Int J Clin Exp Med 2018;11(12):13169-13179
www.ijcem.com /ISSN:1940-5901/IJCEM0082651

Original Article

Comparison of the effects of Shenfu injection and Shenmai injection on doxorubicin-induced chronic heart failure in rats

Yao Zhou[1,2], Senjie Zhong[1], Siyuan Hu[1], Wenbo Dong[1], Tao Yao[1], Hao Liang[3], Lin Li[3], Zhixi Hu[3]

[1]Post-Graduate School, Hunan University of Chinese Medicine, Changsha 410208, Hunan, China; [2]Department of Medicine, Hunan Traditional Chinese Medical College, Zhuzhou 412012, China; [3]Hunan Provincial Key Laboratory of Diagnostics in Chinese Medicine, Hunan University of Chinese Medicine, Changsha 410208, Hunan, China

Received July 16, 2018; Accepted September 13, 2018; Epub December 15, 2018; Published December 30, 2018

Abstract: Shenfu injection (SFI) and Shenmai injection (SMI) are classical Traditional Chinese Medicine (TCM) for chronic heart failure (CHF). SFI is used for Yang Deficiency syndrome, while SMI is used for Yin Deficiency. The present study aimed to investigate the effects of SFI and SMI on doxorubicin (DOX) induced chronic heart failure (CHF) in a rat model. A total of 33 CHF rats were successfully established and received 15 days of SFI and SMI treatment, followed by dividing into the DOX group, SMI group, and SFI group. Another 11 rats were chosen as the control group. Echocardiograms and carotid artery intubations were performed. Blood samples were collected for assessment of cardiac function markers and heart tissue homogenate were used for reactive oxygen species (ROS) and antioxidant enzyme detection. Myocardial tissue was used for histological examinations and apoptosis index analysis. Present results demonstrated that SFI, instead of SMI, improved survival rates of DOX-induced CHF rats. Both SFI and SMI administration improved left ventricle systolic and diastolic function and decreased DOX-induced elevated cardiac function markers. Moreover, SFI showed superior properties in attenuating DOX-induced ROS and inhibition of antioxidant enzyme activities, thus inhibiting DOX-induced cardiac apoptosis. SFI mitigates DOX-induced oxidative stress via activated Keap1/Nrf2/ARE pathways. In conclusion, this study identified that DOX associated CHF may belong to Yang Deficiency in TCM and SFI effectively protects against DOX-induced CHF through activation of Keap1/Nrf2/ARE pathways.

Keywords: Chronic heart failure, doxorubicin, traditional Chinese medicine, Nrf2

Introduction

Chronic heart failure (CHF), a complex clinical syndrome, is a common and lethal syndrome manifested by systemic perfusion insufficient to meet peripheral organ metabolic demands, resulting from attenuated cardiac pump function [1]. It has been considered one of the major challenges in the cardiovascular field in the twenty-first century due to its high incidence and low 5-year survival rates, similar to that of malignant tumors [2, 3]. Traditional Chinese Medicine (TCM) has played an increasingly important role in the treatment of heart failure. The combination of TCM and modern medicine could help improve the symptoms of CHF and quality of patient life [4]. According to the theory of TCM, there are many clinical types of heart failure, including Yang deficiency and Yin deficiency [5]. The main function and efficacy of different kinds of TCM vary considerably and some taboos exist regarding the use of TCM, especially for patients with acute exacerbation of CHF [6]. For example, Shenfu injection (SFI) and Shenmai injection (SMI) are two commonly used TCMs for the treatment of CHF. However, it is recommended that SFI is effective for Yang Deficiency patients, while SMI is effective for Yin deficiency patients [6]. Some studies have suggested that SFI has significant positive inotropic effects. Therefore, it should be contraindicated in diastolic heart failure in the absence of contraction dysfunction [7]. On the other hand, low blood pressure has been correlated with Yang Deficiency [8] and SFI has been commonly used in patients with hypotensive shock, but not for heart failure with hypertension, due to properties in stabilizing blood pressure [8, 9].

Therefore, dialectical treatment is critical during the usage of TCM and exact discrimination of CHF categories could further promote the modernization of TCM and clinical rational drug use for TCM.

Doxorubicin (DOX), an effective anthracycline, is a chemotherapeutic drug commonly used to treat a wide range of cancers [10]. However, its clinical use has been limited by the risk of severe cardiotoxicity, which can lead to progressive irreversible heart failure particularly by multiple cumulative doses [11]. Unfortunately, no single chemical has been applicable in reducing the progressive deleterious actions of DOX without affecting its anti-tumor efficiency. Thus, the search for an effective drug against DOX-associated heart failure remains a critical issue in both cardiology and oncology [12]. Currently, multiple mechanisms are involved in DOX-induced heart failure [13]. Of these, oxidative stress, an excess production of reactive oxygen species (ROS), has been increasingly shown to play a key role in CHF [14]. Nuclear factor erythroid-2 related factor 2 (Nrf2)-Kelch-like ECH-associated protein1 (Keap1) system constitutes the nucleus of signaling pathways in oxidative stress. Pleiotropic protein Nrf2 may have protective effects on CHF via suppressing oxidative stress [15].

The present study aimed to determine the characteristics of the TCM category of DOX-induced CHF in Sprague-Dawley rats, investigating the effects of SMI and SFI on DOX-induced CHF and underlying mechanisms involved in antioxidant properties and activation of Nrf2 pathways. This study further aimed to provide some experimental basis for use of TCM in clinical practice.

Materials and methods

Animals

Sprague-Dawley (SD) rats, male, specific pathogen-free (SPF) grade, 6 weeks old, and 210 ± 10 g in weight, were obtained from Hunan Slack Kingda Experimental Animal Co., Ltd. (Changsha, Hunan, China), license number SCXK9 (Hunan) 2013-0004, animal quality certificate number no. 43004700007659. The rats were bred in the Laboratory Animal Center of Hunan University of Chinese Medicine (HUCM) (Changsha, Hunan, China). They were housed in separate cages and maintained under a 12-hour light/12-hour dark cycle at 24°C with free access to rat chow and water. All animal studies were performed according to guidelines of the Institutional Animal Care and Use Committee of HUCM. Experimental protocols involving the animals and their care were approved by the Experimental Animal Ethics Committee of HUCM. All surgeries were performed under pentobarbital sodium anesthesia.

Drugs and reagents

Doxorubicin was purchased from Zhejiang Haizheng Pharmaceutical Co., Ltd. (Hangzhou, Zhejiang, China, lot number: 130704). SMI was purchased from Zhengda Qingchun Bao Pharmaceutical Co., Ltd. (Hangzhou, Zhejiang, China, lot number: 1402225) and SFI was purchased from Ya'an Sanjiu Pharmaceutical Co., Ltd. (Chengdu, Sichuan, China, lot number: 140205010). Chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lipid Peroxidation MDA Assay Kit, Catalase (CAT) Assay Kit, Total Superoxide Dismutase (SOD) Assay Kit, Glutathione Reductase (GSH) Assay Kit, and Glutathione Peroxidase (GPx) Assay Kit were all obtained from Beyotime (Jiangsu, China). Creatine kinase (CK), creatine kinase-MB (CK-MB), glutamic oxaloacetic transaminase (AST), and lactate dehydrogenase (LDH) diagnostic biochemical assay kits were obtained from Biosino Biotechnology Company, Ltd. (Beijing, China).

Induction of CHF rats and drug treatment

A total of 40 rats received intraperitoneal injections of DOX for 7 weeks at a dose of 1.5 mg/kg (i.e., 0.75 mL/kg, two times per week) to achieve an accumulative total dose of 21 mg/kg. Rats in the control group (n=11) were injected with the same amount of sterile water. After successful establishment of CHF rat models with significantly elevated N-terminal pro-brain-natriuretic peptide (NT-proBNP) and loss of cardiac function detected by echocardiography, the rats (n=33) were randomly divided into three groups: DOX group, SFI group, and SMI group. They were treated daily with intraperitoneal injections for 15 days with SFI (6.0 ml/kg, once a day, equivalent to a human clinical daily dose), SMI (6.0 ml/kg, once a day, equivalent to human clinical daily dose), or 0.5% Polysorbate

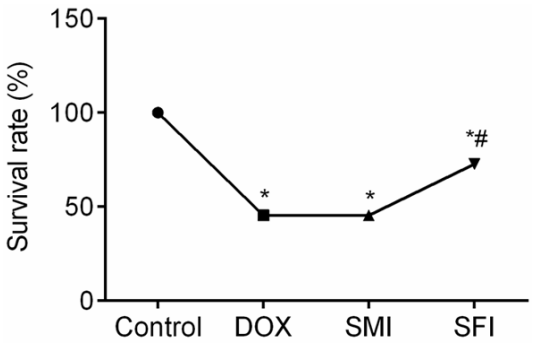

Figure 1. SFI improves survival rates of DOX-treated rats. The number of survival rats in control group, DOX, SMI, and SFI groups was 11, 5, 5 and 8 (n=11 in each group). $^{*}P < 0.05$ vs. control group; $^{\#}P < 0.05$ vs. SMI group.

80 solution (the common solvent of SFI and SMI). General conditions of CHF rats were observed daily and animal survival rates after administration were calculated.

Echocardiographic examination and hemodynamic indexes assay

After administration with DOX and injections with SMI or SFI, echocardiographic examinations were performed to detect cardiac function, according to methods previously described [16]. After anesthesia using 40 mg/kg sodium pentobarbital (Sigma-Aldrich; Merck, Darmstadt, Germany), the rats were fixed in a supine position. The M2 curve was measured in left ventricular long axis and left ventricular papillary muscle level with a linear probe. Left ventricular end-diastolic volume (LVEDD), left ventricular end-diastolic diameter (LVESD), left ventricular end-diastolic volume (LVEDV), and left ventricular end-systolic volume (LVESV) were surveyed for three cardiac cycles and means were obtained. Next, the value of EF and left ventricular shortening scores (LVFS or LVFS), used as a cardiac function parameter index, were calculated by the Simpson method [17, 18].

Hemodynamic indexes were measured using a BIOPAC-MP150 multi-guided physiologic recorder (Biopac systems, inc., Goleta, CA USA). Briefly, after intraperitoneal anesthesia, the rats were fixed in supine position and the right common carotid arteries (CCA) were isolated. A science pressure catheter was slowly inserted into the left ventricle through the CCA until the left ventricular pressure wave could be observed. When the blood pressure curve stabilized for 3 minutes, left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), maximum left ventricular pressure (+dp/dt max), and maximum pressure drop (-dp/dt max) were measured and processed by AcqKnowledge 4.2 (Biopac systems, Inc.).

Assessment of cardiac function indices

Blood samples were then collected from the inner-eye canthus into heparinized capillary tubes. After 1.5 mL blood was collected, the capillary glass tube was removed and the point of insertion was pressed with a medical sterilization cotton ball to stop bleeding. Content of NT-proBNP in the serum was detected by ELISA and the operation was conducted strictly according to ELISA kit instructions. Levels of CK, CK-MB, LDH and AST in serum were measured, according to manufacturer instructions. Subsequently, all rats were immediately euthanized and the hearts were rapidly harvested, washed, and cut longitudinally. These were thoroughly washed with PBS and kept at -80°C.

Histological examinations

After being sacrificed, the left ventricle of each rat was fixed in 10% formalin overnight at -4°C, embedded in paraffin, and cut into sections of 5 μm thickness. Microscopic myocardial changes were observed and analyzed after hematoxylin and eosin (H&E) staining. An *in situ* cell death detection kit, POD (Roche, Basel, Switzerland), was used to detect cell apoptosis in heart tissues. Sections were incubated with proteinase K at 37°C for 20 minutes. Tissues were then incubated with the TdT-mediated dUTP nick end labeling (TUNEL) reaction mixture at 37°C for 1 hour in the dark. Subsequently, the sections were covered with converter-POD at 37°C for 30 minutes, followed by incubation with DAB for 3 minutes. After counterstaining with the nuclear counterstain hematoxylin for 30 seconds, the number of TUNEL-positive cells was counted with a Leica microscope (DM1000, Leica) at 200 × magnification.

Measurement of oxidative stress and antioxidant enzyme activity

A 10% cardiac tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4) using a homogenizer (Tissue Tearor, Biospec, Ba-

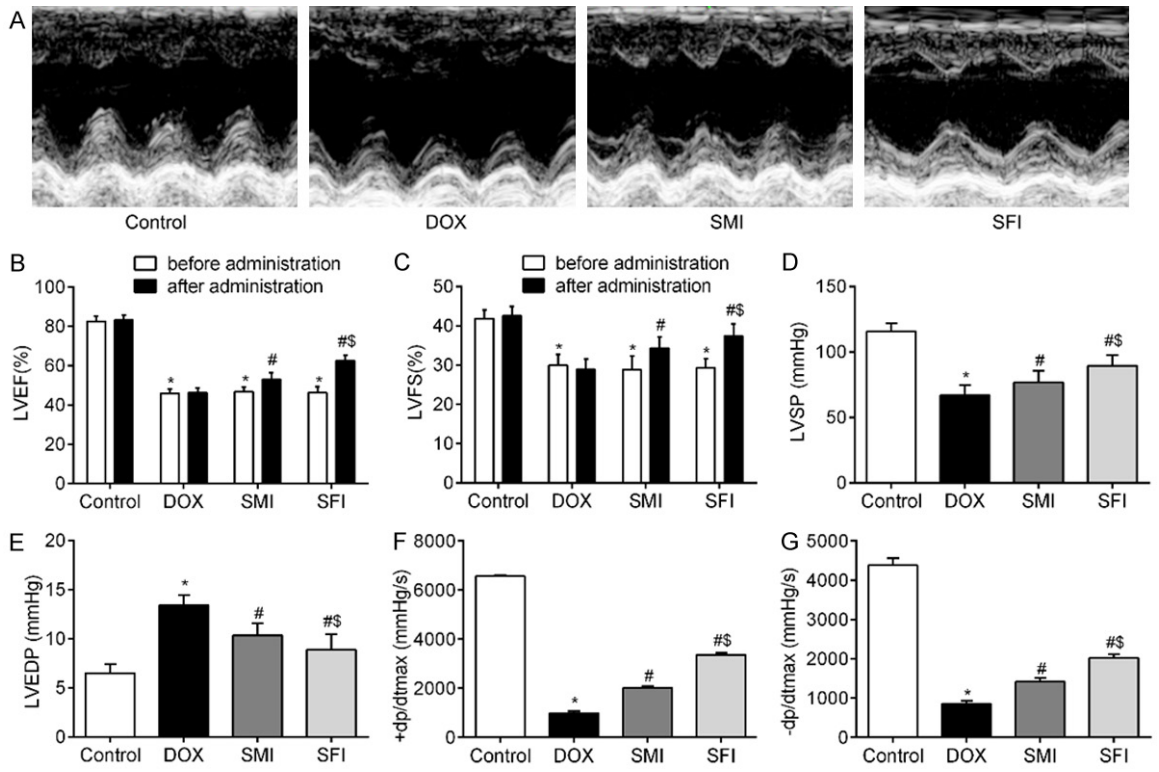

Figure 2. SFI and SMI improves cardiac function and hemodynamic indexes of CHF rats. (A) Representative images of anatomic M-mode echocardiography of Sprague-Dawley rat hearts from control group (n=11), doxorubicin-treated group (DOX group, n=5), DOX-treated rats with SMI treatments (SMI group, n=5), and DOX-treated rats with SFI treatments (SFI group, n=8). Results of (B) LVEF and (C) LVFS were detected before and after SMI or SFI injections in the four indicated groups. The hemodynamic indexes of DOX-induced chronic heart failure (CHF) rats including (D) LVSP; (E) LVEDP; (F) +dp/dt max; (G) -dp/dt max were measured and recorded after administration with SMI or SFI for 15 days. *, $P < 0.05$ vs. control group; #, $P < 0.05$ vs. DOX group; $, $P < 0.05$ vs. SMI group.

rtlesville, OK USA). The homogenate was centrifuged at 14,000 × g for 15 minutes at 4°C to remove cell debris. A fluorometric assay with DCFH-DA was used for reactive oxygen species (ROS) detection in a whole cell of cardiac tissue homogenates. Briefly, cardiac tissue homogenates were loaded with 10 μM DCFH-DA and incubated in the dark for 30 minutes and 45 minutes, respectively, to allow formation of DCF. They were then analyzed for fluorescence (excitation 485 nm/emission 529 nm) using a F-3000 Hitachi spectrophotometer (Hitachi, Ltd., Chiyoda-ku, Tokyo, Japan). Values were expressed as fluorescence intensity normalized/mg protein. Aliquots of the supernatant were used for determining the levels of MDA and activities of CAT, SOD, GPx and GSH, in accordance with manufacturer instructions.

Western blot

Total protein was extracted from heart tissues using RIPA lysis buffer (Beyotime, Jiangsu, China) and equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (Millipore, MA, USA). The membranes were then blocked with 5% skim milk for 1 hour at room temperature and hybridized with primary antibodies against Bcl-2 (1:1000), Bax (1:1000), caspase-3 (1:1000) and cleaved caspase-3 (1:1000), as well as β-actin (1:2000) (antibodies were all obtained from Abcam, Cambridge, MA, USA) at 4°C overnight. Membranes were then treated with corresponding horseradish-peroxidase-conjugated secondary antibodies for 1 hour at room temperature and visualized using an ECL detection kit (Beyotime). Quantity One software (version 4.6) was used for analysis.

Statistical analysis

All experiments were carried out in triplicate and repeated three times. Data are expressed as mean ± standard error SD for the indicated number of independently performed experi-

Table 1. Effects of SMI and SFI on serum NT-proBNP, CK, CK-MB, LDH, and AST levels in DOX-induced rats

	NT-pro BNP (pg/mL)	CK (U/mL)	CK (U/mL)	LDH (U/mL)	AST (IU/mL)
Control (n=11)	98.2 ± 8.3	40.4 ± 2.6	45.4 ± 3.1	59.1 ± 3.5	67.7 ± 4.2
DOX (n=5)	541.2 ± 29.4*	92.7 ± 2.8*	94.6 ± 2.4*	134.8 ± 7.8*	148.6 ± 5.9*
SMI (n=5)	409.6 ± 15.2$^{\#}$	76.3 ± 5.5$^{\#}$	71.7 ± 4.6$^{\#}$	99.4 ± 5.3$^{\#}$	89.5 ± 10.5$^{\#}$
SFI (n=8)	349.7 ± 22.7$^{\#,\$}$	59.6 ± 3.7$^{\#,\$}$	56.2 ± 4.5$^{\#,\$}$	70.9 ± 5.1$^{\#,\$}$	76.0 ± 9.6$^{\#,\$}$

Note: $^{*}P < 0.05$ significantly different from control; $^{\#}P < 0.05$ significantly different from DOX and $^{\$}P < 0.05$ significantly different from SMI (one-way ANOVA followed by Tukey's post hoc test).

ments using SPSS package 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance within the parameters was evaluated by one-way analysis of variation (ANOVA), followed by Tukey's post hoc test. $P < 0.05$ indicates statistical significance.

Results

SFI, but not SMI, improves survival rates of CHF rats

After treatment with DOX for 7 weeks, a total of 33 rats survived from DOX-induced toxicity (1 died from malnutrition, 2 for intestinal obstruction, 2 for pleural effusion, and 2 for heart failure). The success rate of establishing the CHF rat model was 82.5% (33/40). After medicine with SMI or SFI for 15 days, survival rates in control, DOX, SMI, and SFI groups were 100%, 45.45%, 45.45%, and 72.73% (**Figure 1**). SFI significantly improved survival rates, suggesting that SFI plays an effective protection role in the development of DOX-induced CHF.

SFI and SMI attenuate DOX-induced CHF

Subsequently, this study assessed the effects of SMI and SFI on cardiac function. As shown in **Figure 2A**, M-mode images showed significant echocardiographic changes in both SMI and SFI treated groups. Results suggest that LVEF (**Figure 2B**) and LVFS (**Figure 2C**) were significantly reduced before administration. However, LVEF and LVFS were significantly increased in the SMI group after administration and further increased in SFI the group. Moreover, hemodynamic parameters in different groups were then investigated. Compared to the control group, DOX treatment markedly interfered with hemodynamics, as evidenced by reduced LVSP (**Figure 2D**), LVEDP (**Figure 2D**), +dp/dt max (**Figure 2E**), and -dp/dt max (**Figure 2F**), which were ameliorated by SMI treatment. Results showed that hemodynamic parameters in the SFI group were further recovered, compared to the SMI group. Results revealed that SFI showed more excellent properties in improving cardiac function in DOX-induced CHF rats than SMI.

SFI and SMI decrease DOX-induced elevated cardiac function markers

Levels of serum cytokine NT-pro BNP, CK, CK-MB, LDH and ALT of rats in the DOX-treated group and SMI or SFI administration groups were determined. Expectedly, DOX significantly increased NT-pro BNP levels, as well as serum levels of CK, CK-MB, LDH and ALT, compared with those in the control group. There was a significant reduction in the abovementioned serum cytokines between the SMI group and DOX group. Moreover, values of NT-pro BNP and other cardiac function indices in the SFI group were obviously decreased, compared with those in the SMI group (**Table 1**).

SFI and SMI inhibit DOX-induced ROS and inhibition of antioxidant enzyme activities

H&E staining of the heart sections of the DOX group demonstrated a great number of inflammatory cells, massive cardiomyocyte degeneration, and swelling of the myocardial fibers, compared with the control group. Interestingly, after SFI and SMI administration, these injuries were significantly reduced (**Figure 3A**). To understand cellular mechanisms associated with the beneficial effects of SFI and SMI, levels of oxidative stress and endogenous antioxidant enzyme activities were measured. As illustrated in **Figure 3B**, compared with the control group, levels of ROS were obviously increased in the DOX group. In contrast, SMI effectively reduced DOX-induced generation of ROS. Levels of ROS in the SFI group were further reduced, compared to the SMI group. Oxidative stress was characterized by the analysis of lipid peroxidation and levels of MDA were signifi-

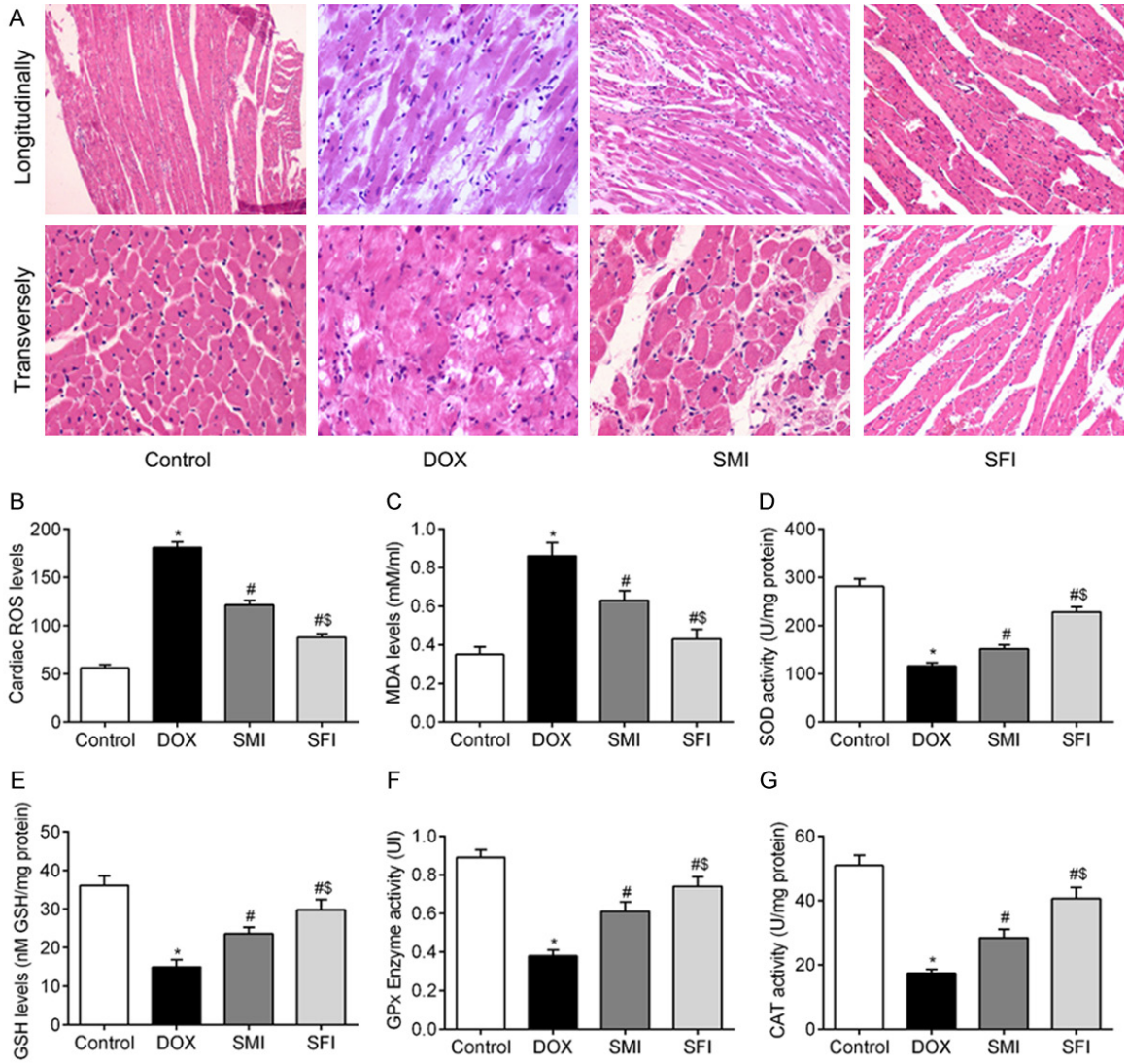

Figure 3. SFI and SMI repress DOX-activated oxidative stress. (A) Histopathological changes of the myocardial tissue of different groups, which cut longitudinally (upper panel) and transversely (low panel), were observed by light microscopes after administration (400 ×). (B) Levels of reactive oxygen species (ROS) in cardiac tissues were measured in DOX-induced rats treated with SMI or SFI treatment. (C) Levels of malondialdehyde (MDA), a marker of lipid peroxidation was detected in the heart homogenates. Cardiac antioxidant enzyme activities of (D) SOD, (E) GSH, (F) GPx, and (G) CAT and were evaluated in the heart homogenates in four groups. Control group: n=11; DOX and SMI groups: n=5, SFI group: n=8. *, $P < 0.05$ vs. control group; #, $P < 0.05$ vs. SMI group.

cantly increased in the DOX groups, but were alleviated by SFI and SMI administration (**Figure 3C**). Moreover, a significant decrease in the activity of antioxidant enzymes, including SOD, GSH, GPx, and CAT, was observed in DOX-treated rats, compared to the control group. In contrast, SFI and SMI remarkably reversed the reduction in antioxidant enzyme activity caused by DOX treatment (**Figure 3D-G**). Results indicate that SFI showed superior antioxidant effects to SMI on DOX-induced oxidative stress and that SFI could markedly enhance antioxidant enzyme activities.

SFI and SMI protect against DOX-induced cardiomyocytes apoptosis

Because apoptosis plays a key role in DOX-induced cardiotoxicity, this study explored the effects of SFI and SMI on DOX-induced myocardial apoptosis. DOX induced cell apoptosis was detected by TUNEL staining in cardiac tissues. Intriguingly, both SFI and SMI administration significantly attenuated DOX-induced apoptosis. Apoptotic rates in the SFI group were significantly lower than those in the SMI group (**Figure 4A**). In addition, the ratio of Bax/

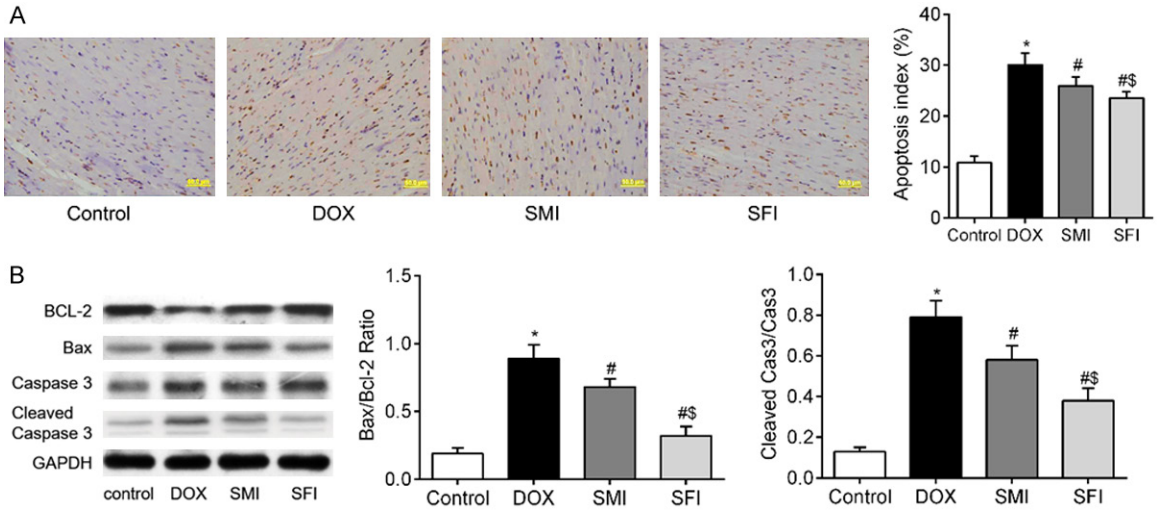

Figure 4. SFI and SMI blunt DOX-induced cardiomyocytes apoptosis. A. Representative pictures of TUNEL assay results (magnification at 400 ×) and TUNEL apoptotic index were determined by calculating the ratio of TUNEL-positive cells to total cells (Control group: n=11; DOX and SMI groups: n=5, SFI group: n=8). B. Representative immunoblots showing Bax, Bcl-2, caspase 3, and cleaved caspase 3 expression. Blots represent the mean ± SD of three separate experiments. Bar diagram showing densitometric analysis for relative expression of Bax/Bcl-2 ratio and cleaved cas3/Cas3 ratio. *, $P < 0.05$ vs. control group; #, $P < 0.05$ vs. SMI group.

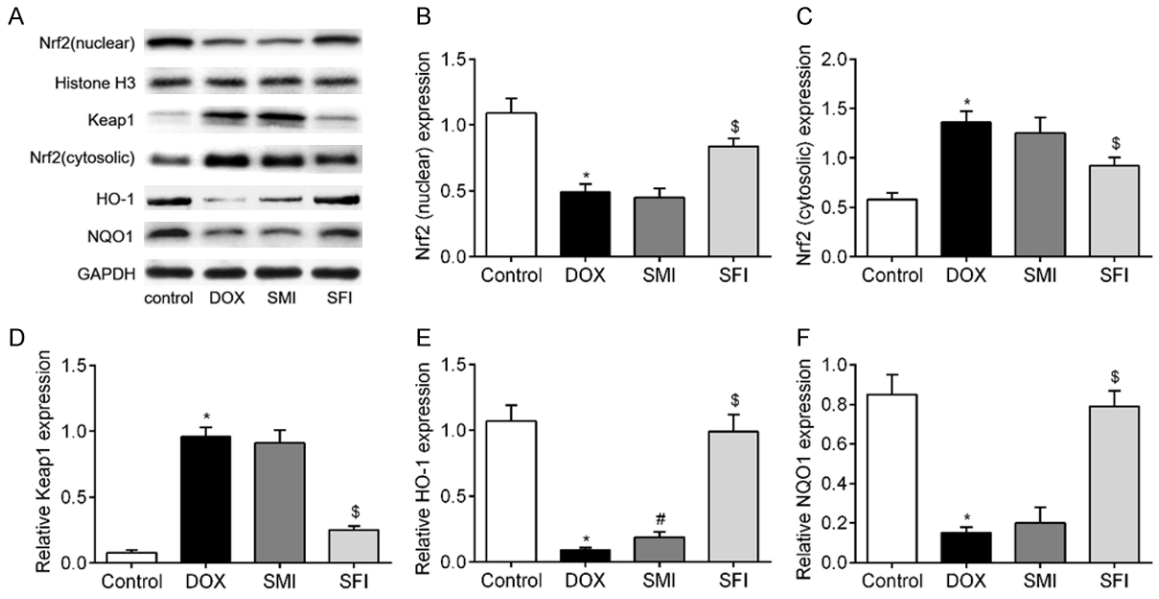

Figure 5. SFI induces Nrf2-mediated activation of ARE pathways in cardiac tissue. (A) Protein expression profile of Nrf2 (nuclear), Histone H3, Keap1, Nrf2 (cytosolic), HO-1, and NQO1 in heart tissues from different groups as indicated. The bar diagrams show relative band intensity of (B) Nrf2 (nuclear), (C) Nrf2 (cytosolic), (D) Keap1, (E) HO1, and (F) NQO1. Results were normalized with Histone H3 for Nrf2 (nuclear), GAPDH for the remaining. *, $P < 0.05$ vs. control group; #, $P < 0.05$ vs. SMI group.

Bcl-2 and cleaved Cas3/Cas3 was dramatically increased in the DOX group but decreased in the SFI and SMI group (**Figure 4B**). These results suggest that both SFI and SMI could prevent DOX-induced apoptosis.

SFI mitigates DOX-induced oxidative stress via activated Nrf2 pathways

Next, the present study investigated potential molecular mechanisms underlying the effects

of SFI on CHF rats. Under normal conditions, Nrf2 is located in the cytoplasm and under oxidative stress. Nrf2 can be released from Keap1 and translocates into the nuclear, which controls the expression of phase2 antioxidant enzymes by binding to the Antioxidant Response Element (ARE) region [19, 20]. To determine whether SFI exerts its function by activating Nrf2, levels of cytosolic and nuclear Nrf2 protein were analyzed. As shown in **Figure 5A-D**, DOX decreased nuclear Nrf2 expression, whereas it increased Keap1 and cytoplasmic Nrf2 expression, compared to the control group. In contrast, SFI, but not SMI administration, increased nuclear translocation of Nrf2, whereas it decreased Keap1 and cytoplasmic Nrf2 expression, compared to the DOX group. Moreover, alteration of Nrf2 downstream target proteins was investigated, including HO-1 (**Figure 5E**) and NQO1 (**Figure 5F**). Results showed that DOX treatment downregulated HO-1 and NQO1 expression in cardiac tissues, compared to the control group. Administration of SFI significantly increased expression of HO-1 and NQO1. Present data indicates that SFI inhibits DOX-induced oxidative stress through stimulation of Nrf2/ARE pathways.

Discussion

DOX is active in many tumors but its clinical use has been limited by the possible development of heart failure. Cumulative doses associated with 5% risk of heart failure have been reported, providing oncologists and cardiologists with limits for safe administration of DOX [21]. In this study, CHF rat models were established by injecting DOX intraperitoneally. Results showed that hemodynamic and pathological changes of CHF rats were consistent with changes of cardiomyopathy and chronic congestive heart failure, as previously described [22-24]. Therefore, inducing a CHF rat model using DOX is a stable, reliable, and repeatable method which could be used for finding novel agents for treatment of CHF. The present study compared the effects of SFI and SMI on DOX-induced CHF, finding that both could be effective in attenuating CHF. However, SFI showed more excellent protective properties than SMI when treating DOX associated CHF. It was assumed that this may largely due to the reason that SFI is suitable for treatment of Yang Deficiency CHF, whereas SMI is for Yin Deficiency [6], leading to the different effects on CHF rats. The excellent properties of SFI, compared to SMI, indicate that the syndrome of DOX-induced CHF may be via Yang Deficiency.

Hemodynamic indexes, such as LVEF, LVFS, LVSP, LVEDP, and ±dp/dt max, can reflect the function of contraction and diastole of the left ventricle [25]. Values of the above markers were reduced after DOX treatment, but significantly improved with the addition of SMI and SFI, suggesting the cardioprotective effects of both SMI and SFI on DOX-induced left ventricular dysfunction. NT-proBNP plays a central role in the diagnosis of DOX-induced cardiac dysfunction, as well as CHF in a rat model [26]. In accordance with previous studies, more than 450 pg/mL was taken as the reference standard of NT-pro BNP in CHF rats [27]. Administration of SFI or SMI significantly reduced its levels. In parallel, it was found that SFI or SMI treatment attenuated levels of serum cardiac injury markers CK, CK-MB, LDH, and AST in DOX-treated mice. This function may largely be due to the fact that SFI or SMI could restrict leakage of cardiac markers into the blood by stabilizing the membranes of myocardium.

Involvement of oxidative stress in the mechanisms of DOX-induced cardiotoxicity has been the subject of many reviews [28]. Oxidative stress is the imbalance between the production of reactive oxygen species and intrinsic antioxidant mechanisms. Lipid peroxidation is the formation of a free radical chain that alters membrane permeability and cell function [29]. The present study found increased levels of MDA, which is the product of lipid peroxidation, in DOX-treated rats, while administration of SFI and SMI significantly reduced MDA levels. Cardiomyocytes are more vulnerable to DOX-induced damage than other organs due to highly active metabolic machinery in the heart as well as lower levels of antioxidant enzymes. Consistent with previous studies [30], present results showed that DOX depleted levels of SOD, GSH, GPx, and CAT. You et al. suggested that Sheng-Mai-San, which owns the same main components as SMI, could protective against DOX-induced cardiomyopathy through attenuation in the DOX-induced increase in MDA levels and reduction in antioxidant enzymes [31]. Similarly, Ma et al. found that Sheng-Mai Yin protects DOX-induced cardiac toxicity through the restriction of myocardial fibrosis [32]. The present study confirmed the

above conclusion. Furthermore, SFI showed superior ability to SMI in reducing oxidative stress. SFI may also act as a stimulator of the activity of antioxidant enzymes. To the best of our knowledge, although previous studies have demonstrated the protective effects of SFI on heart failure [8, 33, 34], this is the first study revealing the remarkable protective effects of SFI on DOX-induced CHF in rats.

The present study explored the deep mechanisms of how SFI exerts its function. Nrf2 is a redox-sensitive transcription factor that transfers to the nucleus to activate expression of antioxidant genes by binding to ARE [35]. Recently, Nrf2 has been reported to play an important role in DOX-induced cardiotoxicity and deficiency of Nrf2 aggravates cardiotoxicity and cardiac function [36]. Activation of Nrf2/ARE signaling attenuates the generation of ROS and apoptosis of cardiomyocytes, indicating protective effects against DOX-induced cardiomyopathy [37], and even CHF. Present data showed that both SMI and SFI administration could inhibit DOX-induced apoptosis in cardiac tissues. However, results showed that only SFI could activate Nrf2/ARE pathways in DOX-induced cardiac injuries. Consistent with previous studies [38], DOX treatment caused significant apoptosis and increased cytoplasmic Nrf2 expression, while decreasing Keap1 and Nrf2 nuclear translocation. Downregulation of Nrf2 target genes, including HO-1 and NQOl levels, was also observed in DOX-treated rats. Interestingly, it was found that SFI, but not SMI treatment, stimulated Nrf2 activity. This may explain the preferable protection of SFI on DOX-induced CHF. The present study revealed that the Nrf2/ARE antioxidant signaling axis was involved in SFI-mediated cardioprotection.

In conclusion, this study demonstrated that SFI showed superior properties to SMI in protecting against DOX-induced CHF, suggesting that DOX association may belong to Yang Deficiency in TCM. SFI not only prevented DOX-induced CHF, but also decreased oxidative stress levels, increased expression of antioxidant enzymes, and restored the activity of Keap1/Nrf2/ARE pathways.

Acknowledgements

We warmly thank the study participants who were also members of the Hunan Provincial Key Laboratory of Diagnostics in Chinese Medicine. This study was funded by a grant from the National Natural Science Foundation of China (No. 81373550, No. 81774208, and No. 81503627). The funding body had no role in this analysis, the writing of this article, or the decision to submit this article for publication.

Disclosure of conflict of interest

None.

Address correspondence to: Zhixi Hu, Hunan Provincial Key Laboratory of Diagnostics in Chinese Medicine, Hunan University of Chinese Medicine, 300C Xueshi Road, Changsha 410208, Hunan, China. Tel: +86-0731-88458217; E-mail: zhixihuuu@yeah.net

References

[1] Metra M and Teerlink JR. Heart failure. Lancet 2017; 390: 1981-1995.

[2] Mosterd A and Hoes AW. Clinical epidemiology of heart failure. Heart 2007; 93: 1137-46.

[3] Meijers WC, van der Velde AR and de Boer RA. Biomarkers in heart failure with preserved ejection fraction. Neth Heart J 2016; 24: 252-8.

[4] Fu S, Zhang J, Gao X, Xia Y, Ferrelli R, Fauci A, Guerra R and Hu L. Clinical practice of traditional Chinese medicines for chronic heart failure. Heart Asia 2010; 2: 24-7.

[5] Wang Q, Yao GZ, Pan GM, Huang JY, An YP and Zou X. Analysis of on medication rules for Qi-deficiency and blood-stasis syndrome of chronic heart failure based on data mining technology. Zhongguo Zhong Yao Za Zhi 2017; 42: 182-186.

[6] Yang X, Liu N, Li X, Yang Y, Wang X, Li L, Jiang L, Gao Y, Tang H, Tang Y, Xing Y and Shang H. A review on the effect of traditional Chinese medicine against anthracycline-induced cardiac toxicity. Front Pharmacol 2018; 9: 444.

[7] Wang KH, Wu JR, Zhang D, Duan XJ and Ni MW. Comparative efficacy of Chinese herbal injections for treating chronic heart failure: a network meta-analysis. BMC Complement Altern Med 2018; 18: 41.

[8] Wen-Ting S, Fa-Feng C, Li X, Cheng-Ren L and Jian-Xun L. Chinese medicine shenfu injection for heart failure: a systematic review and meta-analysis. Evid Based Complement Alternat Med 2012; 2012: 713149.

[9] Yang FJ, Wang ZR, Lin DP, Qu Y, Yin HH, Shi LL, Guo HL, Xiao J, Wang YQ and Liu RT. The influence on hemodynamics of myocardial ischemic dogs and blood pressure of animals with shenfu injection. Zhongguo Zhong Yao Za Zhi 2003; 28: 259-62.

[10] Tocchetti CG, Carpi A, Coppola C, Quintavalle C, Rea D, Campesan M, Arcari A, Piscopo G, Cipresso C, Monti MG, De Lorenzo C, Arra C, Condorelli G, Di Lisa F and Maurea N. Ranolazine protects from doxorubicin-induced oxidative stress and cardiac dysfunction. Eur J Heart Fail 2014; 16: 358-66.
[11] Renu K, Abilash VG, Tirupathi Pichiah PB and Arunachalam S. Molecular mechanism of doxorubicin-induced cardiomyopathy - an update. Eur J Pharmacol 2018; 818: 241-253.
[12] Ahmed LA and El-Maraghy SA. Nicorandil ameliorates mitochondrial dysfunction in doxorubicin-induced heart failure in rats: possible mechanism of cardioprotection. Biochem Pharmacol 2013; 86: 1301-10.
[13] Ojha S, Al Taee H, Goyal S, Mahajan UB, Patil CR, Arya DS and Rajesh M. Cardioprotective potentials of plant-derived small molecules against doxorubicin associated cardiotoxicity. Oxid Med Cell Longev 2016; 2016: 5724973.
[14] Akolkar G, da Silva Dias D, Ayyappan P, Bagchi AK, Jassal DS, Salemi VMC, Irigoyen MC, De Angelis K and Singal PK. Vitamin C mitigates oxidative/nitrosative stress and inflammation in doxorubicin-induced cardiomyopathy. Am J Physiol Heart Circ Physiol 2017; 313: H795-H809.
[15] Kishi T. Disruption of central antioxidant property of nuclear factor erythroid 2-related factor 2 worsens circulatory homeostasis with baroreflex dysfunction in heart failure. Int J Mol Sci 2018; 19.
[16] Fidale TM, Antunes HKM, Alex Dos Santos L, Rodrigues de Souza F, Deconte SR, Borges Rosa de Moura F, Mantovani MM, Alves Duarte PR, Roever L and Resende ES. Increased dietary leucine reduces doxorubicin-associated cardiac dysfunction in rats. Front Physiol 2018; 8: 1042.
[17] Tanaka N, Dalton N, Mao L, Rockman HA, Peterson KL, Gottshall KR, Hunter JJ, Chien KR, Ross J Jr. Transthoracic echocardiography in models of cardiac disease in the mouse. Circulation 1996; 94: 1109-17.
[18] Arenas IA, Mihos CG, DeFaria Yeh D, Yucel E, Elmahdy HM and Santana O. Echocardiographic and clinical markers of left ventricular ejection fraction and moderate or greater systolic dysfunction in left ventricular noncompaction cardiomyopathy. Echocardiography 2018; 35: 941-948.
[19] Konishi M, Baumgarten A, Ishida J, Saitoh M, Anker SD and Springer J. Protein levels in Keap1-Nrf2 system in human failing heart. Int J Cardiol 2016; 225: 62-64.
[20] Tao G, Kahr PC, Morikawa Y, Zhang M, Rahmani M, Heallen TR, Li L, Sun Z, Olson EN, Amendt BA and Martin JF. Pitx2 promotes heart repair by activating the antioxidant response after cardiac injury. Nature 2016; 534: 119-23.
[21] Salvatorelli E, Menna P, Chello M, Covino E and Minotti G. Modeling human myocardium exposure to doxorubicin defines the risk of heart failure from low-dose doxorubicin. J Pharmacol Exp Ther 2017; 362: 263-270.
[22] Guo R, Hua Y, Ren J, Bornfeldt KE and Nair S. Cardiomyocyte-specific disruption of cathepsin K protects against doxorubicin-induced cardiotoxicity. Cell Death Dis 2018; 9: 692.
[23] Kirkham AA, Shave RE, Bland KA, Bovard JM, Eves ND, Gelmon KA, McKenzie DC, Virani SA, Stöhr EJ, Warburton DER, Campbell KL. Protective effects of acute exercise prior to doxorubicin on cardiac function of breast cancer patients: a proof-of-concept RCT. Int J Cardiol 2017; 245: 263-270.
[24] Lee PJ, Rudenko D, Kuliszewski MA, Liao C, Kabir MG, Connelly KA and Leong-Poi H. Survivin gene therapy attenuates left ventricular systolic dysfunction in doxorubicin cardiomyopathy by reducing apoptosis and fibrosis. Cardiovasc Res 2014; 101: 423-33.
[25] Salah EM, Bastacky SI, Jackson EK and Tofovic SP. Captopril attenuates cardiovascular and renal disease in a rat model of heart failure with preserved ejection fraction. J Cardiovasc Pharmacol 2018; 71: 205-214.
[26] Kittiwarawut A, Vorasettakarnkij Y, Tanasanvimon S, Manasnayakorn S and Sriuranpong V. Serum NT-proBNP in the early detection of doxorubicin-induced cardiac dysfunction. Asia Pac J Clin Oncol 2013; 9: 155-61.
[27] Martinez-Rumayor A, Richards AM, Burnett JC and Januzzi JL Jr. Biology of the natriuretic peptides. Am J Cardiol 2008; 101: 3-8.
[28] Shabalala S, Muller CJF, Louw J and Johnson R. Polyphenols, autophagy and doxorubicin-induced cardiotoxicity. Life Sci 2017; 180: 160-170.
[29] Terman A and Brunk UT. Autophagy in cardiac myocyte homeostasis, aging, and pathology. Cardiovasc Res 2005; 68: 355-65.
[30] Ghorbanzadeh V, Mohammadi M, Mohaddes G, Dariushnejad H, Chodari L and Mohammadi S. Protective effect of crocin and voluntary exercise against oxidative stress in the heart of high-fat diet-induced type 2 diabetic rats. Physiol Int 2016; 103: 459-468.
[31] You JS, Huang HF, Chang YL and Lee YS. Sheng-mai-san reduces adriamycin-induced cardiomyopathy in rats. Am J Chin Med 2006; 34: 295-305.
[32] Ma S, Li X, Dong L, Zhu J, Zhang H and Jia Y. Protective effect of Sheng-Mai Yin, a traditional Chinese preparation, against doxorubicin-induced cardiac toxicity in rats. BMC Complement Altern Med 2016; 16: 61.

[33] Ni J, Shi Y, Li L, Chen J, Li L, Li M, Zhu J, Zhu Y, Fan G. Cardioprotection against heart failure by shenfu injection via TGF-beta/smads signaling pathway. Evid Based Complement Alternat Med 2017; 2017: 7083016.
[34] Yan X, Wu H, Ren J, Liu Y, Wang S, Yang J, Qin S and Wu D. Shenfu formula reduces cardiomyocyte apoptosis in heart failure rats by regulating microRNAs. J Ethnopharmacol 2018; 227: 105-112.
[35] Niture SK, Khatri R and Jaiswal AK. Regulation of Nrf2-an update. Free Radic Biol Med 2014; 66: 36-44.
[36] Li S, Wang W, Niu T, Wang H, Li B, Shao L, Lai Y, Li H, Janicki JS, Wang XL, Tang D and Cui T. Nrf2 deficiency exaggerates doxorubicin-induced cardiotoxicity and cardiac dysfunction. Oxid Med Cell Longev 2014; 2014: 748524.
[37] Singh P, Sharma R, McElhanon K, Allen CD, Megyesi JK, Beneš H and Singh SP. Sulforaphane protects the heart from doxorubicin-induced toxicity. Free Radic Biol Med 2015; 86: 90-101.
[38] Bai Y, Chen Q, Sun YP, Wang X, Lv L, Zhang LP, Liu JS, Zhao S and Wang XL. Sulforaphane protection against the development of doxorubicin-induced chronic heart failure is associated with Nrf2 upregulation. Cardiovasc Ther 2017; 35.