of 400 ×. H: Comparison of cardiomyocyte cross-sectional area (CSA) was quantified by automated Image-Pro Plus 6.0 analysis. n ≥ 5. #*P* < 0.05 and ##*P* < 0.01 vs. sham; **P* < 0.05 and ***P* < 0.01 vs. MI. Abbreviations: MI, myocardial infarction; CDE, *Cistanche deserticola* extract.

reduced CVF by 25.9%, 35.8%, and 63.3%, respectively (*P* < 0.05). Treatment with captopril resulted in the same effect on interstitial fibrosis (*P* < 0.05) (**Figure 4**).

Extracellular matrix turnover

Extracellular matrix turnover is a complicated process, in which the degradation of matrix molecules plays an important role. In our study, MMP-2 and MMP-9 quantitative analysis was performed to explore whether the higher collagen density in the peri-infarct region could be explained by decreased matrix degradation. MMP-2 and MMP-9 were detected using ELISA. MMP-9 expression in the serum of rats in the MI group increased by 45.61% (*P* < 0.01 vs. sham). Treatment with CDE (200 mg/kg per day) decreased MMP-9 expression by 57.7% (*P* < 0.01 vs. MI). Captopril treatment had no significant effect on MMP-9 (**Figure 5A**). MMP-2 expression was not significantly different between any of the groups (**Figure 5B**).

Effect of CDE on inflammation

CDE is a negative regulator of inflammatory cytokine signaling [27, 28]. Because the inflammatory response plays an important role in LV remodeling [35, 36], we evaluated whether CDE affected post-MI inflammation. The ELISA results indicated that TNF-α and IL-1β expression in the serum of rats from the MI group was increased by 45.6% and 60.9%, respectively, over that of the sham group (*P* < 0.01 vs. sham). Treatment with CDE (50 mg/kg per day, 100 mg/kg per day, and 200 mg/kg per day) effectively down-regulated TNF-α by 33.1%, 31.4%, and 37.5%, respectively (*P* < 0.01 vs. MI). CDE treatment also down-regulated IL-1β by 32.9%, 44.9%, and 65.7%, respectively (*P* < 0.05 vs. MI). These results demonstrated a

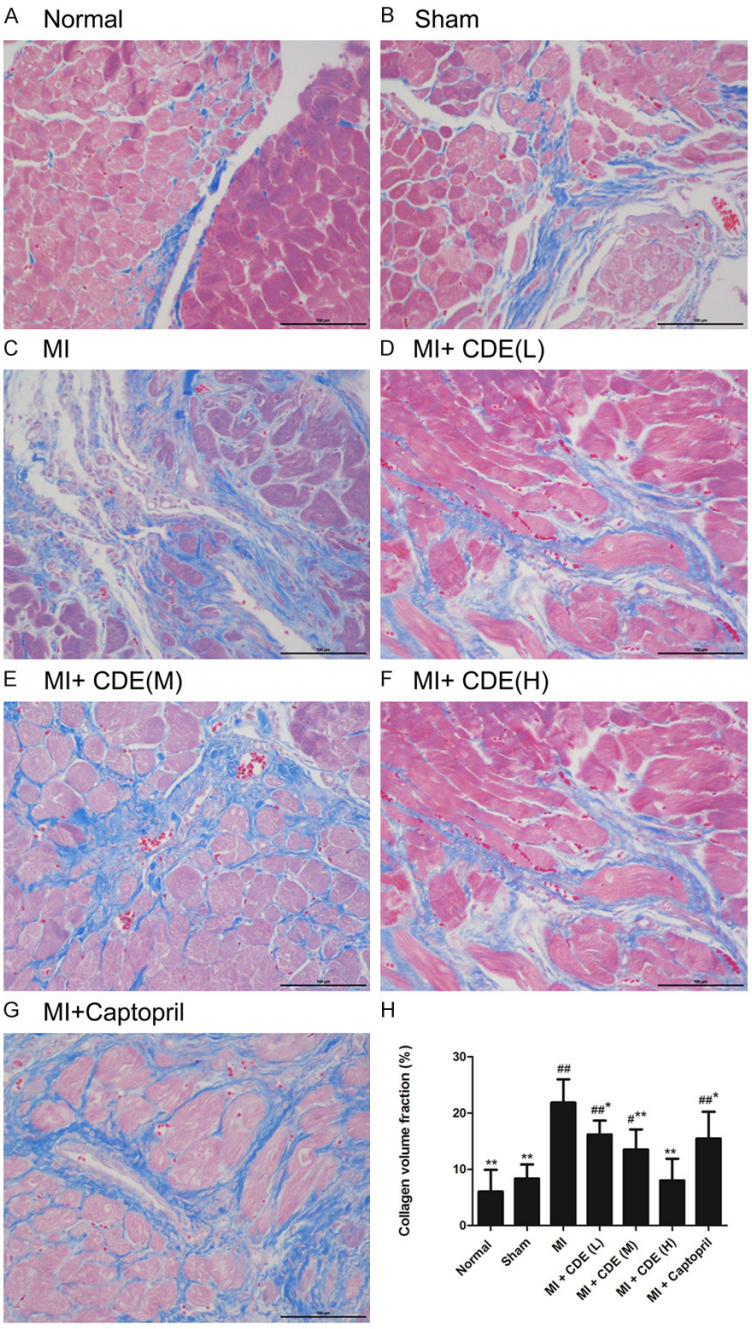

Figure 4. Effect of CDE on the deposition of collagen in the peri-infarct region during MI in rats ($\bar{x}\pm S$). Notes: A-G: Representative section of heart stained with Masson's trichrome viewed at a magnification of 200 ×. The fibrotic area is stained blue, and the viable area is stained red. H: Comparison of collagen volume fraction (CVF) was quantified by automated Image-Pro Plus 6.0 analysis. n ≥ 5. #P < 0.05 and ##P < 0.01 vs. sham; *P < 0.05 and **P < 0.01 vs. MI. Abbreviations: MI, myocardial infarction; CDE, *Cistanche deserticola* extract.

notable anti-inflammatory effect of CDE. Captopril only reduced the expression of TNF-α (P < 0.01 vs. MI group), but had no significant effect on IL-1β (**Figure 6**).

Effect of CDE on the expression of NF-κB p65 and TLR-4 in the peri-infarct cardiac tissues

In chronic inflammation, the TLR-4/NF-κB pathway plays an important role in the development of inflammation [37, 38]. In our study, we evaluated whether CDE affected the TLR-4/NF-κB pathway in the post-infarct hearts. We found that NF-κB p65 gene expression in the non-infarcted myocardium was 1.94±1.22% in the sham group and 8.92±3.9% in the MI group. Treatment with CDE (200 mg/kg per day) and captopril for six weeks significantly decreased NF-κB p65 gene expression by 68.5% and 74.2%, respectively (P < 0.01 vs. MI).

TLR-4 gene expression in the non-infarcted myocardium in the MI group increased by 55% (P < 0.01 vs. sham). Treatment with CDE (200 mg/kg per day) decreased TLR-4 gene expression by 59.5% (P < 0.01 vs. MI). In addition, CDE (100 mg/kg per day and 50 mg/kg per day) had a similar effect on TLR-4 gene expression. Therefore, the effect of CDE on TLR-4 gene expression was dose-dependent. Captopril also significantly suppressed the expression of TLR-4 (P < 0.01 vs. MI) (**Figure 7A**, **7B**). This pattern of changes in gene expression was also seen at the protein level (**Figure 7C**, **7D**), suggesting that CDE suppressed NF-κB p65 and TLR-4 expression in the post-MI hearts.

Discussion

Emerging evidence suggests that uncontrolled chronic immune activation is a major pathogenetic factor for the deleterious remodeling process in the heart after MI [9-13]. Therefore,

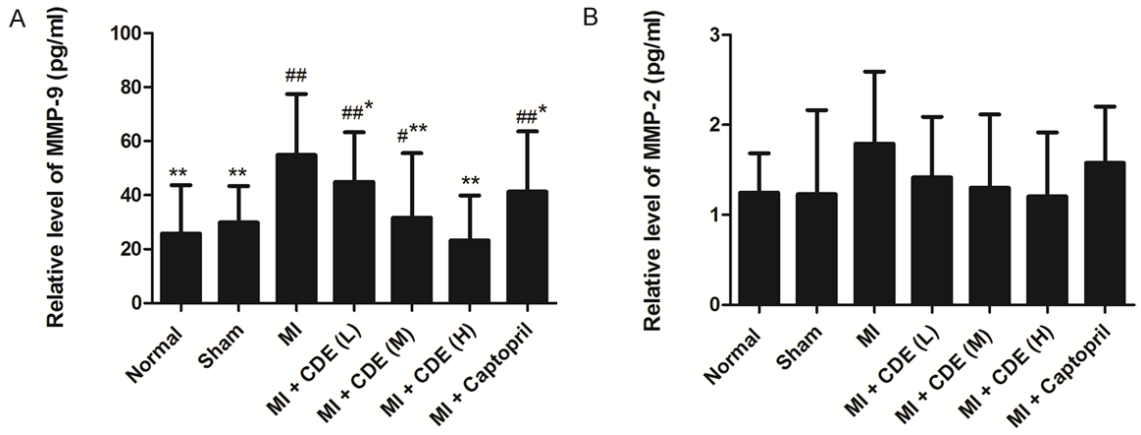

Figure 5. Effect of CDE on the level of MMP-9 and MMP-2 following ligation of LAD for 6 weeks ($\bar{x}$ ±S). Notes: A: Expression of MMP-9 in the serum. B: Expression of MMP-2 in the serum. n ≥ 10. #*P* < 0.05 and ##*P* < 0.01 vs. sham; **P* < 0.05 and ***P* < 0.01 vs. MI. Abbreviations: MI, myocardial infarction; CDE, *Cistanche deserticola* extract.

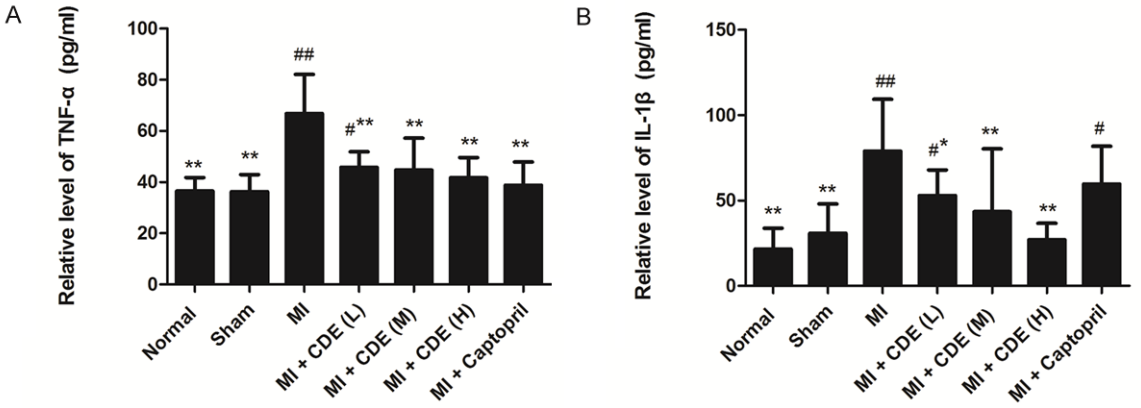

Figure 6. Effect of CDE on the level of TNF-α and IL-1β following ligation of LAD for 6 weeks ($\bar{x}$ ±S). Notes: A: Expression of TNF-α in the serum. B: Expression of IL-1β in the serum. n ≥ 10. #*P* < 0.05 and ##*P* < 0.01 vs. sham; **P* < 0.05 and ***P* < 0.01 vs. MI. Abbreviations: MI, myocardial infarction; CDE, *Cistanche deserticola* extract.

anti-inflammatory strategies for controlling chronic immune activation in the heart are therapeutically relevant in preventing the progression of post-MI heart failure [39-41].

We showed here for the first time that CDE improved post-infarct cardiac remodeling and function in vivo. This outcome was associated with suppression of cardiac inflammation through inhibition of the TLR-4/NF-κB pathway.

These findings indicated that CDE suppressed post-infarct cardiac inflammation and was a potential drug for the treatment of ischemic heart disease.

Extensive experimental studies demonstrated that *C. deserticola* and its extracts could be very useful in the treatment of cardiovascular diseases [29-31].

However, it was still unclear whether post-infarct administration of CDE hindered the progressive deterioration of cardiac function and adverse remodeling after MI.

To investigate the effects of CDE on LV remodeling, we used an animal model and performed permanent coronary artery ligation. Six weeks after surgery, rats treated with CDE (200 mg/kg per day) displayed significantly better cardiac functional and histomorphological parameters compared to untreated MI rats, together with significantly reduced cardiac inflammation.

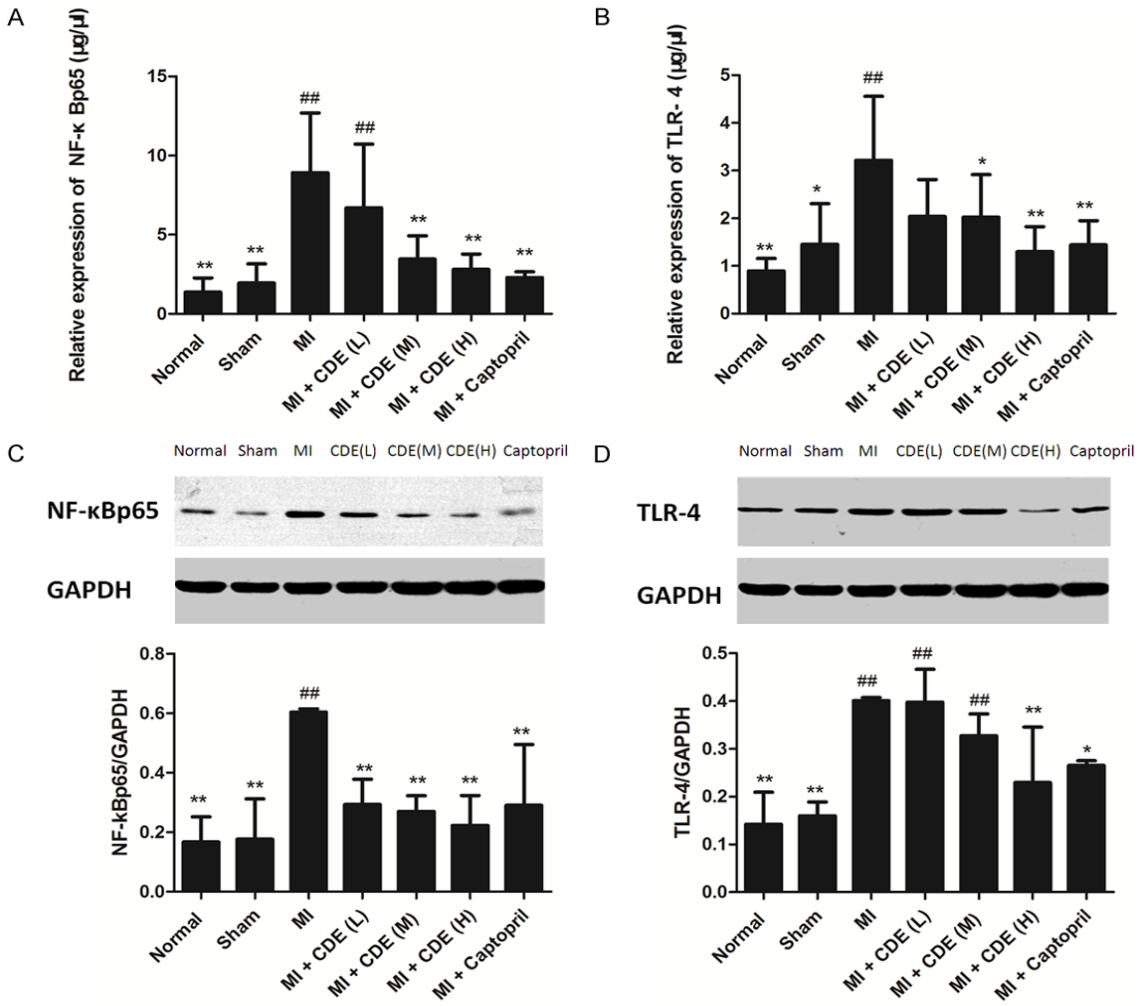

Figure 7. Effect of CDE on the expression of NF-κB p65 and TLR-4 in the non-infarcted myocardium in rats after MI ($\bar{x} \pm S$). Notes: A: Gene expression of NF-κB p65. B: Gene expression of TLR-4. C: Protein expression of NF-κB p65. D: Protein expression of TLR-4. n ≥ 4. #P < 0.05 and ##P < 0.01 vs. sham; *P < 0.05 and **P < 0.01 vs. MI. Abbreviations: MI, myocardial infarction; CDE, *Cistanche deserticola* extract.

These results suggested that CDE suppressed the post-infarct inflammatory reaction and protected the heart from adverse remodeling.

Previous studies indicated that inflammatory cytokines may induce the death of surviving cardiomyocytes in the infarcted myocardium, extending ischemic myocardial injury, contributing to sustained inflammation, and resulting in the development of heart failure [42, 43]. For example, up-regulation of TNF-α in microinfarction after microembolization was associated with ventricular dysfunction [44]. In addition, IL-1β induced cardiomyocyte hypertrophy in vitro in neonatal rat cardiomyocytes. This may induce systolic dysfunction and is correlated with poor exercise tolerance in patients with heart failure [45, 46]. In addition, MMP-9 was induced in response to inflammatory cytokines, whereas enhanced expression of MMP-9 has been linked to post-MI remodeling. Furthermore, MMP-9 gene deletion or treatment with an MMP-9 active site inhibitor has been shown to modulate the cellular inflammatory response and improve post-MI remodeling [47, 48].

In a previous study, CDE was reported to decrease the production of TNF-α and IL-4, both of which are major factors of NO production in the inflammatory pathway [49].

The results of our study indicated that CDE inhibited the expression of proinflammatory cytokines (TNF-α and IL-1β) and attenuated the expression of a pro-fibrotic factor (MMP-9). Therefore, CDE may modulate cardiomyocyte

function via a decrease in inflammatory cytokines and pro-fibrotic factor expression, leading to improved post-MI cardiac remodeling.

Previous studies suggested that the TLR-4/NF-κB pathway played an important role in the chronic development of inflammation [16, 17].

TLR-4, the first and most well-known TLR found in mammals, serves as an important innate immune pattern-recognition receptor (PRR). It is expressed by cells of the myeloid lineage, which are central to innate immune responses and is also expressed in tissues without a recognized immune function, notably the heart and vasculature [50].

The signal transduction pathway of TLR-4 has been clearly elucidated. It is activated primarily through myeloid differentiation factor 88 (MyD88)-dependent pathways and triggers NF-κB. The cumulative activation of NF-κB induces the release of pre-inflammation factors (TNF-α and IL-1). The secondary pathway is an MyD88-independent pathway that proceeds through interfering regulator 3 (IRF3) [51, 52]. In cardiac ischemic injury, TLR-4 has a proinflammatory function during myocardial injury. Timmers et al. demonstrated that TLR-4 played an important role in maladaptive LV remodeling and functional deterioration following MI by inducing inflammatory cytokine production, matrix degradation, and cardiomyocyte hypertrophy [53]. In addition, failing hearts exhibited chronic activation of NF-κB and sustained inflammation [36]. Both in I/R and permanent coronary models, blockade of NF-κB attenuated myocardial injury and LV remodeling [54].

Therefore, we hypothesized that one mechanism for the beneficial effect of CDE in MI hearts was the suppression of the TLR-4/NF-κB pathway. We analyzed the effect of CDE on the TLR-4/NF-κB pathway in the heart following MI by examining TLR-4 and NF-κB p65 subunit protein and mRNA expression in the infarcted myocardium.

Our results clearly indicated that long-term (6 weeks) treatment of MI rats with CDE (200 mg/kg per day) significantly inhibited the up-regulation of TLR-4 and NF-κB p65 in the non-infarcted myocardium at the mRNA and protein levels.

Conclusion

The results of our study indicated that long-term treatment with CDE improved cardiac function in MI rats by suppressing the inflammatory response. The mechanism may involve the inhibition of the TLR-4/NF-κB pathway. These data highlighted the potential of CDE as an anti-inflammatory drug, which may offer a prospect for the prevention of ischemic cardiac injury in the future.

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Disclosure of conflict of interest

None.

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