Exploring the efficacy of Shexiang Tongxin extract pills in severe heart failure

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ORIGINAL RESEARCH

Abstract

The clinical efficacy of Shexiang Tongxin pills (STP) on cardiac function, inflammation, and prognosis was investigated in patients with severe heart failure. A total of 140 patients with severe heart failure, diagnosed and treated at the Shaoxing Central Hospital and its affiliates, were divided into control and experimental groups. Patients in the control group received conventional treatment, while those in the experimental group were given STP plus conventional treatment. The cardiac functions, levels of inflammatory factors, and outcomes of 6-minute walk distance (6MWD) were assessed and compared after 12 weeks of intervention. Besides, the rehospitalization and mortality proportions were also determined during a 1-year follow-up. In addition, oxygen-glucose deprivation and reperfusion (OGD/R) were conducted to mimic the injury of H9c2 cells in heart failure; cell survival, cytotoxicity, apoptosis, and inflammatory factors were also evaluated. Post-intervention, patients in the experiment group demonstrated greater improvement in cardiac functions, especially regarding left ventricular end-diastolic diameter and N-terminal pro-B-type natriuretic peptide, which were significantly decreased, while left ventricular ejection fraction was significantly increased ($P < 0.05$). Further, the levels of inflammatory factors, including tumor necrosis factor-α, hypersensitive C-reactive-protein, and interleukin 6, were significantly lower ($P < 0.05$), and a superior 6MWD outcome was observed in patients from the experimental group, compared to the control group ($P < 0.05$). After 1-year follow-up, both rehospitalization rate and mortality were significantly decreased in the experimental group, compared to the control group (rehospitalization, 38.57% vs. 55.71%, and mortality, 12.86% vs. 27.14%, respectively; $P < 0.05$). Furthermore, STP increased cell survival and reduced cell apoptosis, cytotoxicity, and inflammatory response in cardiomyocytes induced by OGD/R. Shexiang Tongxin pill demonstrated significant and promising clinical effects in treating severe heart failure by ameliorating cardiac functions, reducing inflammatory factor levels, and improving patients' prognosis. In addition, STP ameliorated cellular damage and inflammation at cellular level. Thus, STP demonstrated important clinical values.

Keywords: severe heart failure; Shexiang Tongxin pill; clinical efficacy; cardiac function; inflammatory factors

Introduction

Heart failure remains a global public health issue. As an end-stage clinical syndrome of cardiovascular disease, it is one of the most common causes of morbidity in the elderly population (Borlaug, 2020). According to epidemiological research, in China, heart failure is correlated with the patient's age, which shows an increase in incidence with age. For instance, it was reported that the risk of heart failure could be several times higher with each 10-year increase in age. In addition, mortality because of heart failure remains very high (Truby and Rogers, 2020). In addition to older age, the COVID-19 infection increased the risk of heart failure. Clinical studies have

found that the COVID-19 infection leads to the release of pro-inflammatory cytokines and the recruitment of pro-inflammatory macrophages and granulocytes, resulting in a severe inflammatory storm that could lead to cardiac depression and acute decompensation in new-onset heart failure or chronic heart failure (Bader *et al*., 2021; Tufan *et al*., 2020).

Heart failure can easily progress to severe heart failure despite treatments. Previous studies confirmed that in addition to abnormal cardiac ejection function, severe heart failure is often accompanied by various degrees of inflammatory stresses, with inflammatory conditions shown to be closely associated with cardiac failure (Di Palo and Barone, 2020).

Clinically, cardiotonic, vasodilator, and surgery are commonly used to treat severe heart failure. However, these are often associated with numerous adverse events or contraindications, in addition to a requirement for long-term treatments. Therefore, their clinical effects are limited. In recent years, the development of Traditional Chinese Medicine (TCM) has shown numerous advantages of clinical treatment. For example, TCM compound Si-Miao-Yong-An decoction (SMYAD) attenuated autophagy and apoptosis via PDE5A-AKT and TLR4-NOX4 pathways in an isoproterenol (ISO)-induced heart failure model (Liao *et al*., 2022). Through the Ankyrin repeat domain 1–extracellular signal-regulated kinase–GATA binding protein 4 (ANKRD1-ERK-GATA4) pathway, Baoyuan decoction (BYD) reduced ventricular hypertrophy in heart failure, following an acute myocardial infarction (Meng *et al*., 2021). Shengxian decotion (SXT), a well-known TCM formula composed of Astragali Radix, Bupleuri Radix, Cimicifugae Rhizoma, Anemarrhenae Rhizoma, and Platycodonis Radix, could diminish symptoms of heart failure in a doxorubicin model of chronic heart failure in rats (Huang *et al*., 2021). Shexiang Tongxin pill (STP), as a proprietary Chinese medicine, has demonstrated significant clinical efficacies in treating coronary heart disease, stenocardia, and other cardiovascular diseases (Liu *et al*., 2021; Pan *et al*., 2021). However, its effect on severe heart failure remains unknown, and its effect on patients' cardiac function and inflammation remains to be further investigated.

Heart failure's connection with risk factors, such as type 2 diabetes mellitus (T2DM), chronic inflammation, coronary artery disease, hypertension, sarcopenia, and obesity, indicates that addressing these coexisting conditions through nutritional interventions could play a pivotal role in both preventing and managing heart failure, as pointed out by Billingsley *et al*. (2020). Among the conventional medicinal approaches, STP has gained significant usage in China for addressing cardiovascular issues. STP exhibits the ability to mitigate inflammation, minimize damage to endothelial cells, and enhance the function of coronary microvessels (Lu *et al*., 2020). Pharmacology research has shown that STP decreases the presence of pro-inflammatory cytokines, as demonstrated by Xiong *et al*. (2015). Ability of STP to safeguard endothelial function is closely linked to its anti-inflammatory properties, as indicated by Tian *et al*. (2023). Moreover, STP has the potential to enhance coronary blood flow and cardiac function by mitigating inflammation, as demonstrated Chen *et al*. (2022).

In addition, STP ameliorated cellular damage and inflammation at the cellular level. Thus, STP demonstrated important clinical values. Therefore, this study was conducted to elucidate the clinical value of STP in severe heart failure.

Objects and Methods

Basic information

This study included 140 patients with severe heart failure diagnosed and treated at the Shaoxing Central Hospital Medical Alliance General Hospital and its affiliates from June 2019 to June 2021. Using a random number table, they were randomly divided into control (n = 70) and experimental (n = 70) groups. The baseline characteristics of the patients, including age, gender ratio, cardiac function, and comorbidity of the two groups, are shown in Table 1. Ethical approval was obtained from the Ethics Committee of Shaoxing Central Hospital Medical Alliance General Hospital.

Inclusion and exclusion criteria

The inclusion criteria of the study were as follows: (1) aged 18–80 years (upper limit was not included), without gender limitation; (2) clinical diagnosis with severe heart failure according to relevant standards of the Chinese Guidelines for the Diagnosis and Treatment of Heart Failure 2018 (Heart Failure Group of Chinese Society of Cardiology of Chinese Medical Association *et al*., 2018); (3) left ventricular ejection fraction (LVEF) ≤ 40%; (4) the New York Heart Association (NYHA) cardiac functional grading of II–IV; (5) N-terminal pro-B-type natriuretic peptide (NT-proBNP) level ≥ 1,000 pg/mL; and (6) patients with normal communication and good compliance voluntarily participated and signed the informed consent.

Exclusion criteria of the study were as follows: (1) patients with valvular heart disease, congenital heart disease, and hypertensive heart disease; (2) patients with severe liver and kidney dysfunction that affected drug metabolism; (3) pregnant patients; (4) patients that did not cooperate, intolerant, or dropped out because of various reasons; and (5) allergic to the study drug or its ingredients.

Table 1. Patients' basic information ($\bar{x} \pm s$)/n (%)

Group	Control	Experimental	t/χ^2 value	*P* value
Mean age	60.31 ± 9.36	61.24 ± 8.92	0.600	0.549
Male–female ratio	21/49	30/40	2.498	0.114
Course of disease (years)	5.12 ± 0.98	5.21 ± 0.76	0.6786	0.4985
NYHA grade				
II	8 (11.43)	10 (14.29)	0.366	0.833
III	24 (34.29)	25 (35.71)	–	–
IV	38 (54.28)	35 (50.00)	–	–
Comorbidity				
Hypertension	23 (32.9)	30 (42.3)	1.264	0.531
Diabetes mellitus	32 (45.7)	29 (41.4)	–	–
Cerebral apoplexy	10 (14.3)	8 (11.4)	–	–

Note: Basic information in Table 1 shows no statistical differences, indicating that the experimental data between the two groups are comparable.

Research methods

Following the routine cardiology diagnosis and treatment, patients in the control group were given drug combination therapy, including diuretics, digitalis, nitrates, β-blocker, and aldosterone antagonists. Shexiang Tongxin dropping pills (STDP) were also used in the experimental group based on conventional therapy administered to the control group. The drug, as 35-mg/pill×18 pills (Inner Mongolia Kangenbei Pharmaceutical, Inner Mongolia, China) was approved by the State Food and Drug Administration (SFDA) with an approval No. Z20080018.. It was prescribed as 2 pills to be taken thrice a day (tds) for 12 weeks, followed by 1-year follow-up.

Research contents

Cardiac functions, levels of serum inflammatory factors, 6-minute walk distance (6MWD) outcomes, rehospitalization proportion, and mortality during 1-year follow-up were analyzed before and after the treatment. The predominant indicator for cardiac function was NT-probNP. The secondary parameters were left ventricular ejection fraction (LVEF) and left ventricular end-diastolic diameter (LVEDD). The levels of serum tumor necrosis factor-α (TNF-α), hypersensitive C-reactive protein (hs-CRP), and interleukin-6 (IL-6) were measured to evaluate inflammatory response.

Cell culture and treatment

H9c2 cells are rat cardiomyocytes, and were obtained from American Type Culture Collection (Manassas, VA, US) and maintained in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA, US) with additional GlutaMAX (2 μM; Invitrogen) and fetal bovine serum (FBS, 10%; Invitrogen). The cells were grown in an incubator with 5% CO_2 at 37°C.

Oxygen-glucose deprivation and reperfusion (OGD/R) were conducted to mimic the injury of H9c2 cells in heart failure. OGD was induced by the culture of H9c2 cells in glucose-free DMEM, including sodium dithionite ($Na_2S_2O_4$; 5 μM) for 2 h. The complete medium was used in the supernatant for 6 h for reoxygenation.

Preparation of Shexiang Tongxin pills extract

First, 50.0 g of STP was weighed and decocted twice in eight times the volume of water for 1 h. Second, the decoction was collected and filtered through filter paper. In the third step, eight times the volume of 95% ethanol was added to the remaining filtrate and refluxed twice for 1 h. Finally, two portions of the filtrate were combined and concentrated to a density of 1.0 g/mL by rotary evaporation at 55°C and stored at -20°C. Prior to use, an appropriate amount of the extract was diluted with medium, sonicated for 20 min, and centrifuged at 13,000 rpm for 5 min. The resulting supernatant was filtered for extract through a 0.22-μm filter membrane.

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay

MTT assay was performed to evaluate the viability of H9c2 cell line. H9c2 cells induced by OGD/R were

treated with STP or captopril (positive control drug). Then, they were seeded in 96-well plates. After incubation for 48 h, MTT solution (5 mg/mL; Sigma-Aldrich, MO, US) was supplemented in the culture medium and grown for 4 h in a dark room at 37°C. To dissolve the formazan of MTT, dimethyl sulfoxide (DMSO) was added. The absorbance at 490 nm was read to determine the viability of H9c2 cells.

Lactate dehydrogenase (LDH) measurement

Lactate dehydrogenase level was applied to assess the cytotoxicity of H9c2 cells. The level of LDH was detected using a commercial assay kit (Roche Applied Science, Basel, Switzerland). The Triton X-100 (8 μL, 10%) was filled in and grown for 15 min. Then the culture medium (50 μL) was transferred onto a 96-well opaque-walled assay plate. Further, the LDH detection reagent (50 μL) was added and incubated for 1 h. The absorbance at 490 nm was evaluated via a spectrophotometer.

Flow cytometry

After being induced by OGD/R, H9c2 cells were treated with or without STP or captopril. After incubation for 48 h, the cells were stained with Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) reagent (Vazyme, Nanjing, China) under dark conditions for 15 min. Subsequently, flow cytometry (BD FACSCalibur™ System; BD Biosciences, NJ, US) was used to analyze the percentage of apoptosis.

Western blot (WB) analysis

The total protein was extracted using a Whole Cell Extraction Kit (Epigentek Group, Farmingdale, NY, US). The concentration of total protein was determined by Bicinchoninic acid assay (BCA) protein kit (Beyotime, Shanghai, China). Protein lysates from cells were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to PVDF membranes (Millipore, China) treated with 5% non-fat milk for 1 h at room temperature after washing the PVDF membrane with tris-buffered saline tween (TBST) solution and specific antibodies, such as anti-cleaved caspase-3 (ab2302, 1:1,000), anti-Bax (ab32503, 1:1,000), anti-Bcl-2 (ab32124, 1:1,000), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH, ab8245, 1:1,000) overnight at 4°C. The secondary antibodies goat anti-immunoglobulin G (IgG, ab6721, 1:2,000; Abcam, Cambridge, MA, US) were added onto membranes for 2 h at room temperature. Before imaging, the membranes were washed with an enhanced chemiluminescence reagent (Beyotime), and Image J was used for quantification.

Enzyme-Linked Immunosorbent Serological Assay (ELISA)

H9c2 cells from each group were lysed in radioimmunoprecipitation assay (RIPA) buffer (Beyotime) and centrifuged at 12,000 ×g for 1 h to collect supernatants. The inflammation markers, TNF-α, IL-6, IL-1β, and monocyte chemoattractant protein-1 (MCP-1), as well as creatine kinase-myocardial band (CK-MB) activity, were detected through corresponding ELISA kits (Beyotime) according to the manufacturer's instructions. Finally, the absorbance value was measured at 450 nm.

Statistical analysis

The data were summarized and collated to establish a database. All data were statistically analyzed using the SPSS v24.0 software. Enumeration data are described using n (%) and compared using the Chi-square test. Quantitative data are represented as mean ± standard deviation. For the experimental part, each group was repeated thrice. An independent sample *t*-test and one-way ANOVA were used. For the survival analysis, we analyzed the risk of mortality and rehospitalization for heart failure during follow-up period using multivariate Cox regression analysis. $P < 0.05$ was considered statistically significant.

Results

Comparison of cardiac function between groups

The data for cardiac function parameters before and after intervention in the two groups are shown in Table 2. Before intervention, the levels of LVEDD and LVEF were similar between the two groups ($P > 0.05$). After intervention, the level of LVEDD was significantly lower, while LVEF was observably higher in the experimental group, compared with the control group (LVEDD: 48.42 ± 5.02 vs. 53.78 ± 5.69; LVEF: 52.49 ± 5.98 vs. 43.81±3.94) and the differences were statistically significant ($P < 0.05$).

Comparison of levels of serum inflammatory factors between groups

The levels of serum TNF-α, hs-CRP, and IL-6 before and after intervention in the two groups were not significantly different between the control and experimental groups before intervention (Table 3). After 1-year

Table 2. Comparison of cardiac function between groups ($\bar{x} \pm s$).

Group	Patients		LVEDD (mm)	LVEF (%)
Control	70	Before intervention	60.56 ± 7.60	36.26 ± 5.03
		After intervention	53.78 ± 5.69	43.81 ± 3.94
Experimental	70	Before intervention	61.25 ± 5.76	37.34 ± 3.95
		After intervention	48.42 ± 5.02	52.49 ± 5.98
t value			5.906	10.140
P value			0.000	0.000

Table 3. Comparison of the levels of serum inflammatory factors between groups ($\bar{x} \pm s$).

Group	Patients		TNF-α (ng/L)	hs-CRP (mg/L)	IL-6 (ng/L)
Control	70	Before intervention	30.70 ± 6.44	26.20 ± 6.71	36.52 ± 5.90
		After intervention	21.54 ± 3.40	21.79 ± 3.13	24.81 ± 4.92
Experimental	70	Before intervention	32.21 ± 4.11	26.42 ± 5.89	36.04 ± 5.16
		After intervention	14.30 ± 2.58	13.59 ± 2.78	15.61 ± 3.63
t value			14.208	16.377	12.589
P value			0.000	0.000	0.000

of intervention, the levels of TNF-α, hs-CRP, and IL-6 exhibited various degrees of decrease in both control and experimental groups, with decrease being more significant in the experimental group than in the control group ($P < 0.05$).

Comparison of NT-proBNP and 6MWD between groups

Both NT-proBNP level and 6MWD outcome were tested before intervention, and the results showed that they were not significantly different between the two groups (Table 4). After intervention, the level of NT-proBNP was decreased, while 6MWD was increased in both groups. Importantly, the evaluation of NT-proBNP and 6MWD in the experimental group improved significantly, compared to the control group ($P < 0.05$).

Comparison of proportions of rehospitalization and mortality between groups

During the 1-year follow-up, the number of rehospitalized patients was 27 in the experimental group, accounting for a rehospitalization rate of 38.57% and a mortality rate of 12.86%. Both rehospitalization and mortality rates were significantly lower in the experimental group, compared to the control group, which showed the rehospitalization rate and a mortality rate of 55.71% and 27.14% (χ^2 value = 4.128 and 4.464; P value = 0.042 and 0.035), respectively. The Kaplan–Meier survival curve analysis also showed that the experimental group had lower proportion of heart failure, hospitalization rate, and mortality than the control group (Figures 1A and 1B).

Shexiang Tongxin pills increased OGD/R-induced cardiomyocyte survival

Previous studies have found significant clinical efficacy of STP in improving cardiac function and prognosis in patients with severe heart failure in clinical practice and proved that it had an important clinical value. Next, we further investigated the function of STP in cellular models. Different concentrations of STP (from 0 μg/mL to 1,000 μg/mL) were treated in H9c2 cells, and it was found that the concentration of STP in the range of 0–100 μg/mL had no obvious influence on cell viability and LDH release in H9c2 cells (Figures 2A and 2B). This indicated that the concentration range of 0–100 μg/mL of STP had less effect on the cytotoxicity of H9c2 cell line. In addition, we used OGD/R-induced H9c2 cells as a cardiomyocyte injury model. As shown in Figures 2C–2E, OGD/R markedly decreased the viability of H9c2 cells while significantly increasing LDH release and CK-MB activity in H9c2 cells. In contrast, STP or captopril treatment reversed these indices, where captopril was selected to act as a positive control.

Furthermore, the impact of STP on the apoptosis of H9c2 cells was also elevated by flow cytometry and Western Blot analysis. As shown in Figure 3A, the cell apoptosis rate of H9c2 cells was dramatically increased in the cells treated with OGD/R ($P < 0.001$), while STP or captopril treatment could reduce apoptosis rate in H9c2 cells induced by OGD/R. Additionally, the expression of Bax and cleaved caspase-3 was increased, while the expression of Bcl-2 was reduced in H9c2 cells induced by OGD/R, while STP or captopril treatment reversed these

Table 4. Comparison of NT-proBNP and 6MWD between groups ($\bar{x} \pm s$).

Group	Patients		NT-proBNP (pg/mL)	6MWD (m)
Control	70	Before intervention	3694.25 ± 283.94	366.56 ± 17.40
		After intervention	3251.88 ± 177.29	405.76 ± 23.90
Experimental	70	Before intervention	3701.91 ± 322.74	364.68 ± 15.36
		After intervention	2337.54 ± 136.59	486.06 ± 27.16
t value			34.181	18.570
P value			0.000	0.000

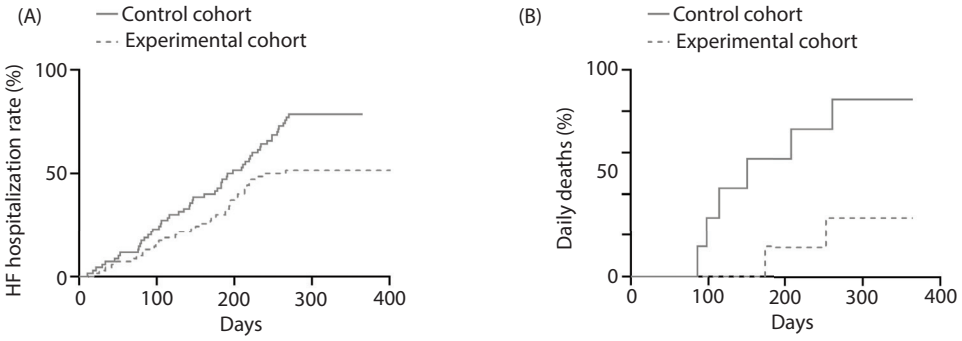

Figure 1. Kaplan–Meier survival analysis of patient rehospitalization and mortality. (A) Kaplan–Meier survival analysis of patient rehospitalization induced by heart failure. (B) Kaplan–Meier survival analysis of patient mortality.

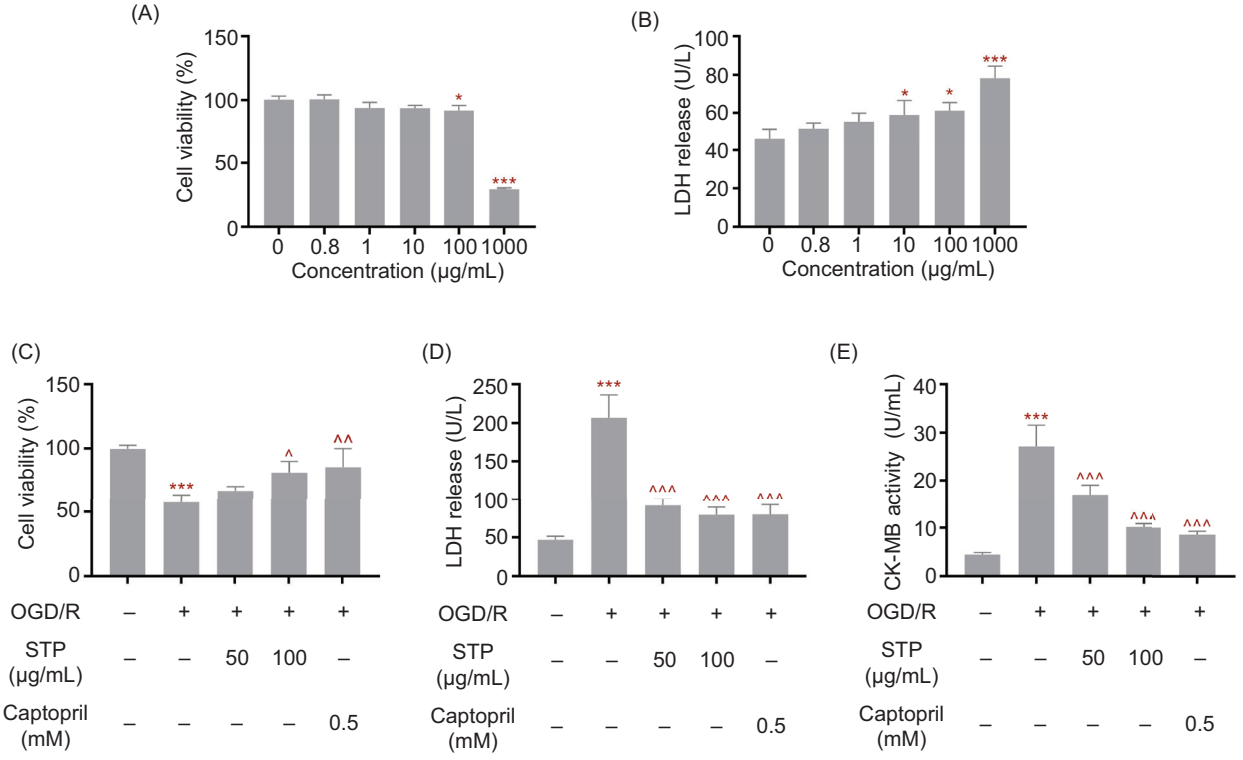

Figure 2. Shexiang Tongxin pills increased OGD/R-induced cardiomyocyte survival. (A) MTT assay was performed to evaluate the viability of H9c2 cells treated with different concentrations of STP (from 0 μg/mL to 1,000 μg/mL). (B) LDH assay was performed to evaluate the cytotoxicity of H9c2 cells treated with different concentrations of STP (from 0 μg/mL to 1,000 μg/mL). (C) MTT assay was performed to evaluate the viability of H9c2 cells induced by OGD/R and treated with STP or captopril. (D) LDH assay was performed to evaluate the cytotoxicity of H9c2 cells induced by OGD/R and treated with STP or captopril. (E) ELISA was performed to evaluate the CK-MB activity of H9c2 cells induced by OGD/R and treated with STP or captopril. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the 0-μM group; $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$, compared to the OGD/R group.

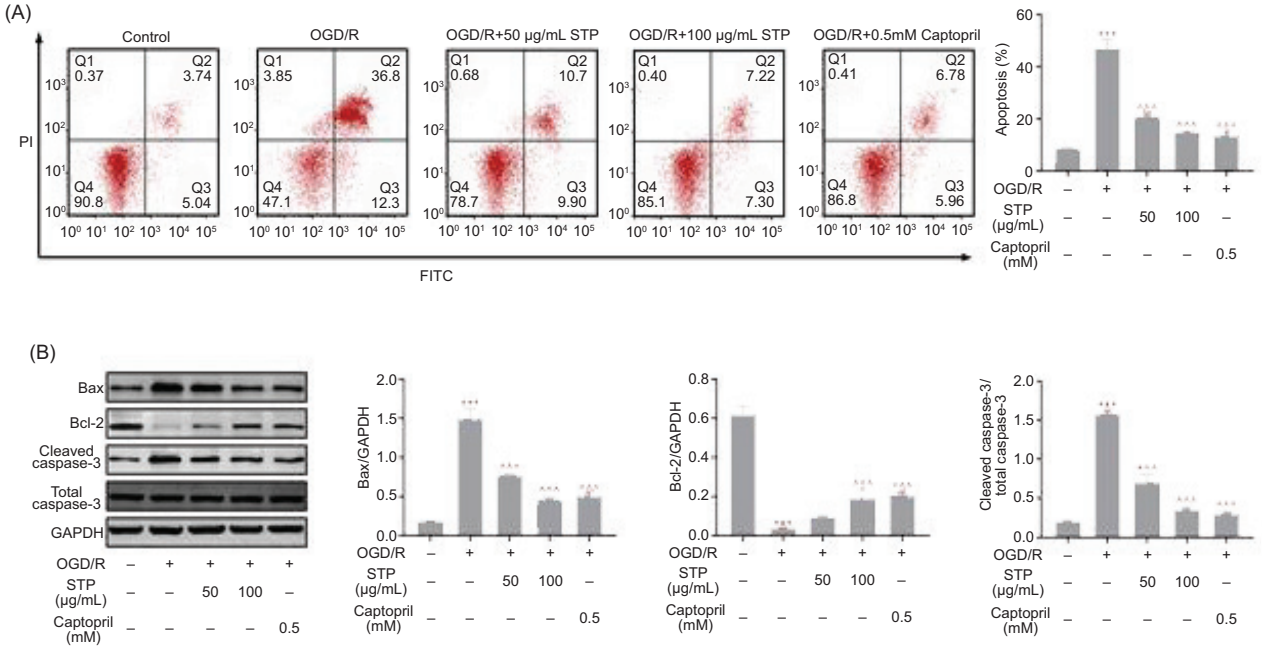

Figure 3. Shexiang Tongxin pills decreased OGD/R-induced cardiomyocyte apoptosis. (A) Flow cytometry assay was performed to evaluate the apoptosis rate of H9c2 cells induced by OGD/R and treated with STP or captopril. (B) The protein level of Bax, cleaved caspase-3, and Bcl-2 was evaluated by Western blot analysis in H9c2 cells induced by OGD/R and treated with STP or captopril. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the 0-μM group; $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$, compared to the OGD/R group.

indices (Figure 3B). These data suggested that STP could increase cardiomyocyte survival and decrease the apoptosis of cardiomyocytes induced by OGD/R.

Shexiang Tongxin pills ameliorated the OGD/R-induced inflammatory response in cardiomyocytes

The measurement of inflammatory indexes in patients before and after the intervention was also done, so we also measured inflammatory index content at cellular level by ELISA. As shown in Figure 4, IL-6, IL-1β, TNF-α, and MCP-1 were significantly upregulated in H9c2 cells induced by OGD/R. At the same time, STP or captopril treatment could downregulate the increase of these inflammatory indexes in H9c2 cells induced by OGD/R. To summarize, STP ameliorated the OGD/R-induced inflammatory response in cardiomyocytes.

Discussion

The increasing aging population in China is causing an increase in chronic diseases, such as diabetes, hyperlipidemia, and cardiovascular diseases, annually. Although the medical level has improved with each passing year, the mortality associated with these diseases remains inevitably high (Murphy *et al*., 2020). The causes of severe heart failure are relatively complicated because they correlate with the body's inflammatory responses, aging, and other organs (Upadhya and Kitzman, 2020). Elderly are more susceptible to severe heart failure because of their age-related decreased metabolism and often have poor prognoses, resulting in high rehospitalization and mortality rates. Therefore, it is of great significance to explore new treatment plans for severe heart failure to reduce mortality and improve the living standard of patients.

Presently, surgery remains the major form of treatment for severe heart failure. However, with an in-depth study of this disease, it was found that combining TCM with Western medicine was associated with better therapeutic benefits. The importance of TCM has been emphasized for treating severe heart failure, with various therapeutic regimens now prescribed in clinics, and different degrees of clinical efficacies achieved with evidences showing enhancement in ventricular reconstruction and improvement in the quality of life and prognosis of patients (Qiu *et al*., 2022; Wang *et al*., 2021). For example, it has been found that TCM monomer astragaloside IV can be used as a potential therapeutic agent for the clinical treatment of heart failure by protecting against myocardial ischemia, regulating the sarcoplasmic reticulum Ca^{2+} pump, promoting angiogenesis, improving energy metabolism, inhibiting cardiac hypertrophy and fibrosis, and decreasing apoptosis of cardiomyocytes (Zang *et al*., 2020).

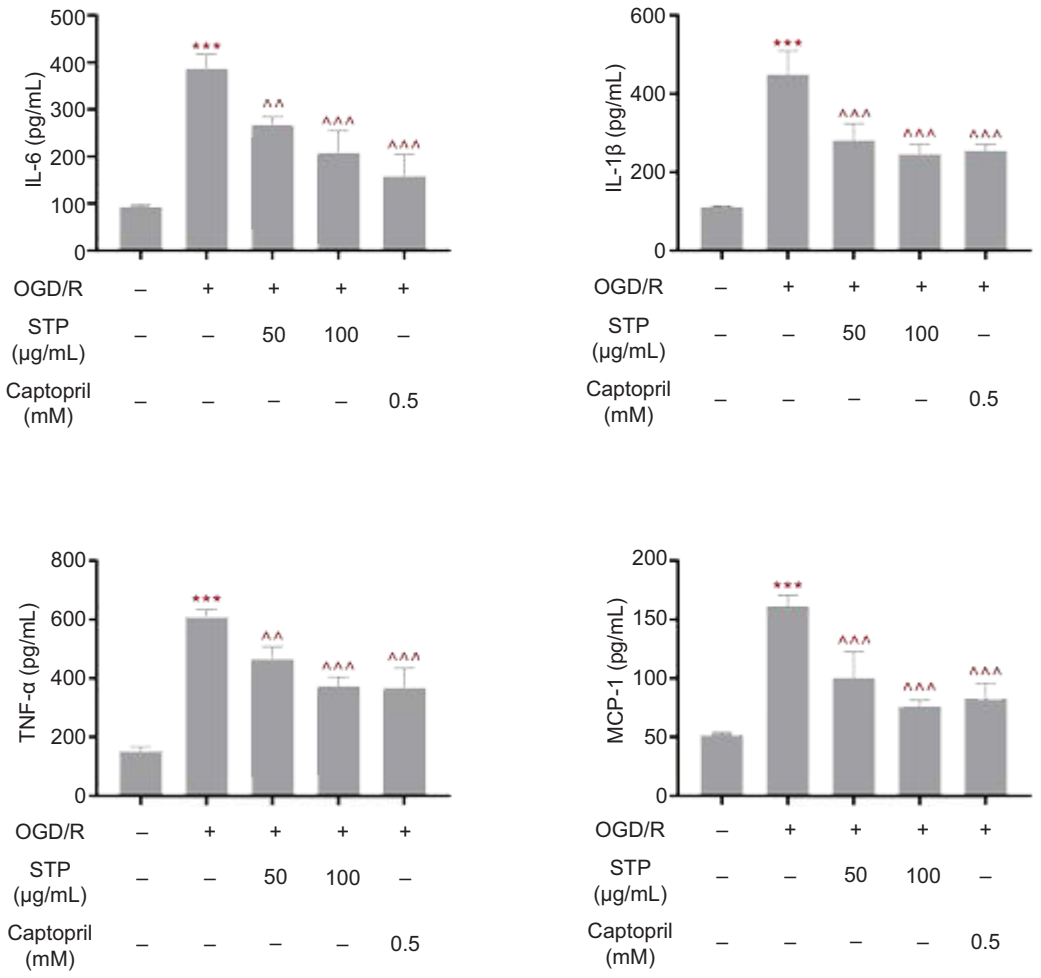

Figure 4. Shexiang Tongxin pills ameliorated the OGD/R-induced inflammatory response in cardiomyocytes. ELISA assay was performed to evaluate the content of IL-6, IL-1β, TNF-α, and MCP-1 in H9c2 cells induced by OGD/R and treated with STP or captopril. *P < 0.05, **P < 0.01, ***P < 0.001, compared to the 0-μM group; ^P < 0.05, ^^P < 0.01, ^^^P < 0.001, compared to the OGD/R group.

Recent clinical studies have also demonstrated that *Aconiti Lateralis Radix Praeparata*, combined with conventional heart failure treatment, has a significant benefit in preventing cardiovascular events and reducing the risk of cardiovascular diseases (Tai *et al*., 2022). All above cases showed that Chinese medicine played an important role in prevention of heart failure and rehabilitation.

Shexiang Tongxin dropping pills mainly comprise artificial musk, total saponins of folium ginseng, venenum bufonis, salvia miltiorrhiza, artificial bezoar, bear bile powder, and borneol. Pharmacological studies showed that the effects of artificial musk were comparable with natural musk, as it could reduce the myocardium's oxygen consumption, induce resuscitation, and relieve swelling and pain. It is also reported that total saponins of folium ginseng could strengthen the heart and improve the body's immunologic functions (Park *et al*., 2022). Besides, other studies found that this drug showed similar reduction in oxygen consumption of the myocardium as artificial musk by slowing the heart rate (Ding *et al*., 2021). Venenum bufonis mainly strengthens the heart, relieves swelling and pain, and relieves internal heat or fever similar to artificial bezoar. Salvia miltiorrhiza can promote blood circulation, dredging the meridian and alleviating pain. Bear bile powder plays an important role in clearing heat and improving eyesight. Additionally, studies have found that this drug significantly regulates the levels of inflammatory factors and oxidative stress in the body (Lin *et al*., 2019). Further, the compatibility of borneol, musk, and bezoar plays a dominant role in clearing heat and restoring consciousness, and the whole prescription functions in tonifying and promoting Qi circulation, invigorating blood circulation, dispersing blood stasis, and relieving pain. Documented evidence demonstrated that adjuvant therapy with STP could improve the clinical effects of patients with heart failure and effectively increase their diastolic and systolic blood pressure (Lin *et al*., 2017, 2022).

This study compared the clinical efficacy of conventional therapy combined with adjuvant therapy supplemented with STP versus conventional therapy only based on patients' cardiac function, levels of inflammatory factors, 6MWD outcomes, and rehospitalization and mortality rates during 1-year follow-up. The results showed that the indicators of cardiac function were significantly improved in patients treated with STP, which was reflected by an obvious decrease in the levels of LVEDD and NT-proBNP and significantly elevated levels of LVEF. These indicators performed best in revealing the patient's cardiac function directly. Consistent with the findings of a previous study (Yao *et al*., 2020), our data indicated that STP could reliably and effectively improve cardiac functions. Further, by comparing the levels of three representative inflammatory factors, we found that the inflammatory stress of patients was significantly ameliorated if treated with STP. This could be related to the role of whole prescription in promoting blood circulation to remove meridian obstruction as well as the working mechanism of bear bile powder. The results of 6MWDS and rehospitalization and mortality proportions during 1-year follow-up showed that STP could positively influence the recovery and prognosis of patients with severe heart failure. However, this study did not involve a clinical sample of Galen malformation heart failure (Reddy and Lucke-Wold, 2022) or infective endocarditis (Boatright *et al*., 2022); these being limitations, more samples of various types of heart failure could be recruited for subsequent studies.

In addition, a study showed that a mice model of chronic heart failure was constructed to investigate the specific effects of STP, and it was found that STP improved cardiac function, reduced cardiomyocyte hypertrophy, and improved myocardial fibrosis in congestive heart failure (CHF) mice by inhibiting ERK–mitogen-activated protein kinase (ERK/MAPK) and transforming growth factor-beta (TGF-β) signaling pathways (Zhang *et al*., 2021). In this study, the effect of STP at cellular level was also evaluated. OGD/R-induced H9c2 cells were conducted to mimic the injury of H9c2 cells in heart failure, and we found that STP increased cell survival and reduced cell apoptosis, cytotoxicity, and inflammatory response in cardiomyocyte induced by OGD/R. However, little is known regarding the specific underlying mechanisms, which should be intensively investigated in the future studies with more patients.

Conclusion

Shexiang Tongxin pills demonstrated satisfying clinical and survival benefits for treating severe heart failure by effectively improving patients' cardiac functions and reducing inflammatory stress, thereby demonstrating important clinical values in reducing rehospitalization and mortality rates in patients with severe heart failure. It could also ameliorate cellular damage and inflammation at cellular level.

Author Contributions

Conceptualization, Yanping Zeng; methodology, Weixing Ma; software, Hua Xue; validation, Yanping Zeng; formal analysis, Weixing Ma; investigation, Xiaohui Ren; resources, Guanfeng Zhu; data curation, Keming Xiao; writing and original draft preparation, Zhenhua Jiang; writing, review, and editing, Yanping Zeng; visualization, Keming Xiao; supervision, Xiaohui Ren; project administration, Weixing Ma; funding acquisition, Yanping Zeng. All authors had read and agreed to the published version of the manuscript.

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Data Availability Statement

All data generated and analyzed during this study are included in this published article.

Conflicts of Interest

The authors declared no conflict of interest.

Consent for Publication

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

References

Borlaug, B.A., 2020. Evaluation and management of heart failure with preserved ejection fraction. Nat Rev Cardiol 17: 559–573. https://doi.org/10.1038/s41569-020-0363-2

Chang, C., Ren, Y., Su, Q., 2022. Exploring the mechanism of Shexiang Tongxin dropping pill in the treatment of microvascular angina through network pharmacology and molecular docking. Annals Of Translational Medicine 10:983. https://doi.org/10.21037/atm-22-3976

Ding, Y., Zhu, H.Y., Zhang, L.Z., Gao, B.B., Zhou, L., et al. 2021. Shexiang Tongxin dropping pill () reduces coronary

microembolization in rats via regulation of mitochondrial permeability transition pore opening and AKT-GSK3β phosphorylation. Chinese Journal of Integrated Medicine 27: 527–533. https://doi.org/10.1007/s11655-019-3176-6

Di Palo, K.E. and Barone, N.J., 2020. Hypertension and heart failure: prevention, targets, and treatment. Heart Failure Clinics 16: 99–106. https://doi.org/10.1016/j.hfc.2019.09.001

Heart Failure Group of Chinese Society of Cardiology of Chinese Medical Association; Chinese Heart Failure Association of Chinese Medical Doctor Association; Editorial Board of Chinese Journal of Cardiology, 2018. Chinese guidelines for the diagnosis and treatment of heart failure 2018. Zhonghua Xin Xue Guan Bing Za Zhi 46(10): 760–789. Chinese. https://doi.org/10.3760/cma.j.issn.0253-3758.2018.10.004

Huang, C., Qiu, S., Fan, X., Jiao, G., Zhou, X., Sun, M., et al. 2021. Evaluation of the effect of Shengxian decoction on doxorubicin-induced chronic heart failure model rats and a multi-component comparative pharmacokinetic study after oral administration in normal and model rats. Biomedicine and Pharmacotherapy 144: 112354. https://doi.org/10.1016/j.biopha.2021.112354

Liao, M., Xie, Q., Zhao, Y., Yang, C., Lin, C., Wang, G., et al. 2022. Main active components of Si-Miao-Yong-An decoction (SMYAD) attenuate autophagy and apoptosis via the PDE5A-AKT and TLR4-NOX4 pathways in isoproterenol (ISO)-induced heart failure models. Pharmacological Research 176: 106077. https://doi.org/10.1016/j.phrs.2022.106077

Lin, S., Chu, J., Zhang, L., Chen, D., Xiao, F., Chen, H., et al. 2017. Protective effects of Shexiang Tongxin dropping pill on pituitrin-induced acute myocardial ischemia in rats. Molecular Medicine Reports 16: 3125–3132. https://doi.org/10.3892/mmr.2017.6963

Lin, Y.J., Jiao, K.L., Liu, B., Fang, L. and Meng, S., 2022. Antiplatelet and myocardial protective effect of Shexiang Tongxin dropping pill in patients undergoing percutaneous coronary intervention: a randomized controlled trial. Journal of Integrative Medicine 20: 126–134. https://doi.org/10.1016/j.joim.2022.01.001

Lin, S., Lin, J.M., Zhang, L., Chen, D.X., Xiao, F., Chen, H.W., et al. 2019. Shexiang Tongxin dropping pill protects against Na(2)S(2)O(4)-induced hypoxia-reoxygenation injury in H9c2 Cells. Chinese Journal of Integrative Medicine 25: 439–445. https://doi.org/10.1007/s11655-018-2976-4

Liu, H., Zhao, J., Pan, S., Zhu, Y., Fu, G., Tang, W., et al. 2021. Shexiang tongxin dropping pill protects against sodium laurate-induced coronary microcirculatory dysfunction in rats. Journal of Traditional Chinese Medicine (Chung i tsa Chih Ying wen Pan) 41(1): 89–97. https://doi.org/10.19852/j.cnki.jtcm.2021.01.011

Meng, H., Du, Z., Lu, W., Wang, Q., Sun, X., Jiang, Y., et al. 2021. Baoyuan decoction (BYD) attenuates cardiac hypertrophy through ANKRD1-ERK/GATA4 pathway in heart failure after acute myocardial infarction. Phytomedicine 89: 153617. https://doi.org/10.1016/j.phymed.2021.153617

Murphy, S.P., Ibrahim, N.E. and Januzzi, J.L., 2020. Heart failure with reduced ejection fraction: a review. Journal of the American Medical Association (JAMA) 324: 488–504. https://doi.org/10.1001/jama.2020.10262

Pan, J., Wang, Z.-H., Wang, X.-M., Zuo, K.-N., Zhou, J.-M., Wang, W., et al. 2021. Pharmacoeconomic evaluation of Shexiang Tongxin dropping pills combined with conventional western medicine treatment for coronary heart disease by Markov model. (Zhongguo Zhong Yao Za Zhi Zhongguo Zhongyao Zazhi China) Journal of Chinese Materia Medica 46: 1832–1838. https://doi.org/10.19540/j.cnki.cjcmm.20210209.501

Billingsley, H.E., Hummel, S.L., Carbone, S., 2020. The role of diet and nutrition in heart failure: A state-of-the-art narrative review. Journal of Progress In Cardiovascular Diseases 63:538–551. https://doi.org/ 10.1016/j.pcad.2020.08.004

Lu, Y., Chu, X., Zhang, J., Zhao, Y., Jin, C., Zhu, J., et al. 2020. Effect of Shexiang Tongxin dropping pill on stable coronary artery disease patients with normal fractional flow reserve and coronary microvascular disease: A study protocol. Journal of Medicine (Baltimore) 99:e22126. https://doi.org/10.1097/MD.0000000000022126

Xiong, M., Jia, C., Cui, J., Wang, P., Du, X., Yang, Q., et al. 2015. Shexiang Tongxin dropping pill attenuates atherosclerotic lesions in ApoE deficient mouse model. Journal of Ethnopharmacology 159:84–92. https://doi.org/10.1016/j.jep.2014.11.013

Tan, Y.F., Fu, Y.H., Zhang, M.Z., 2023. Shexiang Tongxin Dropping Pill Allieviates Heart Failure via Extracellula Matrix-Receptor Interaction Pathways Based on RNA-Seq Transcriptomics and Experimental Studies. Chinese Journal of Integrative Medicine 29:600–607. https://doi.org/10.1007/s11655-023-3633-0

Park, Y.S., Wang, W., Li, Y.D. and Liu, K., 2022. Network meta-analysis of six Chinese patent medicines for replenishing Qi and activating blood in treatment of chronic heart failure. Journal of Chinese Materia Medica (Zhongguo Zhong Yao Za Zhi) 47: 4221–4237. https://doi.org/10.19540/j.cnki.cjcmm.20220510.502

Qiu, R., Zhong, C., Wan, S., Zhang, Y., Wei, X., Li, M., et al. 2022. Developing a core outcome set for assessing clinical safety outcomes of cardiovascular diseases in clinical trials of integrated traditional Chinese medicine and western medicine: study protocol. Trials 23: 1–9. https://doi.org/10.1186/s13063-022-06166-3

Reddy, R. and Lucke-Wold, B., 2022. Primer of vein of galen malformation management. Journal of Pediatric Heath Care Medicine 5: 30–34.

Tai, C.J., El-Shazly, M., Yang, Y.H., Tsai, Y.H., Csupor, D., Hohmann, J., et al., 2022. The effectiveness of Fuzi in combination with routine heart failure treatment on chronic heart failure patients. Journal of Ethnopharmacology 289: 115040. https://doi.org/10.1016/j.jep.2022.115040

Truby, L.K. and Rogers, J.G., 2020. Advanced heart failure: epidemiology, diagnosis, and therapeutic approaches. JACC Heart Failure 8: 523–536. https://doi.org/10.1016/j.jchf.2020.01.014

Tufan, A., Avanoğlu Güler, A. and Matucci-Cerinic, M., 2020. COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs. Turkish Journal of Medical Science 50: 620–632. https://doi.org/10.3906/sag-2004-168

Upadhya, B. and Kitzman, D.W., 2020. Heart failure with preserved ejection fraction: new approaches to diagnosis and management. Clinical Cardiology 43: 145–155.

Wang, L., Xiang, L., Piao, S., Gong, X., Zhou, W., Feng, W., et al. 2021. The efficacy and safety of Chinese medicine fufang zhenzhu Tiaozhi capsule (FTZ) in the treatment of diabetic coronary heart disease: study protocol for multicenter, randomized, double-blind, placebo-controlled clinical trial. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 14: 2651–2659. https://doi.org/10.2147/DMSO.S309419

Yao, Y., Zeng, Z., Zhao, Y., Li, T., Liu, Y. and Chen, R., 2020. Effect of Shexiang Tongxin dripping pills on coronary microcirculation disorder and cardiac dysfunction in a porcine model of myocardial ischemia-reperfusion injury. Nan Fang Yi Ke Da Xue Xue Bao 40: 899–906. Chinese. https://doi.org/10.12122/j.issn.1673-4254.2020.06.19

Zang, Y., Wan, J., Zhang, Z., Huang, S., Liu, X. and Zhang, W., 2020. An updated role of astragaloside IV in heart failure. Biomedicine and Pharmacotherapy 126: 110012. https://doi.org/10.1016/j.biopha.2020.110012

Zhang, S., Liu, H., Fang, Q., He, H., Lu, X., Wang, Y., et al. 2021. Shexiang Tongxin dropping pill protects against chronic heart failure in mice via inhibiting the ERK/MAPK and TGF-β signaling pathways. Frontiers in Pharmacology 12: 796354. https://doi.org/10.3389/fphar.2021.796354