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Protection of Shengmai Recipe on Improving Cardiac Function and Attenuating Kidney Injury in Pressure Overload Rats

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ABSTRACT

Objective Shengmai Recipe (SMR) is a Chinese patent medicine used for the treatment of chronic heart disease. In order to further assess the renal-protective effect against ischemia lesion of SMR, the cardioprotective effect of SMR on pressure overload-induced left ventricular (LV) systolic dysfunction and the potential mechanism on alleviating myocardial damage, myocardial fibrosis, and renal ischemia lesion in chronic heart failure (CHF) rats were investigated. **Methods** Rats with partially ligated abdominal aorta were randomly divided into model, Sham, and SMR groups. One week after recovery from surgery, animals were preventively ig administered with SMR at the dose of 810 mg/kg once daily for 8 weeks. Cardiac function and structure, endogenous biomarkers (CK-MB and LDH), myocardial fibrosis, and organ pathological change were estimated by echocardiography, immunodepression and velocity method, hematoxylin-eosin staining, and masson's trichrome staining, respectively. **Results** The administration of SMR significantly decreased serum CK-MB and LDH levels and reduced myocardial fibrosis. Interestingly, SMR not only improved cardiac function but also ameliorated kidney injury induced by ischemia in CHF rats. **Conclusion** SMR could enhance the LV contractile function, reduce myocardial necrosis, and reverse LV remodeling in CHF rats, and most importantly, SMR could be used to treat the renal ischemia injury in pressure overload rats.

Key words

chronic heart failure; left ventricular systolic function; myocardial fibrosis; renal ischemia lesion; Shengmai Recipe

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1. Introduction

Heart failure (HF) is a highly prevalent chronic disease in older persons. Its prevalence increases with the age, reaching 1% of the population over 65 years old; It is most commonly observed in the patients of 76 years old or older. Unlike other cardiovascular diseases (CVDs), HF is the end stage of a cardiac disease with major impact on morbidity and mortality. HF (75%) cases have antecedent hypertension (Butler, 2011; Lloyd-Jones et al, 2009). To improve the quality of life and prolong life expectancy, the concept of modern medicine in treating chronic heart failure (CHF) has markedly changed in recent years. The prevention of HF should always be a primary objective. Many potential causes of myocardial damage could be treated and the extent of myocardial damage could be reduced, including the prevention of reinfarction, accurate identification, aggressive treatment of hypertension, and so on (Remme and Suedberg, 2001).

In clinic, CHF can be divided into stable stage and acute aggravation stage. According to the New York Heart Association (NYHA) Classification of CHF, it includes four stages such as I-none, II-mild, III-moderate, and IV-severe (Figueroa and Peters, 2006). According to the traditional Chinese medicine (TCM) theory, the stable stage can also be divided into different types, such as insufficiency of heart *qi*, deficiency of both heart *qi* and *yin*, and insufficiency of heart and kidney *yang* (Jiang, 2007). Medicinal herbs are used for the prevention and treatment of CHF with a long history. The characteristics and advantages of traditional herbal medicines are very effective and have few side effects (Castro et al, 2009). Generally, HF at the stable stage can be treated mainly with Chinese materia medica (CMM). Cardiac insufficiency of degree I and II can be simply treated with CMM, and the measures of the western medical treatment should be adopted accordingly (Jiang, 2007). Since 2006, a number of CMM were clinically tested on the CHF patients, including the warming *yang*, nourishing *yin*, and activating blood strategies (Ma et al, 2010). Chinese medicinal formulas have been used to treat heart disease by aiming at arousing the potential recovery of body. Most importantly, some of them can effectively reduce the risk of recurrent disease and increase life expectancy (Chen et al, 2010; Li et al, 2011). To take full advantage of traditional herbal medicines, modern scientific research methods are invaluable to support traditional claims and also to develop traditional remedies as a viable alternative to mainstream pharmaceuticals.

Shengmai Recipe (SMR) is widely used by modification of Shengmai San (SMS), which is derived from *Yixue Qiyuan*, a medical classic written by Yuan-su Zhang in Jin Dynasty. SMR includes three cure strategies of reinforcing *qi*, nourishing *yin*, and activating blood, which consists of six herbs: dry *Ginseng Radix* (*Panax ginseng* C. A. Mey.), *Ophiopogonis Radix* (*Ophiopogon japonicus* (L. f.) Ker-Gawl.), *Schisandrae Chinensis Fructus* (*Schisandra chinensis* (Turcz.) Baill.), *Astragali Mongolici Radix* (*Astragalus membranaceus* Fisch. Bge. var. *mongholicus* Bge. Hsiao), *Salviae Miltiorrhizae Radix* (*Salvia miltiorrhiza* Bge.), and *Chanxiong Rhizoma* (*Ligusticum chuanxiong* Hort.). It has been previously reported that SMS could improve cardiac contractile function in aged rats, inhibit the process of myocardial fibrosis in myocardium of rats with diabetic cardiomyopathy, and reduce adriamycin-induced cardio-myopathy in rats (Zhang et al, 2008a; Ni et al, 2011; You et al, 2006). Modern preparation Shengmai Injection (SMI), the liquid dosage form of Shengmai San, is generally applied in the prevention and treatment of acute coronary heart disease and CHF in China. Clinical studies have revealed a variety of desirable pharmacological effects of SMI such as improving the cardiac function, inhibiting the inflammatory reaction, enhancing the anti-oxidation, etc (Ma et al, 2010; Zhang et al, 2008b; Su et al, 2005). Regarding the cardioprotection of *Astragali Mongolici Radix* in treating myocardial ischemic diseases, the mechanisms underlying may involve improving cardiac function, attenuating the oxidative injury, and reducing the myocardial cell injury induced by free radical (Yang et al, 2012). Moreover, Compound Danshen Formula (CDF) is a widely used Chinese patent medicine (CPM) which has been extensively used as a remedy and clinical prescription to treat CVDs (Li et al, 2012). *Salvia miltiorrhiza* Bunge could also inhibit the progression of diabetic nephropathy and be a therapeutic agent for ameliorating high levels of 24 h urinary protein excretion, decreasing the levels of collagen IV, etc in kidney (Kim et al, 2009). *Chuanxiong Rhizoma* is also an effective medicincal plant which has been extensively applied for many years to treat various diseases, which mainly focuses on cardiovascular and cerebrovascular diseases with other CMM (Ran et al, 2011). Study showed that both *Salviae Miltiorrhizae Radix* and *Chuanxiong Rhizoma* presented different degrees of activating the blood flow and removing blood stasis by dextran (Lu et al, 2008).

Briefly, SMR has been commonly used in the treatment of cardiovascular disease in China. However, whether SMR has a renal-protective effect against ischemia lesion remains unknown. Based on the TCM theory, clinical application, and modern pharmacological research, the effects of SMR on the prevention and treatment of pressure overload induced CHF were evaluated in the present study.

2. Materials and methods

2.1 Ethics statement

All animal protocols were approved by the Institutional Animal Use and Care Committee. All the experiments and animal care were approved by Tianjin Medical Experimental Animal Care Commission and in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation.

2.2 Preparation of SMR extracts

The SMR products was purchased from Yixin Fumai Granule (Tianjin Tasly (Liaoning) Pharmaceutical Co., Ltd.; 20120208). These products were manufactured under GMP

conditions according to the protocol described in *Chinese Pharmacopoeia 2010* with modifications. SMR is usually prescribed at a daily dose of 45 g of Yixin Fumai Granule. When this human dose was converted into an animal dose (a person of 60 kg, and a conversion factor of 0.018 between human and rat), it was equivalent to the dose (4.05g/kg) used in this study.

2.3 Rat model of pressure overload

Male Sprague Dawley rats (220−250 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), and reared under standard laboratory conditions. Pressure overload was induced by constriction of abdominal aorta as described previously with modifications (Hasenfuss, 1998; Smith and Nuttall, 1985). Briefly, SD rats were ip anesthetized with 10% chloral hydrate (3 mL/kg), a midline abdominal incision was made in the abdominal cavity, and a ligation of abdominal aorta between right and left renal artery was made by 4# silk suture. With the appearance of left renal ischemia, the needle was withdrawn. The narrow degree of aorta was about 50% controlled by ligation with a 7# syringe needle. Sham operated animals underwent the same procedure, but the aorta was not banded. One week after arotic banding, the rats were randomly divided into SMR (n = 8) and non-treated (model, n = 7) groups. The Sham-operated rats were regarded as control group (Sham, n = 8). The SMR (4.05 g/kg /10ml) was ig given by tuberculin syringe, once daily for 8 weeks. Normal saline instead of SMR was used in the model and Sham groups. Rats were weighed and inspected weekly. After 8 weeks following up, animals from each group were used for further analysis.

2.4 Echocardiography

After 8 weeks administration, all animals underwent transthoracic echocardiography by VisualSonics Vevo 2100 (Canada). The animals were anesthetized with chloral hydrate. Chests were shaved and the rats were examined in supine position with a 21-MHz phased array transducer (Agilent/Philipps, Germany). Two-dimensional left parasternal long-axis views of the left ventricle (LV) at papillary muscle level were obtained. Two-dimensional guided M-mode tracings were recorded with a sweep speed of 1000 MHz. The following parameters were measured: left ventricular dimension in diastole (LVDd), left ventricular dimension in systole (LVDs), interventricular septal thickness in diastole (IVSd), interventricular septal thickness in systole (IVSs), left ventricular posterior wall thickness in diastole (LVPWd), left ventricular posterior wall thickness in systole (LVPWs), left ventricular end diastolic volume (LVEDV), and left ventricular end systolic volume (LVESV). Based on these measurements, the following parameters were determined: ejection fraction (EF) = (LVEDV−LVESV) / LVEDV, left ventricular endocardial fractional shortening (FS) = (LVDd−LVDs) / LVDd. All data were measured for three times, recording the average.

2.5 Cardiac enzymology

At the end of the observation periods and after echocardiography, animals were weighed and blood samples were collected from the vena ophthalmica, added into 2.5 mL tubes, respectively, and then centrifuged at 3500 r/min for 15 min at 4 ^{o}C. The supernatant obtained was frozen immediately, stored at −80 °C, and thawed before analysis. Serum concentration of creatine kinase isoform MB (CK-MB) and activity of lactate dehydrogenase (LDH) were measured by immunodepression and velocity method, respectively.

2.6 Heart and kidney hematoxylin-eosin staining

After taking blood, animals were sacrificed for organ harvesting. Both hearts and kidneys were taken out immediately and weighed. Hearts and kidneys of each group were fixed in 10% formalin. Heart and renal samples fixed in formalin were embedded in paraffin wax, and 3.5−4 μm slice of histologic sections of tissues was stained with hematoxylin-eosin. The sections were examined under microscope, and photomicrographs were taken.

2.7 Masson's trichrome staining for heart and kidney

Fibrosis or collagen deposition in tissue sections was assessed by Mason's trichrome (Like Trading Co.. Ltd of Guangzhou, China) staining according to the manufacturer's instructions. Collagen appeared as blue color. Quantitation analysis of collagen area was supported by TissueFaxs and HistoQuest System (Tissue Gnostics, Austria).

2.8 Data analysis

Data are expressed as $\bar{x} \pm s$ for each group of animals. Statistical differences among groups were determined using owe-way ANOVA. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effects of SMR on LV structure and function

The results of echocardiography showed that treatment with SMR resulted in a significant improvement in LV systolic function (Figure 1). LV dimension in systole was significantly elevated 9 weeks after banding in the model group, which is indicative of LV dilation. The treatment with SMR mitigated this increase in LV size (Figure 2A). IVSs and LVPWs in the model group had decreased significantly compared with those in the Sham animals, which was indicative of ventricular wall partly being thin, and SMR group significantly improved it comparing with model group (Figure 2A). We assessed the systolic function in Sham operated and model rats compared to those in the pressure overload rats treated with SMR using echocardiography. The change in LVEDD was inversely related to the EF and FS.

Summary data for the ejection fraction and fractional shortening are shown in Figure 2B, depicting a significant improvement in ejection fraction and fractional shortening in pressure overload rats treated with SMR at 8 weeks of follow up compared to pressure overload alone. The dilation of the ventricle and the impairment of EF correlated well with the onset of clinical heart failure symptoms, as evident by dyspnea, rough fur, and inactivity.

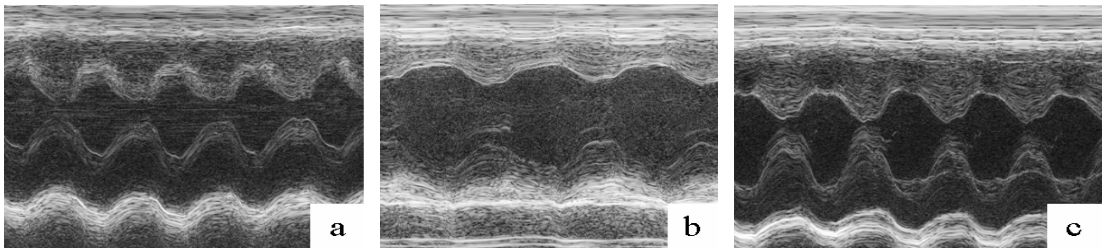

Figure 1 Representative M-mode echocardiograms

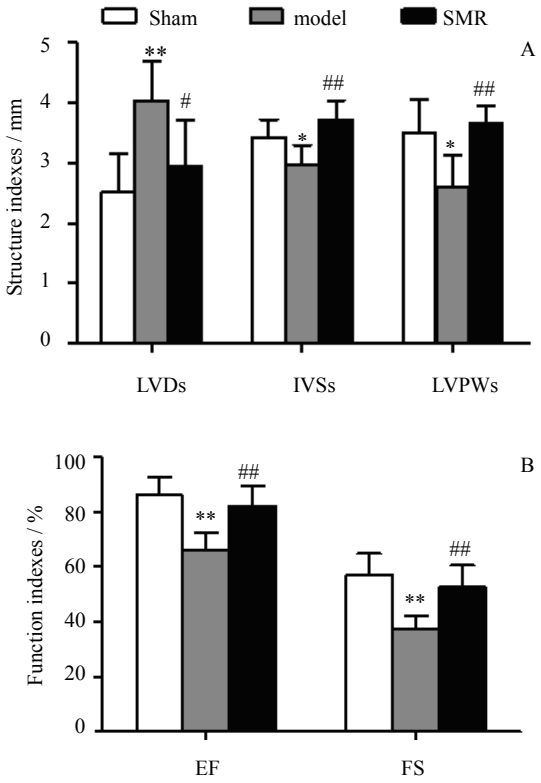

Figure 2 Effect of SMR on cardiac structure (A) and cardiac function (B) parameters in pressure overload rats

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* Sham group; $^{\#}P < 0.05$ $^{\#\#}P < 0.01$ *vs* model group; same as below

3.2 Effect of SMR on myocardial damage

Serum levels of CK-MB and LDH were significantly elevated in model rats compared with those in the Sham group, which indicated a serious myocardial injury. SMR significantly attenuated banding-induced increase in the above enzymes in serum, which was indicative of reduced myocardial necrosis (Figure 3). Heart pathology revealed that the Sham group showed normal morphology. In the model group, severe morphological damages were shown in the heart that the myocardial fibers had losing cross striations, cytoplasmic

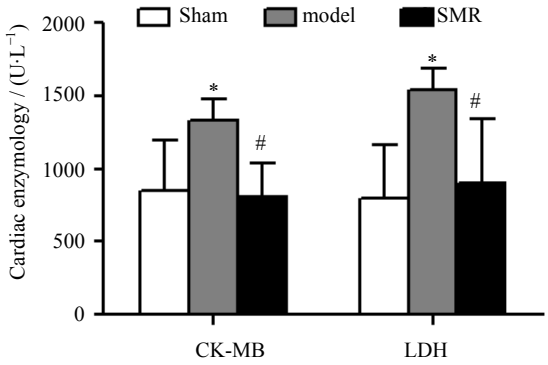

Figure 3 Effect of SMR on CK-MB and LDH activities

condensation, vacuolar degeneration, stromal hyperplasia, and inflammatory infiltration. On the other hand, the histopathological damages in SMR rats were relatively mild (Figure 4). These results demonstrated that SMR would protect the myocardial damage induced by pressure overload.

3.3 Histological assessment of myocardial fibrosis

We investigated the role of SMR in collagen deposition or fibrosis through well-established Masson's trichrome staining. Blue staining represents collagen deposition. There was significant increase in collagen deposition in model group vehicle treated for 8 weeks compared to the Sham group, and SMR treated group decrease the collagen volume fraction. There was robust interstitial and perivascular fibrosis in heart with chronic pressure overload (Figure 5). The percentage of collagen area to Masson's trichrome-stained LV cross-sections analysis was supported by TissueFax and HistoQuest system. Analysis results are shown in Figure 6. These findings suggested that SMR could reduce fibrosis or collagen deposition.

3.4 Renal histopathology and fibrosis

Renal pathology revealed that the rats in the Sham group showed normal morphology (Figure 7). After 8 weeks of vehicle and herbal treatment, the rats in the model and SMR groups showed kidney injuries of different degrees. In the

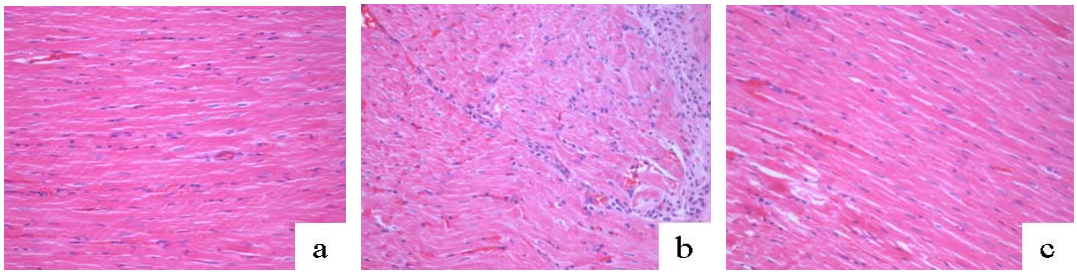

Figure 4 Profiles of heart tissues in rats treated with SMR (HE staining)

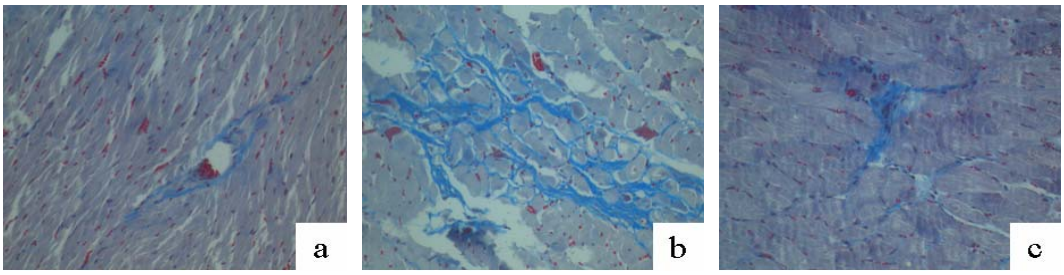

Figure 5 Profiles of heart tissues in rats treated with SMR (Masson staining)

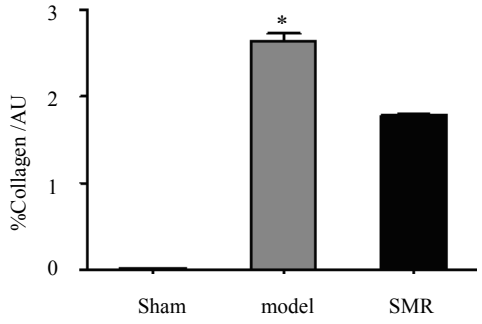

Figure 6 Quantitation of collagen volume fraction in rats after Masson's staining

model group, kidney tubules epithelial cells of rats showed severe malalignment, hydropic degeneration, protein deposition, and protein cast, kidney glomerular capsule parietal layer epithelial cells of rats showed hyperplasia, and glomerulus atrophy and calcification were observed in large parts of kidney. In the SMR treated group, kidneys of rats showed similar slight injury focally compared with age-matched model group, indicative of reduced kidney lesion. Masson's trichrome staining showed the normal kidney of Sham group (Figure 8). Glomerular and tubular destruction, more inflammatory cells infiltration, and extensive areas of fibrosis were observed in the model group. The rats in the SMR-treated group demonstrated less inflammatory cells infiltration and a reduction in the fibrotic area.

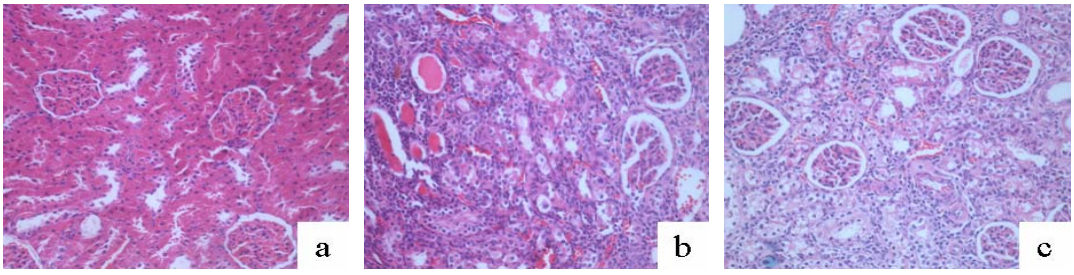

Figure 7 Profiles of kidney tissues in rats treated with SMR (HE staining)

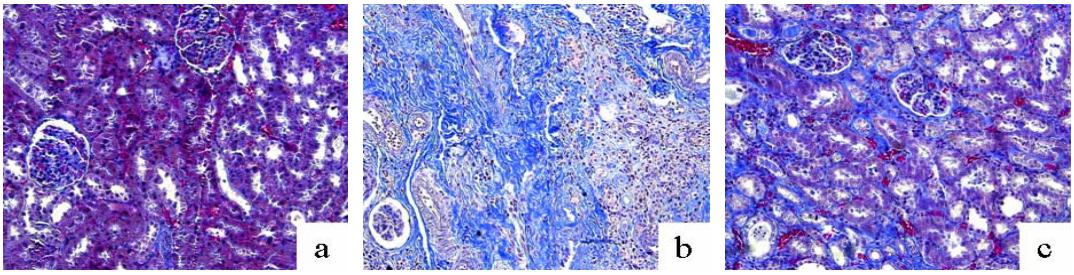

Figure 8 Profiles of kidney tissues in rats treated with SMR (Masson's staining)

4. Disscussion

The purpose of this study was to determine the preventive and therapeutic role of SMR in pressure overload induced HF. Functional studies by echocardiography showed that SMR treatment could ameliorate the LV dysfunction, which was further supported by altering the cardiac enzymology, reducing the heart lesion, and inhibiting the desmoplasia and scar formation. CHF is the end stage of various cadiovascular diseases and is usually induced by the damaged structure or function of the LV (Dickstein et al, 2008). Studies also reported that HF was mainly evaluated by LV systolic dysfunction, and the clinical manifestations and natural history of the syndrome depended primarily on the severity of LV systolic dysfunction. Practical aspects on echocardiographic timing and structured reports are widely used in outpatients with HF (De Gregorio et al, 2011). Newly released systems such as VisualSonics Vevo 2100 provided new tools for researchers to carefully and non-invasively investigate the cardiac function in mice. This system generated the high resolution images and provided the analysis capabilities similar to those used with human patients (Pistner et al, 2010).

Abdominal aorta constriction model which induced pressure loading seems to be well suited for studying the transition from hypertrophy to failure at the level of the hypertension (Hasenfuss, 1998; Smith et al, 1985; Zaha et al, 2003). The significant increase in ventricular chamber diameter and decrease in fractional shortening suggest the ventricular dysfunction during 9 weeks banding in our study. The underlying pathophysiological process is divided into three stages. Firstly, myocardial hypertrophy was an adaptive response of heart to the increased work load with compensated or even enhanced LV systolic function and normal cardiac output (Piano et al, 1998). Cardiac hypertrophy includes not only cardiomyocytes hypertrophy, but also interstitial and perivascular collagen deposition (Sabbah et al, 1993). With persistent existence of pressure loading, excessive accumulation of collagen fibers in myocardium, and remodeling of cardiac collagen network, the heart evolves to a decompensate state with depressed cardiac function, myocardial fibrosis, and increased chamber stiffness (Parmley, 1985). If myocardial fibrosis further aggravates, the cardiac function will be bound to lead to heart dysfunction and even failure (Müller et al, 2007). Therefore myocardial fibrosis is the key of heart function transition from compensation to decompensation, prevent or reverse myocardial fibrosis is an important means of delaying HF progression (Tsutsui, 2004). In our current study, we found that preventive treatment by SMR prevented collagen deposition and myocardial fibrosis as evidenced by the Masson's trichrome staining of heart sections. Collagen area was analyzed in relation to entire myocardium excluding the pericardium and tips of papillary regions.

Moreover, the association between cardiac failure and renal function impairment has gained wide recognition over the last decade. And as it is a systemic disease, it can cause the dysfunction in various organs, but especially in the kidney. The renal failure is often associated with HF, and when present together, the treatment is more complex and the prognosis is worse. This has raised ample interest in the nature of the renal function impairment in CHF and fuelled the hypothesis that specific renal protection might be of benefit in CHF. Both renal hemodynamic factors and structural renal abnormalities contribute to renal function impairment in CHF. As regards hemodynamic factors, renal impairment in CHF is traditionally assumed to be mainly due to a decrease in cardiac output and a subsequent decrease in renal perfusion (Martins et al, 2011; Sinkeler et al, 2012). In our current study, we found that there is increased cardiac ejection fraction and also there is decreased kidney impairment during heart failure stage.

5. Conclusion

Our findings suggest that SMR inhibits myocardial fibrosis, thereby plays a role in ameliorating the LV dysfunction during HF, decreasing the expression of CK-MD and LDH, and also decreases impairment of cardiac structures, thus myocardial damage, minimizing the collagen deposition and inhibiting the kidney ischemia injuries and also interstitial fibrosis.

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