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Preclinical study of Shen Qi Li Xin formula in improving the development of chronic heart failure

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Summary. Chronic heart failure (CHF) is a common clinical heart disease. In recent years, traditional Chinese medicines have shown good outcomes in CHF treatment. We aimed to explore the therapeutic effect of Shen Qi Li Xin formula (SQLXF) in CHF. CHF rats were treated with SQLXF at the doses of 8.48, 16.96, and 33.92 g/kg/d once a day for 4 weeks by intragastric administration. The hemodynamic and cardiac function parameters of the rats were monitored by conduction echocardiography. In our results, SQLXF treatment at the doses of 16.96 and 33.92 g/kg/d significantly improved the haemodynamics and cardiac function of CHF rats by enhancing the levels of LVSP, $+dp/dt_{max}$, $-dp/dt_{max}$, LVEF and LVFS and reducing the levels of LVEDP, LVEDD and LVESD. SQLXF treatment at 16.96 and 33.92 g/kg/d also attenuated the damage of myocardial tissues in CHF rats. In addition, compared with normal rats, the number of pericytes was reduced in myocardial tissues of CHF rats. SQLXF treatment at the doses of 16.96 and 33.92 g/kg/d obviously increased the number of pericytes and proliferation of endothelial cells and promoted angiogenesis in myocardial tissues of CHF rats. *In vitro*, SQLXF impaired low-oxygen-induced inhibition of cell viability and promotion of apoptosis in primary pericytes. Importantly, SQLXF enhanced the adhesion ability of pericytes to endothelial cells. In conclusion, SQLXF improved myocardial injury in CHF rats by enhancing the interaction between pericytes and endothelial cells, suggesting that SQLXF may be a potential drug for CHF treatment.

Key words: Traditional Chinese medicines, Myocardial injury, Shen Qi Li Xin formula, Cell apoptosis, Adhesion ability

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Introduction

Chronic heart failure (CHF) is a common clinical heart disease, and a serious health problem globally. Rheumatic heart disease, hypertensive heart disease, chronic obstructive pulmonary disease and ischemic heart disease are responsible for more than 2/3 heart failure patients (Cho et al., 2016). The disturbances in heart structure and functions of patients result in an abnormal oxygen supply from the heart to other tissues (Alem, 2019). ACC/AHA/HFSA Focused Update on New Pharmacological Therapy for Heart Failure has made a class I recommendation for angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blocker (ARB) combined with β-receptor blocker (BB) and aldosterone antagonist. It can reduce the mortality of some patients with CHB (Yancy et al., 2016). However, the morbidity and mortality of CHF has been increasing in recent years. The incidence of CHF is about 0.1% to 0.9% globally, and the 5-year survival rate for CHB patients is approximately 35% (Bleumink et al., 2004). CHF seriously affects the life quality and socioeconomic status of patients. Moreover, CHF imposes significant costs on the global healthcare system (King et al., 2012; Ziaeian and Fonarow, 2016). Currently, exploring the pathogenesis of CHF and finding an excellent therapeutic idea or target are still very important for CHF treatment.

It is well known that angiogenesis, endothelial dysfunction, and inflammation play a crucial role in the pathogenesis of CHF. Angiogenesis is involved in the repair and maintenance of luminal endothelium in CHF (Vila et al., 2008). Eleuteri et al. reported that physical training contributes to the activation of angiogenesis and attenuates endothelial dysfunction in CHF patients (Eleuteri et al., 2013). Moreover, Duan et al. indicated that inhibition of microRNA 214, which is highly expressed in the serum of CHF patients, could effectively attenuate cardiac dysfunction through promoting angiogenesis by targeting X-box binding protein 1 (Duan et al., 2015). The above studies showed

that improvement of angiogenesis might be helpful for CHF treatment. Additionally, pericytes are perivascular cells and vessel-associated supporting cells that belong to the mesenchymal cell lineage. Pericytes play a crucial role in angiogenesis, they adhere directly to endothelial cells and use the same basement membrane as endothelial cells (Eilken et al., 2017; Chiaverina and di Blasio, 2019). It was reported that angiogenesis can be promoted when the interaction between pericytes and endothelial cells is enhanced (Lee et al., 2017).

In China, traditional Chinese medicines (TCMs) are an important treasure in the treatment of various diseases such as CHF (Wang et al., 2017c). According to the research of Wang et al., 340 CHF patients were recruited and given 6 months of Danhong injection combined with Shenfu injection or Shenmai injection, or Western medication. Follow-up after 12 months found that CHF patients who received Western medication plus TCM treatment had a 34% reduction in mortality and a significant improvement in quality of life (Wang et al., 2017b). Recently, Sui et al. reported that Shen Qi Li Xin formula (SQLXF) combined with routine treatment further effectively improves cardiac function and motor function of the patients with CHF when compared with patients with routine treatment alone (Sui et al., 2018). This study proved the advantages of SQLXF in the treatment of CHF. In this study, we explored the effect of SQLXF on myocardial injury in CHF rats, and investigated the effect of SQLXF on the interaction between pericytes and endothelial cells *in vitro*. Our results will provide more evidence to support SQLXF as a treatment method of CHF.

Materials and methods

Rat CHF model and experimental groups

A total of 30 Sprague-Dawley (SD) rats (gender, male; weight, 200±20 g) were obtained from Charles River (Beijing, China). All rats were raised in a suitable environment with stable temperature at 24°C. After free diet feeding one week, the rats were randomly divided into five groups (n=6): Sham, Model, Low, Middle, and High. CHF model was established as previously reported (Zhao et al., 2019). To establish the rat CHF model, rats were anesthetized by 10% pentobarbital sodium. Then, the rat pericardium was opened to expose the epicardium. Next, the left anterior descending coronary artery of SD rats was ligated between auricular appendix and conus arteriosus using a single 6-0 nylon suture. Rat CHF model was successfully established when the ligated rats with left ventricular ejection fraction ≤45%. The rats in Sham group received the same procedure without ligation.

The rats in Low, Middle, and High groups were treated with SQLXF at doses of 8.48, 16.96, and 33.92 g/kg, respectively as previous described (Jin et al., 2014). Rats received SQLXF by gavage once a day for 4 weeks. Meantime, an equal volume of normal saline was prepared, and was orally administrated to the rats in Sham group and Model group, also once a day for 4 weeks. SQLXF was composed of Panax ginseng C. A. Mey., *Astragalus propinquus* Schischk., *Cinnamomum aromaticum* Nees, *Epimedium brevicornu* Maxim., *Draba nemorosa* L., *Wolfiporia extensa* (Peck) Ginns, *Atractylodes macrocephala* Koidz., *Leonurus japonicus* Houtt., *Salvia miltiorrhiza* Bunge, *Agrimonia pilosa* Ledeb., *Glycyrrhiza uralensis* Fisch. ex DC. SQLXF was provided by the Department of Medication Preparation of First Affiliated Hospital of Heilongjiang University of Chinese Medicine. All animal experiments were approved by the Ethics Committee of First Affiliated Hospital of Heilongjiang University of Chinese Medicine. All protocols were performed strictly in accordance with the requirement of the Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of China. All possible methods were used to avoid animal suffering during the research.

Hemodynamic and cardiac function monitoring

After four weeks of SQLXF administration, rats were accepted conduction echocardiography to monitor the hemodynamic and cardiac function of rats. Briefly, rats were anesthetized with 20% urethane (0.5 mL/100g). A micro-catheter linked with a pressure transducer was inserted into the left ventricular from right common carotid artery. Next, the levels of left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximal positive rate of developed left ventricular pressure ($+dp/dt_{max}$), maximal negative rate of developed left ventricular pressure ($-dp/dt_{max}$), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) of rats were measured using a multichannel physiological recorder.

HE staining

After 4 weeks of SQLXF treatment, the myocardial tissues of rats were obtained, and then the tissues were embedded with paraffin and cut into 4 μm thick slices. Next, the pathological changes in myocardial tissues were ensured using the HE staining kit (Solarbio, Beijing, China) in accordance with the manufacture's protocol. The degree of myocardial tissue lesions was evaluated and scored (0-3 points): 0 points, no tissue damage; 1 point, mild tissue damage, the lesion involved less than 10% of the myocardium; 2 points, moderate tissue damage, the lesion involved 10-50% of the myocardium; 3 points, severe tissue injury, lesions involved more than 50% of the myocardium.

ELISA assay

After 4 weeks of SQLXF treatment, the levels of

serum lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), and brain natriuretic peptide (BNP) were measured according to the manufacture's introduction of rat LDH ELISA assay kit (Invitrogen, Waltham, MA, USA), rat CK-MB ELISA assay kit (Invitrogen), and rat BNP ELISA assay kit (Invitrogen), respectively. Finally, the absorbance at 450 nm was measured with a Synergy H1 Hybrid Microplate Reader (Biotech, USA).

Immunofluorescence staining

Slices of myocardial tissues were subjected to dewaxing and hydration. Then, the slices were washed with cold PBS three times and incubated with 2% BSA for 1 h at 37°C. Subsequently, the slices were maintained with primary antibodies against NG2 (1:200; Abcam), CD31 (1:200; Abcam), and Ki67 (1:200; Abcam) overnight at 4°C. Next day, the slices were incubated with the secondary antibodies for 2 h at 37°C in the darkness. The slices were stained with DAPI to mark nucleus. Finally, the NG2-positive cells, angiogenesis, and CD31-positive cells were observed under a confocal laser scanning microscope.

Preparation for the medicated serum

A total of 40 SD rats were purchased for preparation of medicated serum. All rats were randomly divided into 4 groups (n=10): blank, low-dose, middle-dose and high-dose. In blank, low-dose, middle-dose and high-dose groups, the rats were fed with normal saline, Chinese herbal decoction of SQLXF at the doses of 8.48, 16.96 and 33.92 g/kg, respectively, through gavage administration, once a day for seven days. At 2 h after the last gavage, abdominal aortic blood was obtained from rats under sterile conditions. After that, the blood samples were centrifuged at 3000 rpm for 15 min to obtain serum, the serum was then passed through a 0.22 μm microporous membrane. After inactivation at 56°C for 30 min, the serum was stored at -20°C.

Cell culture and experimental group

Pericytes were isolated from the heart of rats described in a previous study (Špiranec et al., 2018). Endothelial cells were isolated from the heart of rats according to a previous study (Gündüz et al., 2017). Endothelial Cell Medium (Sciencell) and Pericyte Medium (Sciencell) were used to culture the endothelial cells and pericytes, respectively. All cells were cultured at 37°C in an incubator with 5% CO_2. According to the aim of our study, the cells were divided into six groups: control, low-oxygen (LO), LO + blank serum, LO + low-dose serum, LO + middle-dose serum, and LO + high-dose serum. The cells in low-oxygen (LO), LO + blank serum, LO + low-dose serum, LO + middle-dose serum, and LO + high-dose serum groups were cultured at 37°C in an incubator with 94% N_2, 5% CO_2, and 3% O_2. The cells in control group were cultured in normal conditions. Low-oxygen induced pericyte injury. Twenty-four hours later, the cell viability and apoptosis were detected.

For the co-culture system of pericytes and endothelial cells, pericytes and endothelial cells were planted into the same 6-well plate at a ratio of 1:1, and the cells grown in the 1:1 mixture of Endothelial Cell Medium and Pericyte Medium. At 24 h of low-oxygen induction, immunofluorescence assay was performed to examine the percentage of CD31-positive and NG2-positive cells.

CCK-8 assay

Cell viability of pericytes was measured by CCK-8 assay. Pericytes were planted into 96-well plates at a density of 1×10^4/well. After 24 hours of low-oxygen induction, the cells were incubated with 10 μL of CCK-8 solution (MedChemExpress, USA) for another two hours. Next, the absorbance of solution at 450 nm was measured using a Synergy H1 Hybrid Microplate Reader (Biotech, USA).

Flow cytometry

Flow cytometry was carried out to detect cell apoptosis of pericytes. Pericytes were planted into 6-well plates at a density of 1.5×10^6/well. After 24 h of low-oxygen induction, the cells were washed with PBS and resuspended in Biding Buffer. After that, a FITC Annexin V Apoptosis Detection Kit (BD Pharmingen™, USA) was used to detect the rate of apoptotic cells in accordance with the manufacturer's protocol.

Statistical analysis

SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. Data are presented as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's testing was used to analyze the significant difference among multiple groups. $P<0.05$ was considered statistically significant.

Results

SQLXF improves heart hemodynamics and cardiac function of CHF rats

Our data indicated that the levels of LVSP (Fig. 1A), $+dp/dt_{max}$ (Fig. 1C), and $-dp/dt_{max}$ (Fig. 1D) in CHF rats were lower than those in normal rats. However, the levels of LVSP, $+dp/dt_{max}$, and $-dp/dt_{max}$ in CHF rats were significantly increased by SQLXF treatment at the doses of 16.96 and 33.92 g/kg. Moreover, compared with normal rats, the level of LVEDP was increased in CHF rats, while it was then decreased by SQLXF treatment at the doses of 16.96 and 33.92 g/kg (Fig. 1B). Additionally, the cardiac function monitoring uncovered that the levels of LVEDD and LVESD were increased,

while the levels of LVEF and LVFS were decreased in CHF rats as compared with normal rats. SQLXF treatment at 16.96 and 33.92 g/kg notably reduced the levels of LVEDD and LVESD and enhanced levels of LVEF and LVFS in CHF rats (Fig. 2A-D). Overall, 16.96 and 33.92 g/kg of SQLXF effectively improved the hemodynamics and cardiac function of CHF rats. SQLXF had no effect on the hemodynamics and cardiac function of CHF rats at the dose of 8.48 g/kg.

SQLXF attenuates the myocardial injury of CHF rats

Furthermore, we explored the effect of SQLXF on myocardial injury in CHF. The pathological changes of myocardial tissues in rats were assessed by HE staining. In sham-operated rats, myocardial cells were neatly arranged, and myocardial tissues had no obvious tissue damage. CHF rats displayed severe tissue damage, disordered arrangement and increased necrosis of cardiomyocytes, while the tissue injury was effectively attenuated by SQLXF treatment at the doses of 16.96 and 33.92 g/kg. Also, CHF rats had higher myocardial histopathological scores. SQXLF treatment at 16.96 and 33.92 g/kg significantly reduced the myocardial histopathological scores of CHF rats (Fig. 3). In addition, the levels of serum LDH, CK-MB, and BNP in CHF rats were notably higher than those in normal rats. Doses of 16.96 and 33.92 g/kg of SQXLF treatment obviously reduced the levels of LDH, CK-MB, and BNP in serum of CHF rats (Fig. 4A-C). Overall, our results revealed that SQLXF at the doses of 16.96 and 33.92 g/kg effectively attenuated the damage of myocardial tissues in CHF rats. SQLXF had no effect on the myocardial injury of CHF rats at the dose of 8.48 g/kg.

SQLXF promotes angiogenesis in myocardial tissues of CHF rats

To explore whether SQLXF improves the myocardial injury in CHF rats by affecting pericytes and endothelial cells, we detected the numbers of pericytes and endothelial cells and the angiogenesis in myocardial tissues of rats by immunofluorescence staining. Our data showed that the cell number of pericytes in myocardial tissues of CHF rats was lower than that in normal rats, while the reduction in cell number of pericytes was notably inhibited by SQLXF treatment at the doses of 16.96 and 33.92 g/kg (Fig. 5A,D). Importantly, our results displayed that SQLXF treatment at the doses of 16.96 and 33.92 g/kg promoted angiogenesis (Fig. 5B,E)

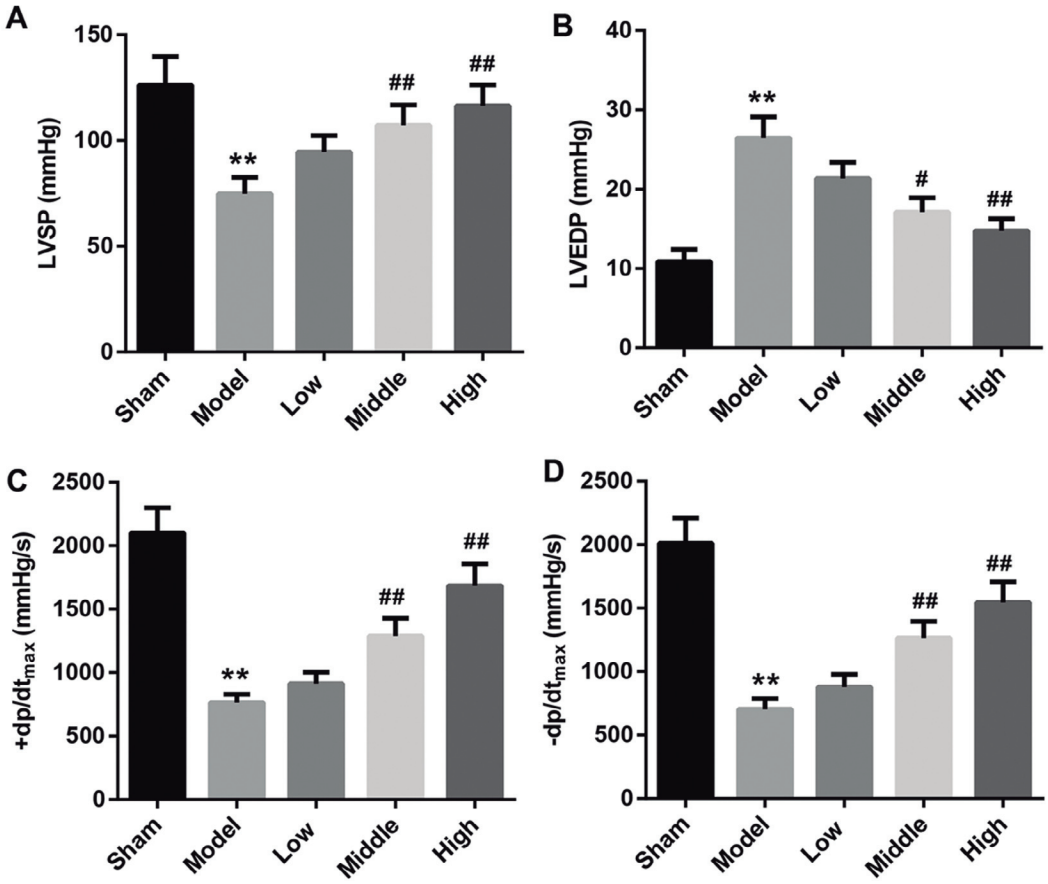

Fig. 1. Effect of SQLXF on heart hemodynamics in CHF rats. CHF rats were administrated with 8.48 g/kg of SQLXF (low-dose group), 16.96 g/kg of SQLXF (middle-dose group), or 33.92 g/kg of SQLXF (high-dose group) by gavage once a day for 4 weeks. After four weeks of SQLXF administration, the levels of LVSP **(A)**, LVEDP **(B)**, $+dp/dt_{max}$ **(C)**, and $-dp/dt_{max}$ **(D)** of all rats were measured by conduction echocardiography. **P<0.01 compared with sham group, and ##P<0.01 compared with model group.

and endothelial cell proliferation (Fig. 5C,F). The number of endothelial cells and angiogenesis in myocardial tissue of CHF rats were slightly increased but not significantly different. All these data proved that SQLXF treatment reduced enhanced cell number of pericytes and promoted proliferation of endothelial cells and angiogenesis in CHF rats.

SQLXF enhances the interaction between pericytes and endothelial cells

Subsequently, we explored the effect of SQLXF on the interaction between pericytes and endothelial cells *in vitro*. Pericytes were cultured at low-oxygen conditions to induce cell injury. The results demonstrated that low-oxygen-resulted in the reduction of cell viability in pericytes, while it was then partly reversed by the treatment of medicated rat serum containing 16.96 and 33.92 g/kg of SQLXF (Fig. 6A). In addition, the results of flow cytometry proved that low-oxygen significantly induced apoptosis of pericytes. The medicated rat serum containing 16.96 and 33.92 g/kg of SQLXF effectively suppressed apoptosis of pericytes (Fig. 6B). Importantly, we established a co-culture system of pericytes and endothelial cells, and then stained the CD31-positive endothelial cells and NG2-positive pericytes. Our data showed that the percentage of both CD31-positive and NG2-positive cells was lower in low-oxygen-treated cells than that in control cells. The percentage of double positive cells was increased in the co-culture system of pericytes and endothelial cells following the treatment of medicated rat serum containing SQLXF at the doses of 16.96 and 33.92 g/kg (Fig. 6C). Thus, these findings suggested that overall, SQLXF enhanced the adhesion of pericytes to endothelial cells.

Discussion

CHF is a complex clinical syndrome that greatly affects the life quality of patients. Over the past decades, many drugs have been applied to treat CHF. However, CHF patients usually respond poorly to Western medicine. Compared with Western medicine, TCMs have many advantages, such as multi-targets, and few side-effects (Fu et al., 2010). Wang et al. demonstrated that Qishen granules, a Chinese herbal formula, might further improve CHF efficacy and safety based on the standard treatment (Wang et al., 2017a). Contrasted with standard treatment, Fuling Sini decoction has a better outcome in CHF treatment (Huang et al., 2018). Sui et al. have confirmed that SQLXF could effectively improve the cardiac function of CHF rats by reducing

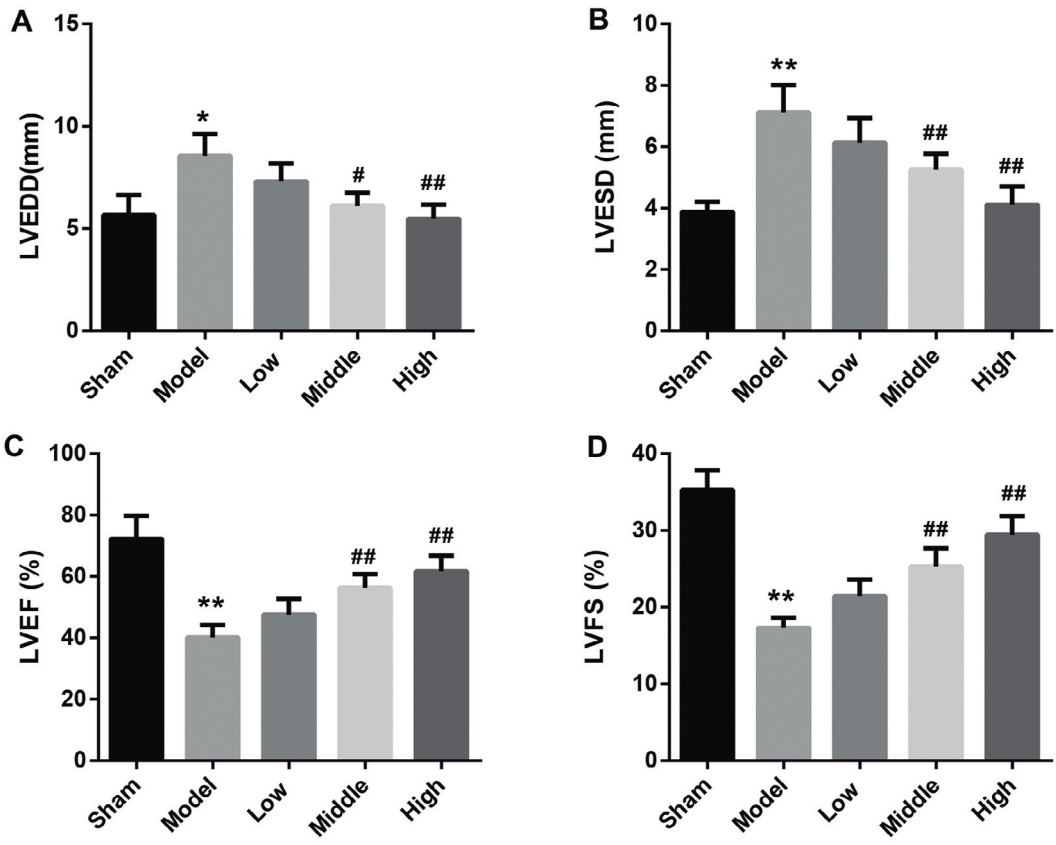

Fig. 2. Effect of SQLXF on cardiac function in CHF rats. The levels of LVEDD **(A)**, LVESD **(B)**, LVEF **(C)**, and LVFS **(D)** of rats were measured by conduction echocardiography. *P<0.05,s**P<0.01 compared with sham group, and #P<0.05, ##P<0.01 compared with model group.

oxygen glucose deprivation/reoxygenation-induced apoptosis of cardiac myocytes (Sui et al., 2021). Consistently, we further confirmed the therapeutic mechanism of SQLXF, also a Chinese herbal formula, in the development of CHF. We found that a certain dose of SQLXF effectively improved the indexes of hemodynamics and cardiac function, and attenuated myocardial injury in CHF rats. Although multiple TCMs are the alternative drugs of CHF treatment, the action mechanism of TCMs in CHF remains unclear. We found that SQLXF improved CHF through promoting angiogenesis via enhancing the interaction between pericytes and endothelial cells.

Cardiac fibrosis and vasculogenesis impairment are the important characteristics of heart failure. Angiogenesis is mainly regulated by angiopoietin signaling and vascular endothelial growth factor, and it is closely associated with endothelial cells. Ang-I and Ang-II are two major members of angiopoietins, which are expressed mainly on endothelial cells (Bansal et al., 2019; Di Matteo et al., 2020). Endothelial progenitor cell-derived microvesicles protect primary rat kidney cells from Ang II induced oxidative stress and inflammatory response (Song et al., 2022). Additionally,

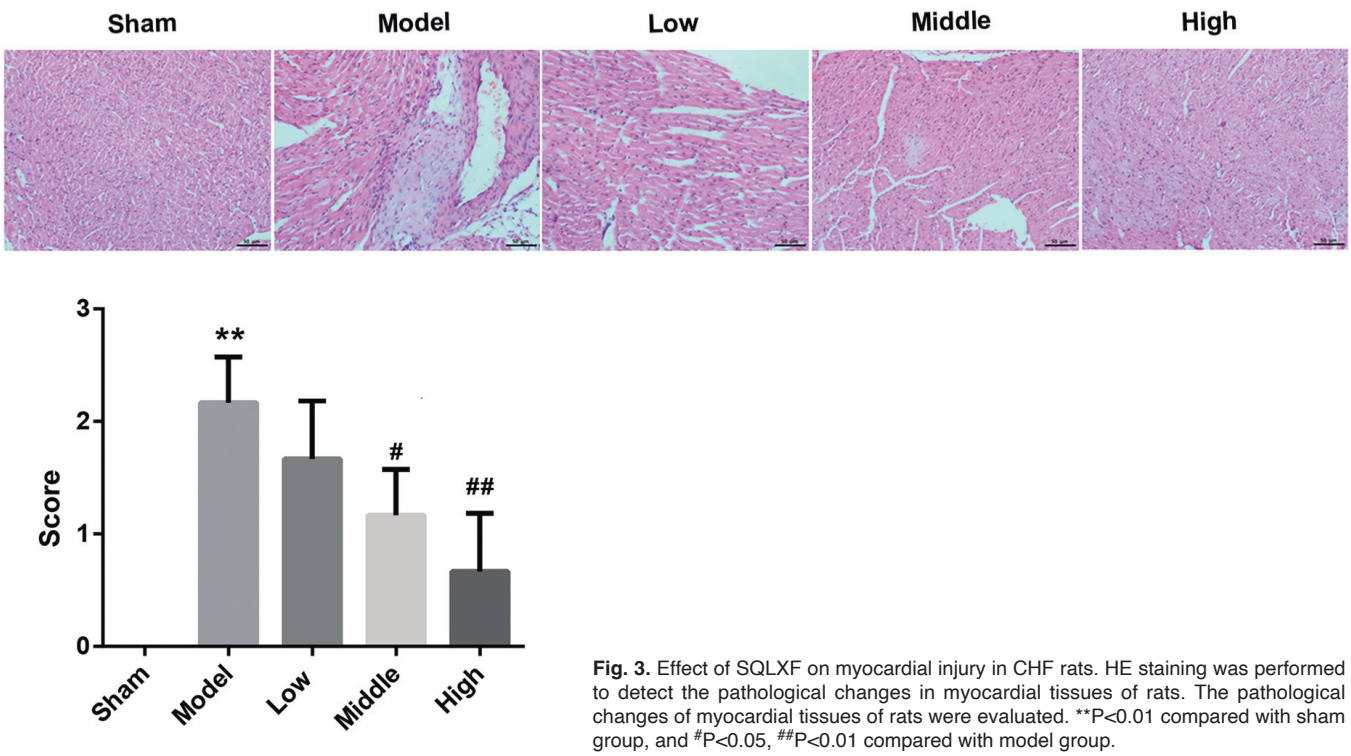

Fig. 3. Effect of SQLXF on myocardial injury in CHF rats. HE staining was performed to detect the pathological changes in myocardial tissues of rats. The pathological changes of myocardial tissues of rats were evaluated. **P<0.01 compared with sham group, and #P<0.05, ##P<0.01 compared with model group.

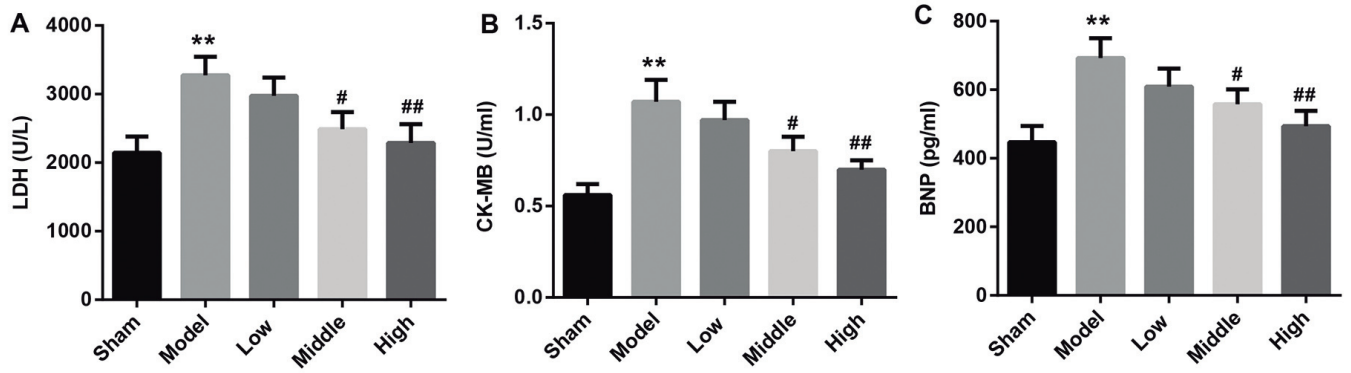

Fig. 4. Effect of SQLXF on the levels of LDH, CK-MB and BNP in CHF rats. ELISA assay was carried out to examine the concentrations of LDH **(A)**, CK-MB **(B)**, and BNP **(C)** in serum of rats. **Pz0.01 compared with sham group, and #P<0.05 and ##P<0.01 compared with model group.

several factors have been implicated in the pathophysiology of reperfusion injury (RI) including vascular/microvascular injury, endothelial dysfunction, accelerated cell necrosis, granulocyte activation and modulation of NO/Ang II axis. Pazoki-Toroudi et al. demonstrated that captopril or enalapril prevents RI-induced kidney lesions in rats, which may be attributed to inhibition of Ang II formation (Pazoki-Toroudi et al., 2003). Furthermore, David Polhemus et al. revealed that the numbers of CD31-positive and Ki67-positive cells are increased in the myocardial tissues of CHF mice after hydrogen sulfide treatment, suggesting that hydrogen sulfide improves the heart function of CHF mice via induction of angiogenesis (Polhemus et al., 2013). Growing evidence has demonstrated the importance of vasculogenesis in improvement of CHF. In our present study, we found that a certain dose of SQLXF significantly facilitated the proliferation of endothelial cells and angiogenesis in myocardial tissues of CHF rats. Moreover, we also found that a certain dose of SQLXF also promoted the proliferation of pericytes in myocardial tissues of CHF rats.

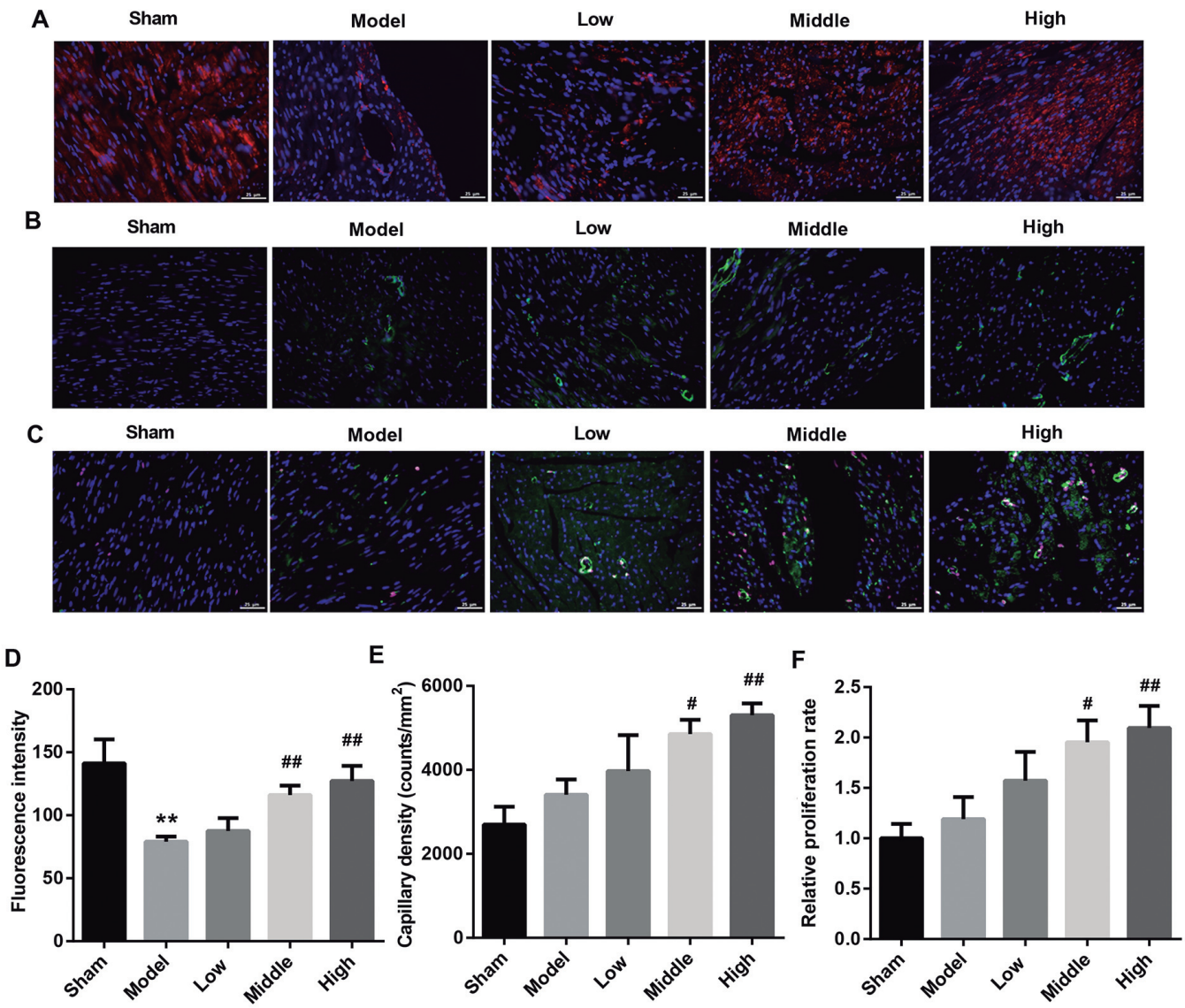

Fig. 5. Effect of SQLXF on the loss of pericytes, endothelial cell proliferation, and angiogenesis in CHF rats. **A, D.** The cell number of pericytes in myocardial tissues of rat was examined using immunofluorescence staining NG2, and the fluorescence intensity was analyzed. NG2 is a specific surface marker protein of pericytes. **B, E.** The angiogenesis in myocardial tissues of rats was measured by immunofluorescence staining CD31, and the capillary density was analyzed. CD31 is a marker of endothelial cells. **C, F.** The proliferation of endothelial cells in myocardial tissues of rats was examined using immunofluorescence staining CD31 and Ki67, and the relative proliferation rate of endothelial cells was analyzed. Ki67 is a common marker for the detection of cell proliferation. $^{**}P<0.01$ compared with sham group, and $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared with model group.

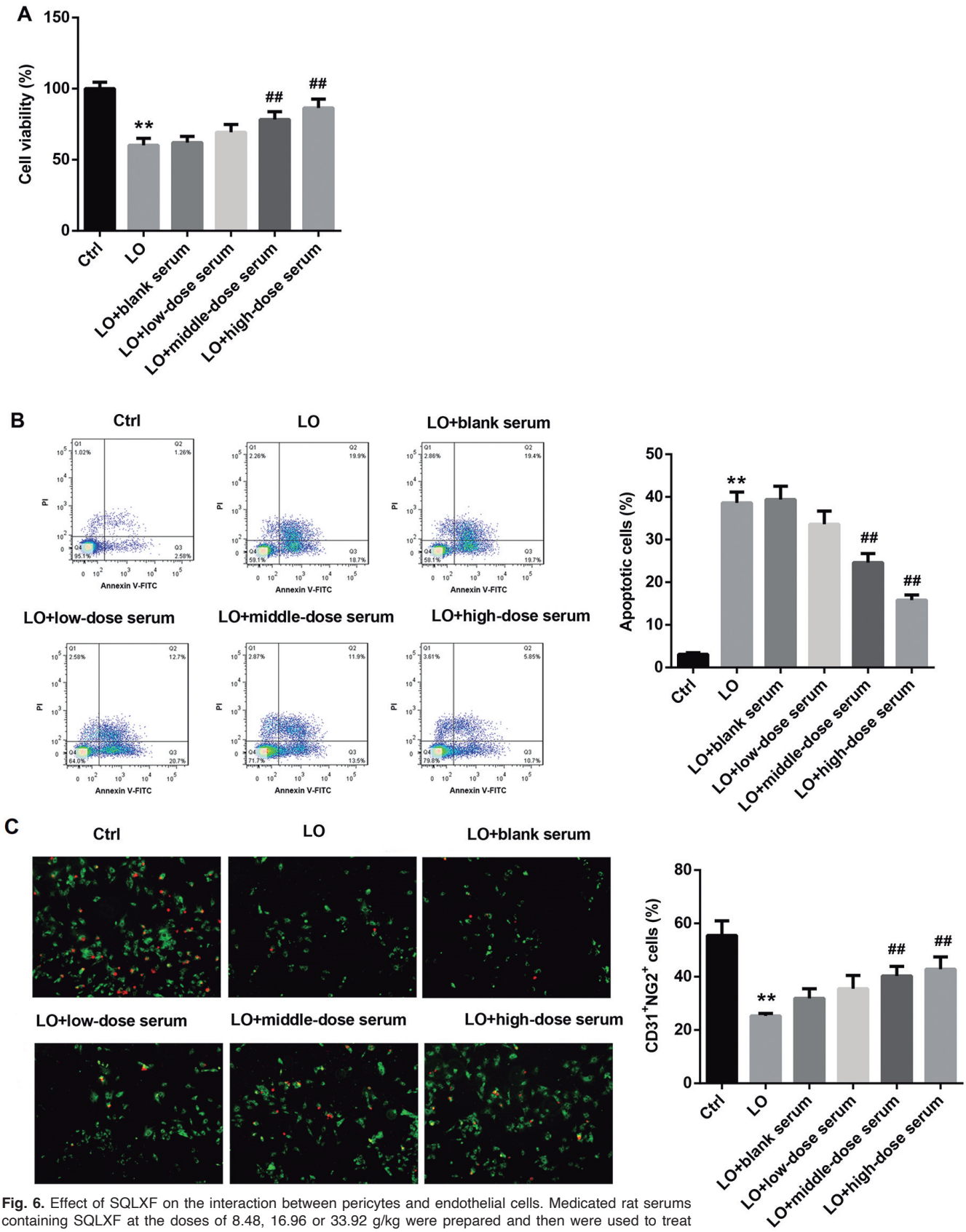

Fig. 6. Effect of SQLXF on the interaction between pericytes and endothelial cells. Medicated rat serums containing SQLXF at the doses of 8.48, 16.96 or 33.92 g/kg were prepared and then were used to treat low-oxygen-stimulated cells. **A.** CCK-8 assay was performed to detect the cell viability of pericytes. **B.** Flow cytometry was carried out to examine the cell apoptosis of pericytes. **C.** Both CD31-positive and NG2-positive cells were detected using immunofluorescence. NG2 is a specific surface marker protein of pericytes and CD31 is a marker of endothelial cells. **P<0.01 compared with Ctrl group, and $^{\#\#}$P<0.01 contrasted with LO group.

Pericytes are a fundamental part of the mural cell populations, and play a crucial role in angiogenesis. Pericyte dysfunction has been reported to be involved in the development of multiple disorders, such as diabetic retinopathy and CHF (Cuervo et al., 2017). During the process of angiogenesis, pericytes adhere to the inner wall of vessels and embed into the vascular basement membrane, which they share with endothelial cells (Chiaverina and di Blasio, 2019). At present, NG2 has been recognized as a suitable marker of pericytes. In many studies, NG2 is used to mark pericytes to study the arterial biology and vascular growth (Ozerdem and Stallcup, 2003). Here, we found that the number of NG2-positive cells was decreased in myocardial tissues of CHF rats, while a certain dose of SQLXF treatment markedly increased the number of NG2-positive cells. Moreover, we further found that medicated serum containing a dose of SQLXF treatment enhanced the cell viability of pericytes and inhibited low-oxygen-induced pericyte apoptosis. Importantly, our data proved that a certain dose of SQLXF contributed to the adhesion of pericytes to endothelial cells.

This work revealed that SQLXF alleviated myocardial injury in CHF rats by regulating the interaction between pericytes and endothelial cells through *in vivo* and *in vitro* assays. However, the molecular mechanism by which SQLXF reduces CHF is still unclear. Many molecules are involved in angiogenesis to regulate the progression of CHF. For instance, Li et al. confirmed that inhibition of miR-221-3p represses angiogenesis of endothelial cells by targeting HIF-1α, which contributes to improve cardiac function of CHF mice (Li et al., 2021). Pinocembrin ameliorates collagen fiber deposition and apoptosis, and facilitates angiogenesis by activating the Nrf2/HO-1 pathway, thereby alleviating CHF in rats (Chen et al., 2021). Thus, we speculated that SQLXF may regulate angiogenesis to alleviate CHF by regulating miR-221-3p/HIF-1α or Nrf2/HO-1 pathway, which requires further research.

Our results demonstrate that SQLXF, a TCM, could effectively reduce the myocardial injury in CHF rats by enhancing the interaction between pericytes and endothelial cells. Our data provide new evidence to support the potential of SQLXF in CHF treatment.

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Conflicts of interest. The authors declare that they have no conflict of interest.

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