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Danshen injection prevents heart failure by attenuating post-infarct remodeling

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ABSTRACT

Ethnopharmacological relevance: Danshen Injection (DSI) is a traditional Chinese medicine extracted from Danshen, prepared from the dried root and rhizome of *Salvia miltiorrhiza Bunge*. Danshen is an ancient antipyretic traditional Chinese medicine which is mostly used to improve blood circulation and dispel blood stasis. Danshen decoction or liquor-fried Danshen (with grain-based liquor) which is cool in nature is traditionally used to 'cool the blood' and reduce the swelling of sores and abscesses.

Aim of study: The present study aimed to examine the effect and mechanism of DSI in LAD induced heart injury.

Materials and methods: One day after LAD surgery, adult male Sprague-Dawley rats were randomized to 3 groups: MI group; DSI group (1.5 ml/kg/d, intramuscular); and Valsartan group (10 mg/kg/d, intragastric). Echocardiography and hemodynamic measurements (Pressure-Volume loop) were performed to evaluate cardiac function. Pathological methods (Masson, and Sirus red staining) were used to check myocardial fibrosis. Western blotting assay was used to detect the protein expression of MMP-2. RT-PCR was used to detect the gene expression of MMP-9, MPO, iNOS, Bcl-2 and Bax.

Results: DSI administration to LAD rats resulted in improved cardiac functions, hemodynamic parameters and normalized ventricular mass. Furthermore, DSI-treated group demonstrated potential regulation of myocardial collagen I and III deposition associated with MMP-2 expression. Also, DSI administration decreased gene expression of iNOS, MPO and MMP-9, and increased Bcl-2/Bax ratio.

Conclusion: Myocardial fibrosis, cardiac hypertrophy, hemodynamic deterioration as well as systolic and diastolic dysfunctions which characterize a failing hearts were significantly prevented by DSI. Our study may provide future directions to focus on the anti-hypertrophic mechanisms of DSI and pathological roles played by MMP-2 in myocardial hypertrophy. Meanwhile, DSI also performed the effect of anti-inflammation by the way of decreasing iNOS and MPO. The way Danshen Injection increasing Bcl-2/Bax presented the possibility that it may has the effect of inhibiting cell death.

1. Introduction

Post-myocardial infarction response including, myocardial fibrosis, and cardiac remodeling remains a major challenge in the effective management of heart failure (HF), with the few available therapeutic agents. Thus, prevention of post-myocardial infarct mal-adaptive process is an ultimate therapeutic goal to reduce HF disease burden. Identification of potential pharmacotherapies in the treatment of this syndrome is imperative to maintain cardiac function and structure. The use of herbal products as the topic of research is increasingly becoming more apparent.

Heart failure maybe a ramification of left ventricular remodeling after myocardial infarction (MI) and continues to be a critical source of morbidity and mortality (Chen-Scarabelli et al., 2015). Recently, prevention of this syndrome by attenuating MI complications has been the focal point for researchers. Heart failure is no longer viewed as just

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Table 1
Hemodynamic parameters of heart function after 14 days ligation.

	Sham	MI	Valsartan	Danshen Injection
LVPed (mmHg)	11.92 ± 3.44	20.68 ± 1.59##	13.84 ± 3.97**	13.66 ± 1.07**
LVPes (mmHg)	101.1 ± 5.4	87.33 ± 10.7##	87.13 ± 8.95	92.68 ± 5.42
LVPdev (mmHg)	86.74 ± 7.8	70.98 ± 12.64#	77.26 ± 13.43	84.27 ± 4.45*
SV(μl)	89.4 ± 26.4	48.25 ± 27.9##	86.96 ± 18.47*	88.95 ± 24.52**
Ved(μl)	192.13 ± 35.1	245.77 ± 53.87#	195.87 ± 27.63*	185.03 ± 25.65*
MAP(mmHg)	63.42 ± 13.94	74.29 ± 15.55	70.75 ± 11.52	68.99 ± 4.97
+dp/dt(mmHg/s)	7759.5 ± 495.18	4664.3 ± 1323.39##	5833.67 ± 556.36*	5855.17 ± 1198.56*
-dp/dt(mmHg/s)	−7752.17 ± 1634.9	−4215 ± 1230.1##	−5621.83 ± 722.6*	−5859.83 ± 675.14*
Tau (ms)	9.9 ± 1.7	14.45 ± 3.4##	11.52 ± 2.05*	11.47 ± 2.48*
EDPVR	0.0708 ± 0.05	0.2967 ± 0.23	0.0772 ± 0.02	0.12 ± 0.06
ESPVR	2.21 ± 1.15	0.41 ± 0.17#	2.6 ± 1.7	0.64 ± 0.46
Ea	1.25 ± 0.69	1.7 ± 0.95	1.14 ± 0.17	1.05 ± 0.40
PRSW	490510429 ± 4905	3121 ± 2182##	5146 ± 1604	5697 ± 2551

$P < 0.01$ compared with the sham group.
* $P < 0.05$ compared with the MI group.
** $P < 0.01$ compared with the MI group.
# $P < 0.05$ compared with the sham group.

a problem with excessive salt and water retention nor increase in total peripheral resistance (Sherwi et al., 2012), but also as a complication of post-myocardial adaptive processes such as left ventricular remodeling (Joki et al., 2015). Studies have shown that myocardial infarction portends a deleterious outcome (Asakura and Kitakaze, 2010). During post-infarct response, non-infracted myocardium increases stroke volume (SV) by increasing left ventricular end diastolic volume (LVEDV) through Frank-Starling mechanism to compensate for the weakening heart (Kyhl et al., 2013). However, a failing heart after LAD increases end diastolic volume (EDV) using Laplace mechanism and therefore decreases the pumping ability of the ventricles (Lorell and Carabello, 2000). This therefore can be interpreted to suggest that myocardial infarction leads to increased SV, LV dilatation, increase in cardiac muscle mass, enlargement of left ventricles, decline of systolic and/or diastolic function and ultimately, heart failure (Bang et al., 2014; Wang et al., 2010). Study to prevent heart failure depends on our ability to identify drugs that could alleviate post-myocardial infarction-induced remodeling (Magrini et al., 2005).

It is widely documented that incidence of myocardial remodeling inoperably leads to heart failure that continues to strain health care system and results in increased mortality rate (Xu et al., 2015). The semantic difficulty is the proven beneficial effects of the present standard therapies on one hand, and the increased disease burden of heart failure syndrome on the other hand. Additionally, recent reports suggesting a high mortality rate caused by heart failure is at least in part, an evidence of the insufficient pharmacotherapy needed to prevent myocardial remodeling processes that lead to heart failure (Rabkin and Moe, 2015; Wehling, 2013). Thus, scientific and medical approach is to prevent the pathogenesis of this syndrome by identifying and discovering natural products that could complement the existing therapies.

Traditional Chinese medicine (TCM) has garnered much interest as a multitargeted therapy. Danshen, the extraction of the dried root and rhizome of *Salvia miltiorrhiza Bunge* (Piao and Liang, 2012; Wang et al., 2016), is a well-known Chinese herbal medicine which is frequently used to dispel heat and improve blood circulation. Chinese herbalists use Danshen decoction which is cool in nature to treat insomnia and palpitation (Cho et al., 2010). As a traditional Chinese medicine, Danshen has been used for the treatment of various diseases, such as Alzheimer's disease, Parkinson's disease, renal deficiency, hepatocirrhosis, cancer and bone loss (Su et al., 2015). Danshen has also been shown to be effective against ischemic disease such as angina pectoris (Yao et al., 2015), myocardial infarction (MI) (Zou et al., 2015) and stroke (Cao et al., 2015) in China (Ji et al., 2000; Su et al., 2015). Thus, there is a substantial agreement about the potential cardioprotective effects conferred by Danshen (Lu et al., 2014) and their ability to interact well with western medicines (Luo et al., 2013; Wang et al., 2016). This may partly be responsible for the apparent gain of prominence in western countries in the clinical management of diseases. Danshen formulae (Yin et al., 2015) and Danshen extract (Zhou et al., 2012) have the effect of reducing cardiac fibrosis induced by isoprenaline and Danshen injection attenuates myocardial fibrosis in chronic iron-overloaded mice (Zhang et al., 2015). Danshen also maintains cardiac function induced by LAD by up-regulating HIF1 alpha and VEGFA expression (Ai et al., 2015). Danshen injection ameliorates iron overload-induced cardiac function injury in mice (Zhang et al., 2013). In vitro studies have demonstrated that Danshen has cardioprotective effect in adult rat cardiac myocytes by inhibiting L-type calcium current and attenuating calcium transient and contractility (Gao et al., 2014) following hypoxia injury (Zhu and Deng, 2012).

Network pharmacology analysis revealed that Danshen is mainly used as sovereign herb or minister herb in the application of cardiovascular disease by inhibiting the inflammatory response (Lu et al., 2015). A major complexity and unexplored therapeutic function for DSI is the paucity of information regarding its ability to prevent heart failure by reducing post-myocardial infarction remodeling and hypertrophy (Yang et al., 2011).

Herein, we examined the therapeutic effect of DSI on cardiac remodeling after coronary ligation. Valsartan as a blocker in ANG II receptor has widely discussed in the cardiovascular injury, especially heart failure and cardiac hypertrophy. Valsartan has the effect of inhibiting LV hypertrophy, dilatation and protecting cardiac function (Sui et al., 2015). For this reason we chose valsartan as positive control in this study. We observed myocardial infarction and fibrosis in rat model after 14 days of ligating the left anterior descending coronary artery. We further provided evidence to support our contention that DSI could prevent post-myocardial infarction-induced cardiac remodeling and hypertrophy as evidenced by the parameters studied. We analyzed results from PV loops (Table 1), echocardiography (Table 2) and LV collagen volume fraction (LVCVF) to determine cardiac function and structure. Matrix metalloproteinases (MMPs) have been associated with the development of left ventricular remodeling after MI (Wagner et al., 2006). Hence, we have demonstrated a novel focus and ability of DSI to prevent cardiac fibrosis, remodeling and heart failure through a mechanism involving the repression of MMP-2 and MMP-9 activity. In view of this observation, we determined whether DSI targeting of MMP-2 and MMP-9 are a mechanistic process for the anti-hypertrophic effect. The findings further suggest that sustained activation of MMP-2 and MMP-9 has a causal association with post-

Table 2
Echocardiographic parameters of heart function after 14 days ligation.

	Sham	MI	Valsartan	Danshen Injection
IVS;d (mm)	1.47 ± 0.19	1 ± 0.4#	1.48 ± 0.47*	1.4 ± 0.22*
IVS;s (mm)	2.47 ± 0.25	1.27 ± 0.35##	2.33 ± 0.46**	2.19 ± 0.49**
LVID;s (mm)	4.3 ± 0.42	6.65 ± 0.97##	4.64 ± 0.67**	5.5 ± 0.8**
LVID;d (mm)	7 ± 0.64	8.37 ± 0.87#	7.45 ± 0.67*	7.75 ± 0.79
LVPW;d (mm)	1.89 ± 0.41	1.77 ± 0.29	1.7 ± 0.25	1.59 ± 0.38
LVPW;s (mm)	2.3 ± 0.25	2.35 ± 0.24	2.26 ± 0.11	2.26 ± 0.3
LV Mass (mg)	619.17 ± 70.19	702 ± 64.04##	595.1 ± 70.8**	632.19 ± 44.76*
AV peak(mm/s)	1190.2 ± 210	880.13 ± 270##	1275.7 ± 225**	1136.7 ± 211.4*
EF(%)	71.81 ± 7.43	34.09 ± 13.79##	68.03 ± 8.0**	54.72 ± 8.8**
FS(%)	41.74 ± 7.6	13.65 ± 4.59##	34.92 ± 5.48**	28.06 ± 5.75**

# $P < 0.05$ compared with the sham group.
* $P < 0.05$ compared with the MI group.
$P < 0.01$ compared with the sham group.
** $P < 0.01$ compared with the MI group.

myocardial infarction induced remodeling and heart failure after LAD. Hence, we provided data for anti-hypertrophic effect and potential mechanism of DSI and set the stage for further research. The immune system also plays a significant role in ventricular remodeling, and it's persistent activation may lead to long-term cardiac injury (Burchfield et al., 2013). Specifically, there is amount of study about the role of inflammatory cells and pathways during acute ischemic injury and subsequently activated recovery processes (Dick and Epelman, 2016). Cardiac inflammation also has may deleterious effects, including of blood vascular rarefaction and dysfunction and stimulation of cardiac fibrosis, leading to remodeling and finally heart failure (Henri et tal.,2016). MPO activity, which is accepted as an indicator of neutrophil infiltration, and mediates dysregulation of vascular tone in heart failure (Adam et al., 2015) as part of inflammation response.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (220 ± 20 g) were purchased from Beijing Vital River Lab Animal Technology Co. Ltd. This study was carried out in accordance with the recommendations in the Guidance for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of China and the protocol approved by the Laboratory Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (Permit Number: TCM-LAEC2014004) (Ren-An et al., 2014). The rats were housed in cages at a temperature of 22 °C ± 2 °C and humidity of 40% ± 5%, under a 12-h light/dark cycle, and received standard diet and water ad libitum. Before experiment the animals were fasted for 12 h, but free access to water. The experimental procedures were according to the European Union (EU) adopted Directive 2010/63/EU, and all animals were administrated following the guidelines of Tianjin University of TCM Animal Research Committee (TCM-LAEC2014004).

2.2. HPLC analysis

Danshen Injection was diluted with methanol at the ratio of 1:4. The solution was centrifuged at 14,000 rpm for 10 min. Aliquot of the supernatant solution was injected into HPLC for analysis (Duan et al., 2015). An Agilent HPLC System (Agilent Co., USA) equipped with a VWD was used to separate the components in Danshen Injection. All components were separated by Kromasil C_{18} column (250 mm×4.6 mm, 5 μm) and a C18 guard column. The flow rate was 0.8 ml/min. The column temperature was 40 °C. Wavelength was set at 288 nm. The mobile phase comprised (A) acetonitrile and (B) trifluoroacetic acid solution (0.05%, v/v) using a gradient elution of 2–30% A at 0–65 min, 30–2% A at 65–66 min, 2% A at 66–68 min.

The quality control standard for DSI according to the national drugs surveillance administrative bureau is that total amount of danshensu ($C_9H_{10}O_5$) should not be lower than 0.6 mg, Protocatechuic aldehyde ($C_7H_6O_3$) should not be lower than 0.2 mg, rosmarinic acid ($C_{18}H_{16}O_8$) should not be lower than 0.1 mg and salvianolic acid B ($C_{36}H_{30}O_{16}$) should not be lower than 0.1 mg.

2.3. Reagents

DSI was purchased from Chiatai Qingchunbao Pharmaceutical Co., Ltd. (Hangzhou, China) (drug approval number: Z33020177; batch number: 1208162). Valsartan was purchased from Beijing Novartis Pharma Ltd. (Beijing, China) (drug approval number: H20040217; batch number: x1739). Chloral hydrate was purchased from Tianjin Kermel Chemical Reagent Co., Ltd. (batch number: Q/12HB 4218-2009) freshly prepared to 5% solution with saline before experiment. Masson staining kit was purchased from Sigma-Aldrich Co., LLC. (Santa Clara, USA). Red staining kit was purchased from Nanjing Senbeijia biological technology Co., Ltd (Nanjing, China). Primary antibodies against rat MMP-2 was brought from Abcam (Cambridge, MA, USA). Primary antibodies against rat GAPDH was purchased from Cell Signaling Technology (Beverly, CA, USA). Protein lysis buffer and protein inhibitor (RIPA, R0020) was purchased from Solarbio (Beijing, China).

2.4. MI model and drug administration

Anesthesia was induced with 5% chloral hydrate (300 mg/kg) intraperitoneal injection. After the operation of tracheal intubation, the ventilation was conducted with a small animal mechanical ventilator (Shanghai Precision Scientific Instrument Co., Ltd. ALC-V8 CHINA). A midsternal thoracotomy was operated to expose the heart in the supine position. Via ligation of the left anterior descending (LAD), we obtained myocardial infarction in rat model (Yoon et al., 2013), then closed the chest at once. The chest of the sham group was opened and threaded but was not ligated, other operation was the same with the MI. Animals with ejection fraction between 35–50% were used in the experiment after 24 h of LAD. The MI rats were randomized into 3 groups: MI, Valsartan (10 mg/kg) and DSI (1.5 ml/kg). Sham and MI groups were orally administered with normal saline for 14 days.

2.5. Echocardiography assessment of left ventricular function

Transthoracic echocardiography was performed under light isoflurane inhalation anesthesia (1%), with the animals breathing spontaneously. Measurements were obtained after 14 days of administration; 1 day before PV loop analysis and subsequent death. Short-axis M- and B-mode images of the left ventricular (LV) were obtained using a

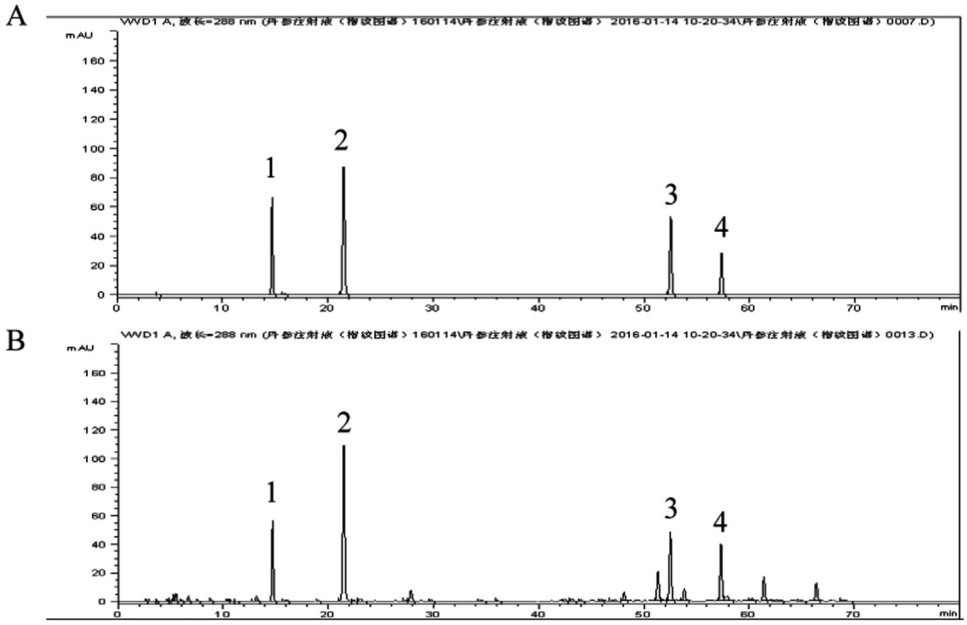

Fig. 1. The typical chromatograms of standard compounds (A) and sample(B). (1) Danshensu sodium, (2) Protocatechuic aldehyde, (3)rosmarinic acid, (4) salvianolic acid B.

vevo2100 ultra-high resolution small animal ultrasound imaging system in real time (VisualSonics Vevo 2100, Canada) with a MS-250 ultrasound scanning transducer (model C5) to determine heart rate (HR), end-diastolic dimension (EDD), end-systolic dimension (ESD), diastolic anterior wall thickness, AV peak, diastolic posterior wall thickness, and percent fractional shortening (%FS), which was calculated [as follows: %FS=100%*(EDD-ESD)/EDD], ejection fraction[%; 100*(LVEDV-LVESV)/LVEDV] were calculated (Jia et al., 2014). These parameters were averaged 3 cardiac cycles.

2.6. Measurement of LV pressure-volume loop

The volume and pressure were calibrated by MVPS-Ultra system (Millar Instruments). Tidal volume was set at 119–289 μl. Rats were anesthetized with chloral hydrate as above-mentioned. The anesthetized animal was placed in a supine position, and a 10- to 15-mm incision was made in the anterior midline of the neck to expose the trachea. The left external jugular vein was dissected free and catheterized with Miller catheter. The right carotid artery was dissected and exposed, and a Millar-Tip (2 F tip size) conductance catheter (model SPR-869, Millar Instruments, Houston, TX) was introduced into the artery and advanced into the LV via the aortic valve. Once steady-state hemodynamics was achieved, PV loops were recorded and processed. For all animals, parallel conductance was determined individually using a 15- to 30-μl bolus of 15% saline given through the right venous catheter. The PV loop data were processed to compute cardiac parameters with chart7 software. At the end of the experiment, the animals were killed under anesthesia to obtain blood, and the hearts were excised and kept in paraformaldehyde (Naghshin et al., 2009).

2.7. Measurement of protein expression of MMP-2

Western blotting assay was performed to determine protein expression of MMP-2 (n=3 per group). The myocardium tissues were homogenized in the lysis buffer using an ultrasound homogenizer at 50 Hz. The lysates were then centrifuged. The protein concentration of the supernatants were measured with the BCA protein assay. The concentration of the final protein was 4 μg/μl, 7.5 μl protein were loaded into 8% SDS-polyacrylamide gels and transferred to PVDF membranes. After blocking in non-fat milk, the membranes were incubated with primary antibody against MMP-2 (1:1000) or GAPDH (1:1000) overnight at 4 °C. After incubation with HRP-linked secondary antibodies, the immune complexes were visualized with chemiluminescence (ECL Blotting Analysis System; Amersham, Arlington Heights, IL) and exposed in a dark box, and the protein signal was quantified by scanning densitometry in the X-film using a multifunctional imaging analysis system (Versadoc MP 5000;Bio-Rad Laboratories Inc., Hercules, CA,USA), and normalized to GAPDH.

2.8. RT-PCR

Total RNA was isolated from myocardium tissues with TRIzol (15596026; Roche, USA). Primers were designed and synthesized as previously mentionad: MMP-9 (Fwd 5′-CAAACCCTGCGTATTTCCAT-2′; Rev 5′- GTCATAGTTGGCGGTGGTG-3′), Bax (Fwd 5′-ACGCATCCACCAAGAAGC-2′; Rev 5′- GCCACACGGAAGAAGACCT-3′). Bcl-2 (Fwd 5′- GGTGGACAACATCGCTCTG-2′; Rev 5′-ACAGCCAGGAGAAATCAAACA-3′), iNOS (Fwd 5′- GAAGGCGT-AGCTGAACAAGG-2′;Rev5′-GTGCTAATGCGGAAGGTCAT-3′), Myeloperoxidase (Fwd5′-ACCCTCATCCAACCCTTCAT-2′;Rev5′-CCA-CCTTCCAGCACAACTCT-3′), GAPDH (Fwd 5′- ATGATTCTA-CCCACGGCAAG-2′; Rev 5′-CTGGAAGATGGTGATGGGTT-3′). First-strand cDNA was synthesized using Transcriptor First strand cDNA Synthesis kit (04379012001; Roche, USA) as follows: 25 °C 10 min ; 37 °C 120 min ; 85 °C 5 min. The cDNA was amplified with a volume of 20 μl by PCR using Fast Start Universal SYBR Green Master Mix (04913914001; Roche, USA) follows the RT-PCR approach as follows: 95 °C 10 min, 95 °C 15 s and 60 °C 40 s (40 cycles). The PCR process was perfprmed using the (C1000; BIO-RAD,USA), and the relative Mrna expression was calculated using $2^{-\Delta\Delta CT}$.

2.9. Measurement of histology

After catheterization, hearts were quickly removed, weighed and then fixed in buffered paraformaldehyde. The hearts were embedded in paraffin, and were then cut into 4 μm serial sections using microtome. Several sections were stained with Masson to observe any histopathological changes (Ren-An et al., 2014). Collagen content in the infarct region was shown by picrosirus red staining and the level of fibrosis was observed by Masson staining.

2.10. Statistical analysis

All values were shown as the mean ± SD with the one-way analysis of variance and multiple comparisons between the groups was performed using LSD method by SPSS17.0 statistical software. Difference was considered to be significant when $p < 0.05$.

3. Result

3.1. HPLC analysis

The contents of the four compounds were analyzed by using the HPLC method. Representative chromatograms of the mixed four standards and Danshen Injection are shown in Fig. 1.

3.2. DSI improves cardiac function: Echocardiography Parameters

Valsartan and DSI improved cardiac performance of the infracted hearts. The cardiac function of rats in different groups was evaluated by echocardiography at 1 and 14 days after LAD. The left ventricular fractional shortening (FS) and ejection fraction (EF) were calculated (Fig. 2). Representative echocardiographic images from at least three cardiac contractile cycles of the hearts from control and experimental rats were provided. The mean percentages of EF and FS of the indicated groups were provided. Ultrasonic detection displayed that one day after ligation, EF and FS values in the ligation groups were prominently decreased ($p < 0.01$, data not shown). After 14 days of LAD, hemodynamic studies suggested a marked improvement of cardiac function in Valsartan and DSI groups in the context of EF and FS parameters ($p < 0.01$), while MI group went deteriorated. The left ventricular outlet tract velocity peak of blood was dramatically decreased in MI group, and there was no significant difference between the DSI, valsartan administered and sham groups at different degrees.

3.3. DSI can inhibit morphological changes

Effect of DSI treatment on morphological changes of systolic IVS, LVPW and LVID (Fig. 3).

Compared with the sham group, after 14 days of ligation, the IVS was significantly thinner and LVID was longer in the MI group whiles valsartan and DSI group both recovered dramatically. There was no significant difference among groups on LVPW both in systole and diastole.

3.4. DSI can improve hemodynamic parameters

The view of P-V loop (Fig. 4) shows the changes of volume and pressure on MI rats. After 14 days of ligation, MI group represented a significant deterioration on pressure and pressure-related maximum positive left ventricular pressure development (+dp/dt max) and maximum negative left ventricular pressure development (-dp/dt max) compared with the sham group (Fig. 5). The LV end-diastolic pressure was significantly decreased in administration group compared with the MI group, while the end-systolic pressure did not show any significant changes. The developed pressure of DSI group markedly rose compared with the MI group (Ped). The ± dp/dtmax shows that DSI increased cardiac systolic function. DSI administrations recorded lower end-systolic volume, volumes-related stroke volume (SV) and cardiac output (CO) were similar to the sham and valsartan groups, but significantly different from the MI group (Fig. 5).

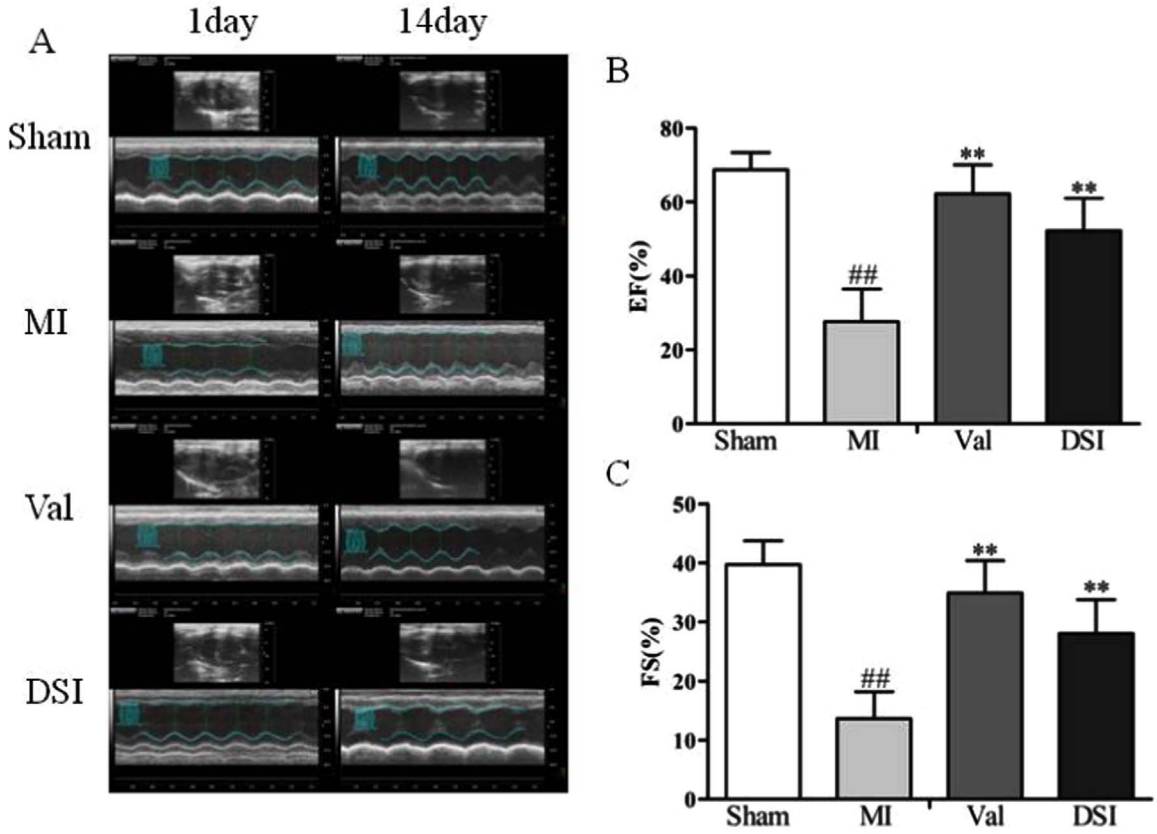

Fig. 2. Effects of treatments on cardiac function. (A) Representative echocardiographic images from at least three cardiac contractile cycles of the hearts from control and Experimental rats are provided. (B)The Left ventricular ejection fraction (EF) and (C) fractional shortening (FS) were calculated. ##$p < 0.01$ compared with the sham group;**$p < 0.01$ compared with the MI group.

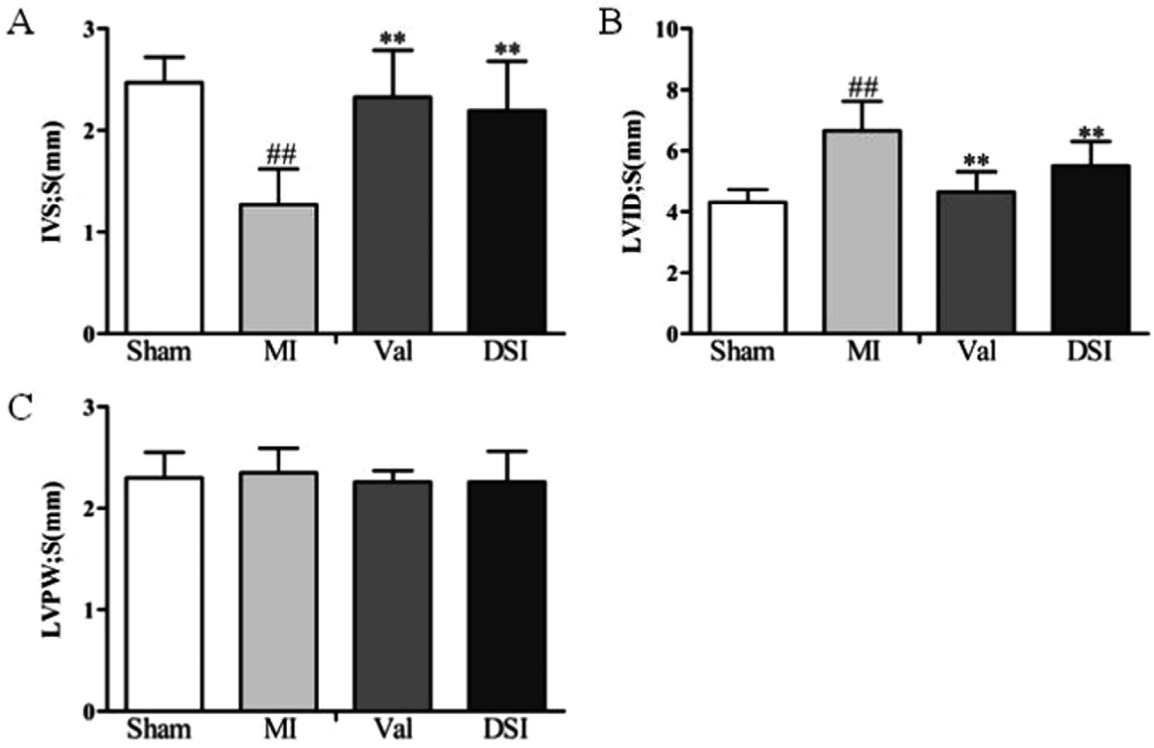

Fig. 3. Effects of treatments on morphological changes. (A) IVS;s (B) LVID;s and (C) LVPW;s. ##$p < 0.01$ compared with the sham group;**$p < 0.01$ compared with the MI group.

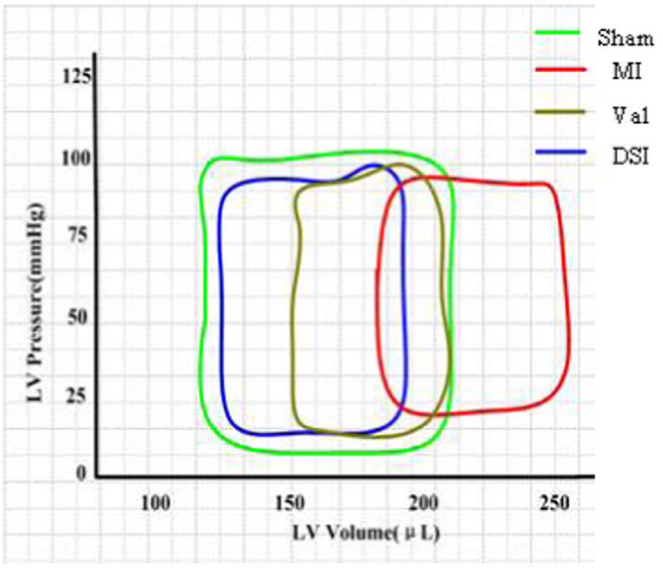

Fig. 4. The view of Pressure-Volume loop.

3.5. Effect of DSI on heart ratio after MI

Effects of treatments on heart weight/body weight and computed LV mass. As shown in Fig. 6, Compared with the sham group, the MI group had a significantly increased heart index (HW/BW) and LV mass which were obtained by echocardiography. The heart index and LV mass induced by 1.5 ml/kg Danshen Injection and 10 mg/kg valsartan were both reduced markedly.

3.6. DSI reduced MMP-2 protein levels after MI

We observed that MI model group significantly increased MMP-2 levels compared to sham-operated animals. However, DSI injection (1.5 ml/kg) and valsartan treatment (10 mg/kg) markedly decreased MMP-2 expression suggesting that it may associated with anti-inflammatory effect ($p < 0.01$) (Fig. 7).

3.7. DSI reduced the expression of hypertrophy and cell death genes

We assessed MMP-9, iNOS, Myeloperoxidase and Bcl-2/Bax gene levels by using qRT-PCR. We found that these genes were significantly increased by MI model compared to sham-operated animals. However, DSI injection (1.5 ml/kg) and valsartan treatment (10 mg/kg) markedly decreased these expression implicating that it may prevent of inflammation (Fig. 8).

3.8. DSI can inhibit cardiac fibrosis

In polarized microscope, sirius red staining clearly showed that type I collagen significantly increased in MI group demonstrated by the red and yellow colors. Green color was observed in the administration groups indicating the presence of type III collagen. The myocardial fibers of sham, DSI and valsartan treated groups were normally arranged and were visible within a large number of capillaries compared with the MI group. Endocardial myocardial fiber necrosis with more infiltration of inflammatory cells was observed in the MI group. The infarction ratio was significantly reduced after DSI administration ($p < 0.05$) (Fig. 9).

4. Discussion

In the present study, we showed that DSI has a favorable preventive effect on heart failure by attenuating cardiac remodeling to maintain both systolic and diastolic function. The findings further reinforce the contention that cardiac fibrosis, remodeling and heart failure are associated with post-myocardial infarction maladaptive processes after LAD (Li et al., 2014). In vivo induction of post-myocardial infarction-induced ventricular remodeling and fibrosis suggests significant deterioration of cardiac function following LAD (Fan et al., 2015). We found marked reduction in left ventricular diastolic and systolic

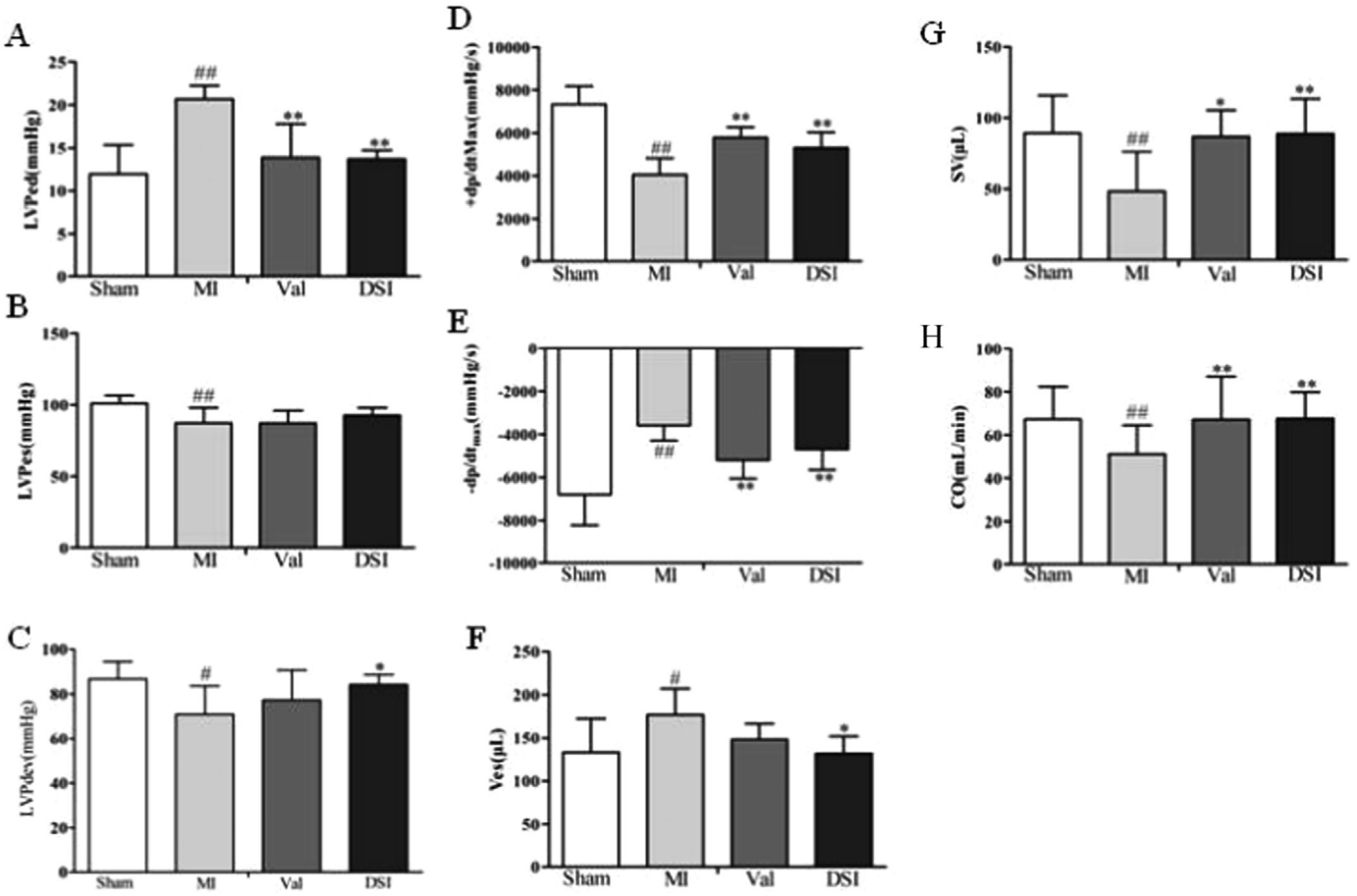

Fig. 5. Effects of administrations hemodynamic. Pressures about (A)LVPed, (B)LVPes, (C)LVPdev, pressure-related Maximum positive left ventricular pressure development (D) and Maximum negative left ventricular pressure development(E). Effects of administrations on end-systolic volume(F), volumes-related stroke volume(SV) (G)and cardiac output (CO)(H). $##p < 0.01$ compared with the sham group; $#p < 0.05$ compared with the sham group; $*p < 0.05$ compared with the MI group; $**p < 0.01$ compared with the MI group.

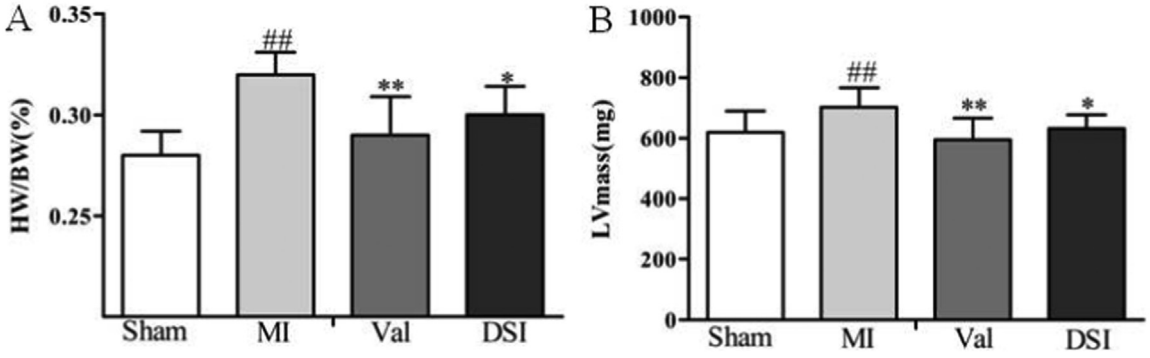

Fig. 6. Effects of treatments on heart weight/body weight (A)and computed LV mass(B). (HW/BW, n=6;LV mass, n=8). $##p < 0.01$ compared with the sham group; $*p < 0.05$ compared with the MI group; $**p < 0.01$ compared with the MI group.

function after 14 days of LAD in the MI group. Findings from the hemodynamic assessments in this group revealed marked increase in left ventricular end diastolic pressure and reduced ejection fraction compared to the sham, valsartan and DSI treated groups. Contractile dysfunction was revealed by the presence of a dilated left ventricular cavity and fibrosis. We further observed significant mala-adaptive response which was characterized by increase heart index (HW/BW) and ventricular mass in the myocardium of the MI group. These observations coupled with the reduced systolic and diastolic functions observed in rats belonging to the MI group characterize a heart in a failing state. Thus, we have identified a natural compound that can protect a failing heart and substantially increase EF (Fig. 10).

Intriguingly, we observed consistent virtual normalization of all the echocardiography parameters studied after LAD by DSI administration which were markedly different from those obtained in the MI group. At the dose of 1.5 ml/kg/d,i.m, DSI increased ejection fraction of 43.5% to 54.7 ± 8.8 whiles EF in the MI group continued deteriorating 45.2% to 34.1 ± 13.7. DSI further prevented ventricular dilatation, maintained ventricular mass (thickening and enlargement of ventricles) and prevented both systolic and diastolic dysfunctions that were present in the MI group. These observations, together with reduced heart index (HW/BW) and ventricular mass obtained in DSI treated rats suggest a prevention of heart failure through the reduction of remodeling and hypertrophic program by DSI. The findings can be interpreted to suggest that 14 days administration of DSI into the LAD rats, results in the ability of the traditional Chinese medicine to normalize ventricular

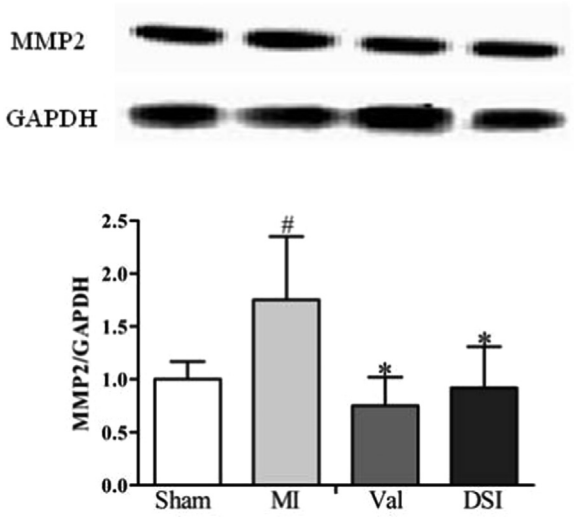

Fig. 7. Effects of treatments on expression of MMP2. #$p < 0.05$ compared with the sham group; *$p < 0.05$ compared with the MI group.

mass and contractility.

As seen in the results, rats administered with DSI demonstrated normalized hemodynamic properties that were similar to the sham and valsartan administered groups demonstrating important beneficial properties of DSI. The cardiac function was evaluated by the measurement of stroke volume (SV), ejection fraction (EF), cardiac output (CO) and cardiac index (CI) (Knaapen et al., 2007). Pressure-Volume loop is the relationship between the ventricular pressure and volume in each moment of the cardiac cycle (Unsold et al., 2015). The height and width of pressure-volume loop reflect the systolic pressure and stroke volume, respectively Fig. 4. The indices of diastolic function are assessed by Left ventricular end diastolic pressure (LVEDP), ± dp/dt, Tau, filling volume, filling rate and filling fraction (Abraham and Mao, 2015). Our results suggest that marked left ventricular systolic and diastolic dysfunction after LAD is well attenuated by treatment with DSI. We have thus, for the first time reported in the present study that DSI could alleviate LAD-induced post-myocardial infarction remodeling.

Another interesting finding in this study was that changes in the studied parameters after DSI treatment were associated with reduced MMP-2 expression levels. It should be emphasized that activation of MMP-2 concurrently occurred with excessive collagen deposition that resulted in fibrosis and altered contractile function observed in the MI group. This suggests that unrestrained MMP-2 activity, could lead to fibrosis, myocardial remodeling and ultimately, heart failure. Inhibition of MMP-2-dependent processes therefore represents a key mechanistic strategy for the prevention of cardiac remodeling (Gu et al., 2015). Interestingly, DSI reduction of myocardial collagen deposition and remodeling involves the inhibition of MMP-2. Therefore, our findings, strengthen the contention that DSI by targeting MMP-2 regulatory pathway may be an important molecular mechanism underlying its anti-hypertrophic properties. Hence, identification of DSI molecular targets in details and deciphering the myriad of mechanisms responsible for mediating remodeling and hypertrophic program is a worthwhile pursuit to effectively manage heart failure. Notwithstanding, we recommend further studies to delineate in broad terms, the possible molecular targets responsible for reduction of cardiac remodeling.

It has been widely reported that Danshen showed anti-inflammatory effect in vitro. Inflammatory mediators such as nitric oxide which produced by inducible nitric oxide synthase (iNOS). INOS induced by inflammatory cytokines in macrophages, is responsible for prolonged production of larger amounts of NO (Wu et al., 2015). It was previously demonstrated that the water-soluble extract contributed less than lipid-soluble extract to the reduction of NO production in LPS-induced RAW264.7 cell (Li et al., 2012). Salvianolic acid B represents one of the most bioactive compounds that can be extracted from the water-soluble fraction of Danshen. Danshen and salvianolic acid B both induced the expression of heme oxygenase-1 and inhibited nitric oxide production and iNOS expression (Joe et al., 2012). Myeloperoxidase is an enzyme stored in azurophilic granules of polymorphonuclear neutrophils and macrophages and released into extracellular fluid in the setting of

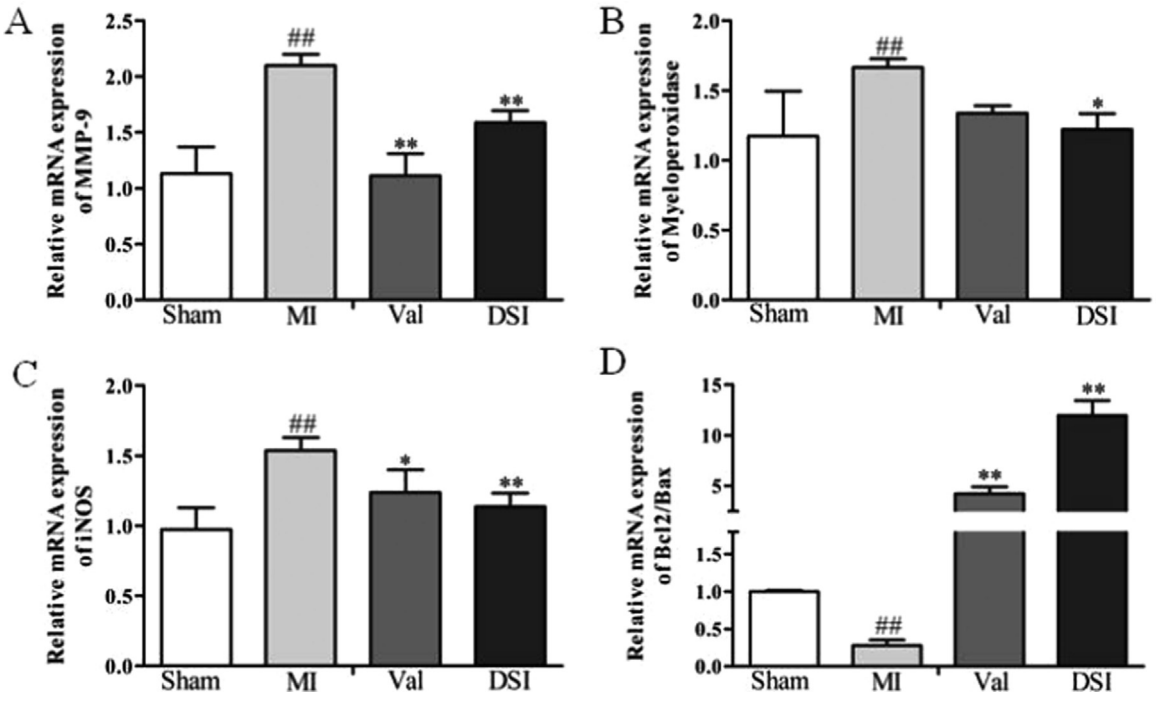

Fig. 8. The gene expression on rat of post-ischemic. RT-PCR analysis shows significantly up regulated MMP-9, inos and MPO and decreased Bcl2/Bax in MI group as compared to sham group, administration groups significantly decreased the expression of MMP-9, MPO and iNOS and increased Bcl2/Bax, n=3 in each group (#$p < 0.05$ compared with the sham group; *$p < 0.05$ compared with the MI group).

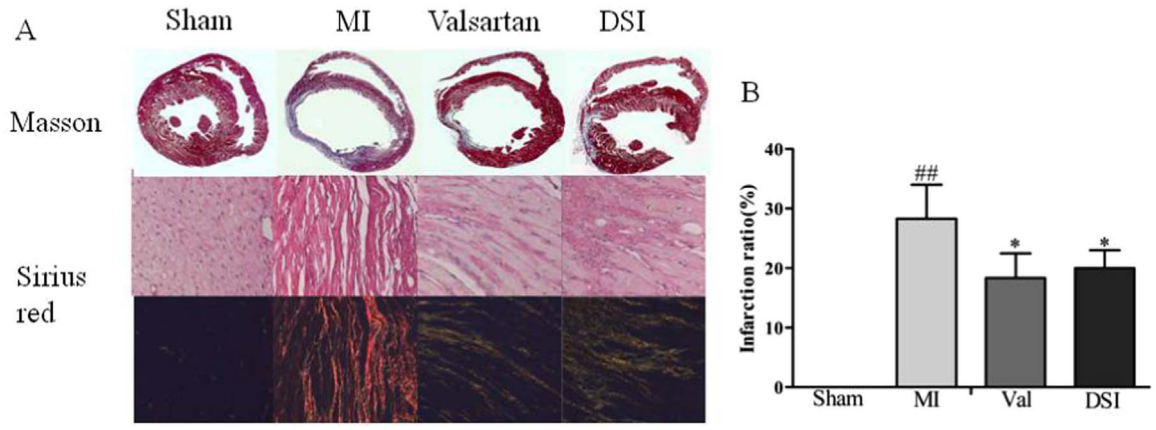

Fig. 9. Effects of treatments on Histological. (A)Masson and Sirius red staining. (B)Infarction ration calculated with Masson staining(n=3). #$p < 0.05$ compared with the sham group;*$p < 0.05$ compared with the MI group.

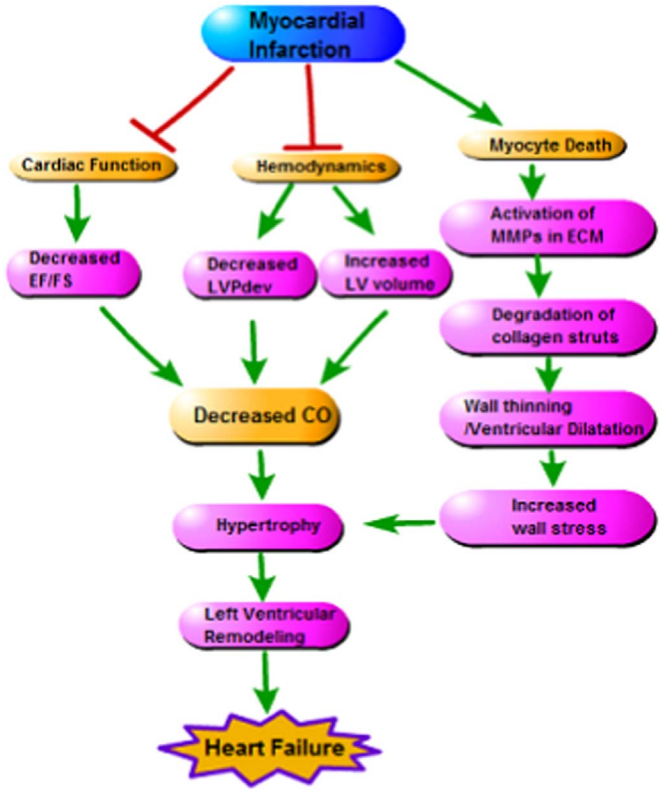

Fig. 10. DSI has the effect of prevent Left Ventricular Remodeling by improving EF, LVPdev and LV stroke volume and inhibiting activation of MMPs.

inflammatory process. Also, our study showed that the gene expression of iNOS and MPO increased in MI group and can be significantly inhibited by DSI. We also have question why sham-operated animals show slightly increased RNA and protein levels compared to DSI treated group. It has some limitation of MI model between sham and DSI group which can be proved by cardiac function displayed by echocardiography and hemodynamic parameters. DSI may have the effect specific target proteins and RNAs which contributes to heart dysfunctions. Although, DSI partially restored some proteins and RNAs levels but still not enough to complete rescue of heart dysfunction into the normal levels.

Danshen extract dilates the coronary artery and increases coronary blood flow, which is beneficial for reducing myocardial infarct size (Xu et al., 2009). It has been demonstrated that tanshinone IIA treatment can recover cardiac function and markedly reduce myocardial infarct size through different mechanisms, such as via the PI3K/Akt-dependent pathway associated with decreased cardiac apoptosis and inflammation (Jiang et al., 1981; Zhang et al., 2010). Danshen extract exerts an anti-apoptotic role against myocardial ischemia reperfusion injury by regulating ERK1/2/JNK pathway (Xu et al., 2014). Cryptotanshinone markedly inhibited the phosphorylation of mitogen-activated protein kinases (MAPKs), including ERK1/2, p38MAPK, and JNK, which are crucially involved in the regulation of pro-inflammatory mediator secretion (Tang et al., 2011). Danshen extract, Tanshinone IIA induced apoptosis, as demonstrated by DNA fragmentation and caspase-3 cleavage, decreased Bcl-2/Bax protein ratio, and depolarization of mitochondrial membranes to facilitate cytochrome c release into the cytosol (Che et al., 2010). The cardioprotective effects of tanshinone IIA have been investigated in cardiac myocyte apoptosis induced both by the in vitro incubation of neonatal rat ventricular myocytes with H_2O_2 and by in vivo occlusion and reperfusion of the left anterior descending coronary artery in adult rats (Fu et al., 2007).

T-lymphocytes in plaques are mostly CD4+ T-lymphocytes which belong to Th1 subtype. Most of CD4+ T-lymphocytes are active and can induce “cytokine waterfall” as well as secrete proinflammatory cytokines, eventually leading to inflammation. Moreover, T-lymphocytes are activated to express CD40L, and the expression of tissue factor and matrix metalloproteinases (MMPs) is then promoted by interaction between CD40L and CD40. The activation of T lymphocytes plays a promoting role in the inflammatory processes of cardiovascular diseases. Fang et al. (2008) found that Tan IIA could reduce both the expression of CD40 and the activity of MMP-2. The anti-inflammation effect of Tan IIA might be one of its potential mechanisms in treating Atherosclerosis. Sal A and Sal B shared the core structure of rosmarinic acid (Sperl et al., 2009), the high affinity to SH2 domains of Src-family kinases and CD36 suggested the role of immune modulator in the cardiovascular protective effect of salvianolic acids. Interestingly, there also emerge a new concept relates to immunity-related protective mechanism of the heart. TNF-α, Toll-like receptor 4 (TLR4), sphingosine-1 phosphate, and activation of specific miRNAs all involved in SAFE (“Survivor Activating Factor Enhancement”) pathway which considered as a cardioprotective signaling pathway. Notably, TNF-α can initiate the induction of SAFE pathway plays main role in immune system (Cabrera-Fuentes et al., 2016).

In summary, DSI has the effect of preventing Left Ventricular Remodeling by improving EF, LVPdev and LV stroke volume and inhibiting activation of MMP-2 and MMP-9. DSI also showed the effect of anti-inflammatory by inhibiting iNOS and MPO expression. The way DSI inhibited ischemic injury may also caused by decreasing cell death signaling because Bcl-2/Bax ratio was significantly increased by DSI. Notably, the prevention of post-myocardial mal-adaptive responses is a pivotal therapeutic goal in the clinical management of heart failure. In

view of this, we contend that discovery of more pharmacological agents is crucial to broaden the spectrum of heart failure therapies. It is therefore, important for clinicians to realize the treatment options in the present study and assimilate them for effective management of heart failure.

List of authors and their respective contribution

1. Wang Lingyan and Lingyan Li performed the LAD surgery.
2. Wang Lingyan, Patrick Fordjour Asare and Xing Xiaoxue performed the western blot, hemodynamics and echocardiography.
3. Jiahui Yu performed RT-PCR.
4. Wang Lingyan and Gao Hui performed the PV loop studies.
5. Wang Lingyan and Patrick Fordjour Asare wrote the manuscript.
6. Wang Lingyan, Yanyan Li, and Jiahui Yu performed the masson and sirus staining.
7. Guanwei Fan and Yan Zhu analyzed the results and reviewed the manuscript.
8. Guanwei Fan and Xiumei Gao conceived and designed the experiments.

Disclosure

The authors report no conflicts of interest in this work.

Acknowledgments

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