

## Original Research Article

# Cardioprotective effects of *Dan-Yang-Fu-Xin* decoction on chronic heart failure in rats

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### Abstract

**Purpose:** To evaluate the cardioprotective effects and possible mechanisms of *Dan-Yang-Fu-Xin* decoction (DYFX) in a rat chronic heart failure (CHF).

**Methods:** A CHF rat model induced by ligation of the left anterior descending coronary artery was used to investigate the cardioprotective effects of DYFX. After intragastric administration for 8 weeks, several functional cardiac indices, including fractional shortening (FS), ejection fraction (EF), heart rate (HR) and cardiac output (CO) were assessed by ultrasound examination. Subsequently, inflammatory markers, viz, interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), myocardial enzymes, namely, lactate dehydrogenase (LDH) and creatine kinase (CK), were also assessed by enzyme-linked immunosorbent assay (ELISA).

**Results:** Intragastric administration of DYFX (200, 400 and 600 mg/kg) significantly reversed the decrease in body weight and increase in cardiac weight ( $p < 0.05$ ) induced by CHF. Treatment with DYFX also significantly reversed EF, FS, HR, and CO changes in CHF rats. In addition, DYFX inhibited the two inflammatory cytokines (TNF- $\alpha$  and IL-6) and myocardial enzymes (CK and LDH), suggesting that these effects may include the mechanisms of cardioprotection involved in attenuation of CHF.

**Conclusion:** DYFX possesses cardioprotective effects involving CHF. The protective mechanisms may include the suppression of expression of inflammatory mediators and myocardial enzymes.

**Keywords:** *Dan-Yang-Fu-Xin* decoction, Cardioprotection, Chronic heart failure, Inflammatory mediators, Myocardial enzymes

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## INTRODUCTION

Chronic heart failure (CHF) is a complex clinical syndrome involving impairment of cardiac pump function, resulting in a heart that cannot pump sufficient blood to meet the metabolic needs of the body [1,2]. CHF involves ventricular remodeling and hypertrophy, reduced cardiac ejection fractions (EFs) and impairment of active relaxation and contraction of the left ventricle [3]. CHF represents a major public health problem in

the world because of its high morbidity, mortality and cost [4].

Currently, angiotensin receptor antagonists,  $\beta$ -blockers, mineralocorticoid receptor blockers and diuretics have been used for treatment of CHF. However, these treatments are because of their side effects including damage to the liver, kidney, and gastrointestinal tract [5,6]. Traditional Chinese medicine (TCM) has shown efficacy in treating complex multi-factor diseases, because

TCM is a unique medical system that uses drugs with multiple components that have synergistic therapeutic efficacies [6-8]. *Dan-Yang-Fu-Xin* decoction (DYFX) is a TCM prescription consisting of *Salvia miltiorrhiza*, *Epimedium brevicornu*, *Ligusticum wallichii*, *Astragalus membranaceus* and *Glycyrrhiza uralensis* (Table 1).

It has been widely used in cities northern China, such as *Linyi*, *Yanzhou* and *Tengzhou* to treat coronary heart disease, anemia, chronic bronchitis and osteoporosis. DYFX is also beneficial for asthma and allergies. However, to the best of our knowledge, there have been no reports describing the pharmacological effects of DYFX.

The aim of the present study was to investigate the cardioprotective effects of DYFX in a CHF rat model by determining several cardiac function indices as well as the relationship between DYFX treatment and inflammatory markers.

## EXPERIMENTAL

### Chemicals and reagents

Interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Shanghai Bluegene Biotech (Shanghai, China). Creatine kinase (CK) and lactate dehydrogenase (LDH) ELISA kits were purchased from R&D systems (Beijing, China). Chloral hydrate was purchased from Kermel (Tianjin, China). All other chemicals and reagents used were of analytical grade.

### Preparation of DYFX water extracts

Because DYFX is prepared by directly decocting with water in traditional folk medicine, all preparations of DYFX (Table 1) were in powder form and were soaked for 8 h before decocting with distilled water six times and extracted three times for 30 min each time. The decoction were filtered and concentrated under reduced pressure at 55 °C with a vacuum rotary

evaporator. The resulting w/w yield of DYFX was 11.2 %. Distilled water was used to dissolve and dilute the extract to the appropriate concentrations for intragastric administration.

### CHF animal model

Male Sprague-Dawley rats weighing  $220 \pm 20$  g were housed under controlled conditions at a temperature of  $22 \pm 1$  °C and humidity of  $45 \pm 5$  % with a 12 h light/dark cycle. The rats had free access to standard pallet diet (solid rodent chow) and tap water. The experiments were performed after 1 week acclimatization period. The experimental procedures were approved by the Committee of Animal Care and Use of our institute.

The rats were anaesthetized by intraperitoneal injection of chloral hydrate (300 mg/kg). Myocardial infarction and heart failure were induced by ligation of the left anterior descending coronary artery as previously described [9,10]. The surviving rats were randomly divided into five groups (n = 14): 1) the control group, 2) the CHF group, 3) the high dose DYFX group (600 mg/kg/d), 4) the medium dose DYFX group (400 mg/kg/d), 5) the low dose DYFX group (200 mg/kg/d). The control group received the same surgery but without ligating the vessel. The treatment was continued for 8 weeks via intragastric administration. At the end of the procedure, cardiac function was examined, blood samples were drawn from the abdominal aorta, and the serum was stored at -80 °C.

### Determination of cardiac function

Animals were anesthetized intraperitoneally with chloral hydrate (300 mg/kg). A portable ultrasound (Vivid I, GE Healthcare, Little Chalfont, UK) equipped with a 12-MHz linear transducer (12 L) was used to determine the fractional shortening(FS), ejection fraction (EF), heart rate (HR) and cardiac output (CO). An ultrasound image workstation (GE Healthcare) was used to transfer data online for subsequent analysis.

**Table 1:** Composition of *Dan-Yang-Fu-Xin* Decoction (DYFX)

Plant	Plant part	Weight (g)
<i>Salvia miltiorrhiza</i>	Roots	40
<i>Epimedium brevicornu</i>	Whole herbs	30
<i>Ligusticum wallichii</i>	Roots	15
<i>Astragalus membranaceus</i>	Roots	10
<i>Glycyrrhiza uralensis</i>	Roots	5

### Determination of TNF- $\alpha$ and IL-6

The myocardial tissues were homogenized in RIPA lysis buffer containing 50 mM Tris-HCl (pH=7.4), 150 mM NaCl, 1 % Triton X-100, 1 % sodium deoxycholate, 0.1 % SDS, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride and 1 mM NaF, (myocardial tissue - RIPA lysis buffer ratio was = 1:10, v/v) and then centrifuged (6,000  $\times$  g, at 4 °C for 15 min). The supernatants were collected to analyze TNF- $\alpha$  and IL-6 using ELISA kits according to the manufacturers' instructions.

### Determination of serum LDH and CK

Serum was separated from whole blood using centrifugation (6000 g, 4 °C and 15 min). Serum levels of LDH and CK were measured using ELISA kits according to the manufacturer instructions.

### Statistical analysis

Statistical analysis of data were performed using SPSS software, version 13.0 (SPSS, Chicago, IL, USA). All data are expressed as the mean  $\pm$  SD. One-way ANOVA was used for statistical analyses of observed values. A value of  $p < 0.05$  was considered statistically significant.

## RESULTS

### Effects of DYFX on body weight and cardiac weight

As shown in Table 2, the body weight of animals in the CHF group significantly decreased ( $p < 0.05$ ) compared with animals in control group. Furthermore, the whole heart weight, left ventricular weight, index of heart weight to body weight and index of left ventricle weight to body weight were significantly increased ( $p < 0.05$ ) in CHF rats. However, intragastric administration of DYFX (200, 400, and 600 mg/kg) significantly reversed these changes ( $p < 0.05$ ), indicating that DYFX could reduce cardiac hypertrophy in CHF animals (Table 2).

**Table 2:** Effects of DYFX on body weight and cardiac weight

Group	Body weight (g)	Whole heart weight (g)	Left ventricular weight (g)	Index of Heartweight to body weight	Index of left Heartweight to body weight
Control	289.26 $\pm$ 10.27 *	0.86 $\pm$ 0.09 *	0.55 $\pm$ 0.04 *	0.30 $\pm$ 0.03 *	0.19 $\pm$ 0.03 *
CHF	268.75 $\pm$ 8.53	1.06 $\pm$ 0.10	0.78 $\pm$ 0.08	0.39 $\pm$ 0.04	0.29 $\pm$ 0.02
200	279.32 $\pm$ 9.76 *	0.97 $\pm$ 0.09	0.63 $\pm$ 0.06 *	0.35 $\pm$ 0.03	0.23 $\pm$ 0.03 *
400	284.72 $\pm$ 8.92 *	0.95 $\pm$ 0.08 *	0.59 $\pm$ 0.05 *	0.33 $\pm$ 0.03 *	0.21 $\pm$ 0.02 *
600	283.91 $\pm$ 7.98 *	0.92 $\pm$ 0.07 *	0.58 $\pm$ 0.03 *	0.32 $\pm$ 0.02 *	0.20 $\pm$ 0.02 *

Data were expressed as mean  $\pm$  SD; \* $p < 0.05$ , compared with CHF group

### Effect of DYFX on cardiac functions

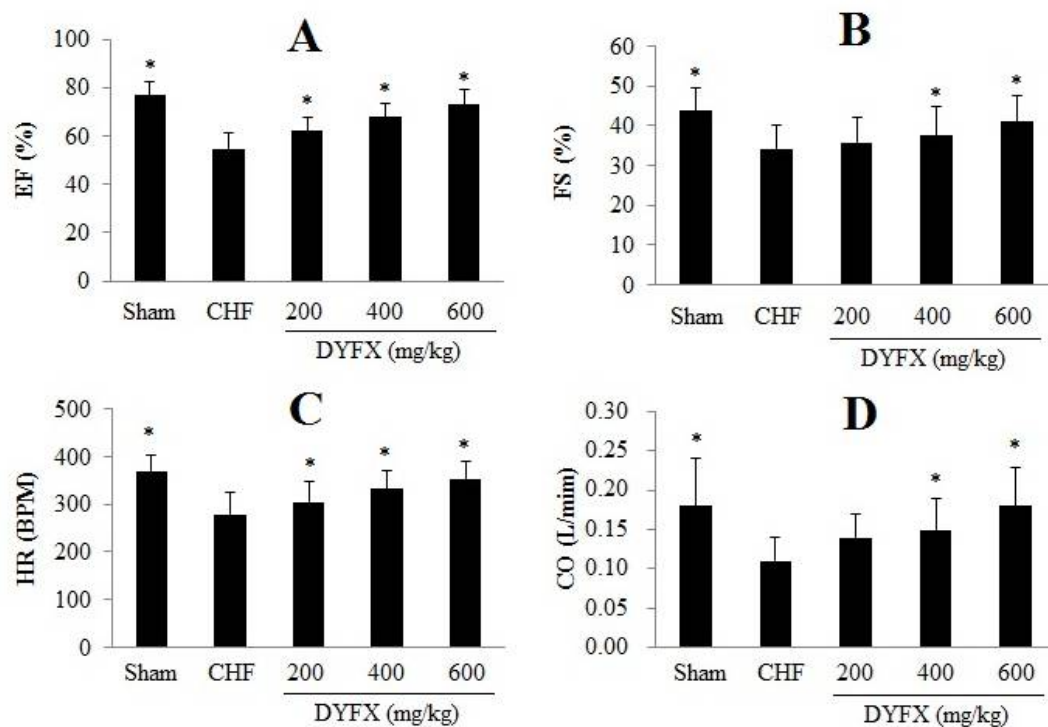
Figure 1A and 1B showed that the EF and FS of the CHF group significantly decreased ( $p < 0.05$ ) compared with the control group. However, the EF in DYFX treated groups increased significantly ( $p < 0.05$ ) compared with the CHF group, and the FS in the DYFX treated groups (400 mg/kg and 600 mg/kg,  $p < 0.05$ ) significantly increased compared with the CHF group. HR and CO are shown in Figure 1C and 1D. The HR and CO of CHF rats were significantly decreased compared with the control group ( $p < 0.05$ ). However, the HR in the DYFX treated groups significantly increased ( $p < 0.05$ ) compared with the CHF group, and the CO in the DYFX treated groups (400 mg/kg and 600 mg/kg,  $p < 0.05$ ) significantly increased compared with the CHF group. Taken together, the results showed that DYFX significantly reversed EF, FS, HR and CO changes in CHF rats.

### Effect of DYFX on TNF- $\alpha$ and IL-6 levels

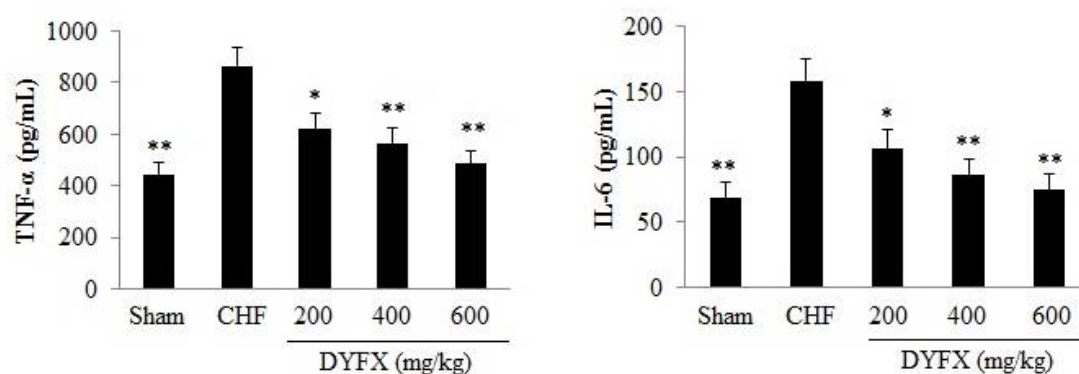
Figure 2 shows that myocardial production of TNF- $\alpha$  and IL-6 increased significantly in the CHF group compared with the control group ( $p < 0.01$ ). The levels of the two cytokines decreased in groups treated with DYFX compared with the CHF group (200 mg/kg,  $p < 0.05$ ; 400 mg/kg and 600 mg/kg,  $p < 0.01$ , respectively). The results showed that there was a close relationship between CHF and inflammation, and treatment with the DYFX effectively inhibited expression of the two inflammatory cytokines induced by CHF.

### Effect of DYFX on LDH and CK

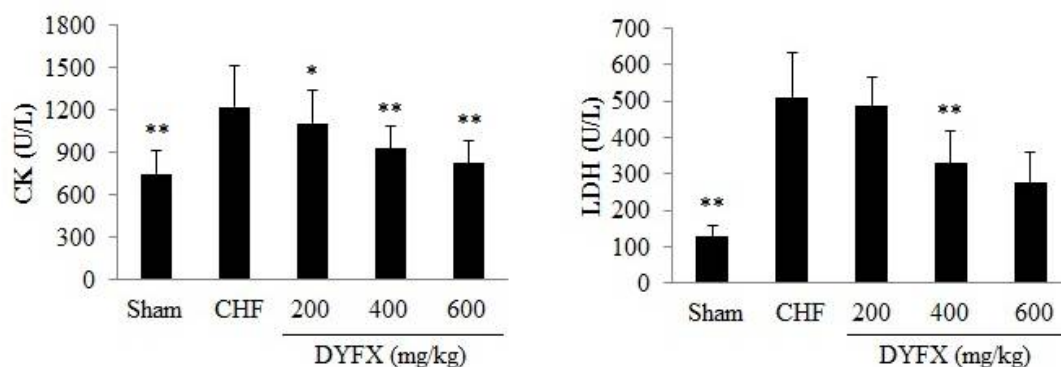
Figure 3 shows that, there was a significant increase ( $p < 0.01$ ) in serum LDH levels of the CHF group compared with the control group. Treatment with 400 mg/kg DYFX resulted in a significant decrease ( $p < 0.01$ ) in serum LDH levels as compared with the CHF group, and a significant increase ( $p < 0.01$ ) in serum CK levels in the CHF groups compared with Control group.



**Figure 1:** Effects of DYFX on EF, FS, CO and HR of the CHF rats. Data were expressed as Mean  $\pm$  SD; \* $p < 0.05$ , compared with CHF group



**Figure 2:** Effect of DYFX on TNF- $\alpha$ , IL-6 in CHF rats. Data were expressed as Mean  $\pm$  S.D.. \* $p < 0.05$ , compared with CHF group. \*\* $p < 0.01$ , compared with CHF group



**Figure 3:** Effects of DYFX on CK and LDH in serum of CHF rats. Data were expressed as Mean  $\pm$  SD; \* $p < 0.05$ , compared with CHF group; \*\* $p < 0.01$ , compared with CHF group

Treatment (200 mg/kg,  $p < 0.05$ ; 400 mg/kg and 600 mg/kg,  $p < 0.01$ ) resulted in a significant decrease in serum CK levels compared with the CHF group.

## DISCUSSION

In present study, a CHF rat model induced by ligation of the left anterior descending coronary artery was used to investigate the cardioprotective effects of DYFX. The results showed that DYFX reduced cardiac hypertrophy and reversed EF, FS, HR and CO changes in CHF rats. The possible mechanism of these cardioprotective effects might be related to the inhibition of inflammatory cytokine levels and myocardial enzymes levels. It is well-known that CHF is associated with immune activation, and its development and progression were closely related to inflammatory cytokines [11]. The overproduction of inflammatory factors, especially TNF- $\alpha$  and IL-6, could cause a decrease in cardiac systolic potential and cardiac output. The two factors could cause necrosis and apoptosis of cardiomyocytes and contribute to myocardial remodeling, resulting in worsening of CHF [5, 12]. The levels of TNF- $\alpha$  and IL-6 were evaluated in our study, showing that treatment with DYFX significantly suppressed the inflammation response via by decreasing the levels of TNF- $\alpha$  and IL-6 in CHF rats.

CK and LDH are marker enzymes of cardiac damage. There have been many reports describing the relationship between the two myocardial enzymes and cardioprotective effects [13-15]. The CK and LDH are released into the blood stream after myocardial injury. These myocardial enzymes levels were determined in the serum of CHF rats in our study, showing that DYFX could decrease the levels of CK and LDH.

## CONCLUSION

The present study shows that DYFX possesses potent cardioprotective effects on CHF animals. The protective mechanisms may involve suppression of the expressions of inflammatory mediators and myocardial enzymes. However, further investigations are required to identify the chemical constituents and complete mechanisms of DYFX action.

## CONFLICT OF INTEREST

No conflict of interest associated with this work.

## CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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