Effect of Qiangxin Huoli decoction on rats with adriamycin-induced chronic heart failure

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Abstract

OBJECTIVE: To investigate the effect of Qiangxin Huoli decoction on rats with chronic heart failure (CHF) induced by adriamycin (ADR), and to investigate the underlying mechanism of this effect.

METHODS: Ninety-six healthy Wistar rats were divided into six groups: control, CHF model, CHF treated by Shenfu injection, and three CHF groups treated with Qiangxin Huoli decoction at high, medium, and low doses, respectively. Qiangxin Huoli decoction was administered orally to protect the stomach in the three Qiangxin Huoli decoction groups, while the control group and the CHF model group were administered the same volume of 0.9% physiological saline, and the Shenfu group were administered the same volume of Shenfu injection. Ten days later, the CHF model was then induced in all groups except the control group by intraperitoneal injection of ADR at gradient dose intervals. The bodyweights were recorded on days 10, 20, 30, and 40. Hemodynamic indices were recorded, including left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum increase in left ventricular pressure (+dp/dtmax), maximum decrease in left ventricular pressure (-dp/dtmax), heart rate (HR), and electrocardiogram using an eight-channel physiological recorder with LabChart software monitoring. The plasma brain natriuretic peptide (BNP) concentration was determined by enzyme-linked immunosorbent adsorption. The expressions of B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X protein (Bax) were detected by immunohistochemical methods.

RESULTS: The CHF model group were in poor condition, and the mean bodyweight was significantly decreased compared with the control group. Furthermore, compared with the control group, the CHF groups had significantly decreased LVSP, +dp/dtmax, and -dp/dtmax, and significantly increased LVEDP. The CHF groups also showed significant increases in HR, S-T segment elevation, and plasma BNP levels compared with the control group. Compared with the CHF model group, the treatment groups had significantly increased Bax expression (P < 0.05) and significantly decreased Bcl-2 expression (P < 0.01), indicating less apoptosis. The high
dose Qiangxin Huoli decoction and the Shenfu group showed the most significant improvements.

CONCLUSION: In the rat model of CHF, Qiangxin Huoli decoction significantly reduces the abnormal hemodynamics, improves cardiac function, reduces plasma BNP concentration, regulates the expression of apoptosis proteins, inhibits the apoptosis of myocardial cells, and plays a protective role.

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Keywords: Heart failure; Hemodynamics; Doxorubicin; Natriuretic peptide, brain; Genes, bcl-2; Bcl-2-associated X protein; Qiangxin Huoli decoction

INTRODUCTION

Chronic heart failure (CHF) results from the loss of myocardial cell function, leading to a deficiency in ventricular blood ejection and a decline in heart function. A variety of factors have been implicated in the pathogenesis of CHF, which results in changes to cardiac structure and function. CHF results from the worsening pumping function of the heart, hemodynamic disorders, abnormal changes in neurohumoral factors, and dysfunction of the myocardial cells, all of which promote the occurrence and development of ventricular remodeling.

In recent years, patients with CHF have been managed by the use of both Western Medicine and Traditional Chinese Medicine (TCM). The Yiqi Wenyang Huoxue Lishui treatment for CHF uses analysis from the TCM perspective. The pathogenesis of CHF is investigated in terms of Qi and Yang deficiencies, which are characterized by blood stasis and water retention. The TCM prescription called Qiangxin Huoli decoction addresses these deficiencies to restore cardiac activity after heart failure. Qiangxin Huoli decoction has been used in clinical practice for nearly 40 years, and has been granted Chinese national patents. The present study investigated the effects of the Qiangxin Huoli decoction on the hemodynamics, electrocardiogram (ECG), plasma brain natriuretic peptide (BNP) changes, and expression of the apoptosis proteins B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X protein (Bax) in a rat CHF model.

METHODS

Drugs
Doxorubicin hydrochloride for injection [adriamycin (ADR)] was purchased from Shenzhen Million Pharmaceutical Co., Ltd. (lot No. H44024359, specifications 10 mg/branch). The composition of the Qiangxin Huoli decoction was Huangqi (Radix Astragali Mongolici) 20 g, Fuzi (Radix Aconiti Lateralis Preparata) 15 g, Dansheng (Radix Codonopis) 15 g, Danshen (Radix Angelicae Sinensis) 10 g, Chuanxiong (Rhizoma Chuanxiong) 10 g, Danshen (Radix Salviae Miltiorrhizae) 15 g, Sanqi (Radix Notoginseng) 5 g, Fuling (Poria) 15 g, Baizhu (Rhizoma Atractylodis Macrocephalae) 15 g, Guizhi (Ramulus Cinnamomi) 10 g, Tingli (Semen Lepidii Apertii) 15 g, stir-frying with liquid adjuvant Gancao (Radix Glycyrrhizae) 10 g (provided as Jiangxin non-dried particles by the Affiliated Hospital of the University of Traditional Chinese Medicine, Changchun, China). The liquid decoction was made in three concentrations (high, medium, and low) in accordance with the conversion coefficient from human to rat. The Shenfu injection was purchased from Ya’an 39 Pharmaceutical Co., Ltd. (lot No. Z51020664, specifications 10 mL/branch, Ya’an, China).

Reagents and instruments
Rat plasma BNP detection kits were obtained from Abcam Company (lot No. 20151024076, Cambridge County, UN). The rat Bcl-2 kits (lot No. 2015121439), rat Bax kits (lot No. 201403215), and dianminobenzene color agent (lot No. 2011052604) were purchased from Zhongshans Jinqiao Co. (Beijing, China). The PowerLab 8/35 Data Acquisition and Analysis System, including Quad Bridge Amplifier, Dual Bio Amplifier and Stimulus Isolator were produced by AD Instruments (New South Wales, Australia). The TDZ5-WS centrifuge was provided by Changsha Willcom Co. (Changsha, China). The thermo enzyme standard instrument and HM340E paraffin section machine were provided by American Thermo Co. (Walham, MA, USA). The TB-718D automatic tissue embedding machine was manufactured by Hubei Taiva Technology, Co., Ltd. (Hubei, China). The Motic Mike Audi microscope was manufactured by MOTIC CHINA GROUP Co., Ltd. (Xiamen, China). The JT202N electronic balance was manufactured by Jingtian Electronic Instrument Co., Ltd. (Shanghai, China).

Animals and congestive heart failure model establishment
Ninety-six healthy Wistar rats (48 males, 48 females) weighing (210 ± 10) g were supplied by the Experimental Animal Center of Jilin University (Certificate of quality No. SCXK[Jl] 2014-0003). The study was approved by the experimental animal ethics committee of Changchun University of Traditional Chinese Medicine. The rats were fed for 1 week before they were divided into six groups by random number table method: control group, model group, Shenfu group, and the high, medium, and low dose Qiangxin Huoli decoction groups. Each group contained 16 rats (eight males, eight females) that were kept in cages. Before the CHF model was created, the high, medium,
and low dose Qiangxin Huoli decoction groups were orally administered Qiangxin Huoli decoction at doses of 3.6, 1.8, and 0.9 g·kg⁻¹·d⁻¹, respectively, to protect the stomach. The control group and the CHF model group were orally administered the same volume of 0.9% physiological saline, and the Shenfu group were administered the same volume of Shenfu injection (3 g·kg⁻¹·d⁻¹). Ten days later, all rats (except the controls) were injected intraperitoneally with ADR (1 mg/mL in normal saline) using the gradient dose interval method to establish the CHF model. The dosages and frequencies of the administration gradient were 1 mg/kg (× 2), 2 mg/kg (× 2), 3 mg/kg (× 2), and 4 mg/kg (× 1) at 2 d intervals. The cumulative ADR dose for each rat was 16 mg/kg, and the modeling time was 19 d. The control group received the same treatment, but with intraperitoneal injections of physiological saline. At the end of the modeling, the treatment groups continued to receive separately intragastric administration of Qiangxin Huoli decoction or Shenfu injection and the control and model groups received saline for another 11 d, making the total duration of the experiment 40 d.

**Index detection**

Each rat was weighed on an electronic weighing tray once every 10 d.

**Hemodynamic monitoring**

At the end of the experimental period, the rats were fasted for 12 h but were allowed water before being anesthetized by an intraperitoneal injection of 10% hydrochloric acid ethanol (3 mL/kg). The rats were then fixed in the supine position, and information was collected using an eight-channel physiological recorder. Number 7 metal needles were inserted into the right upper limb and both sides of the lower limbs in each rat to provide ECG connections. The right carotid artery in the neck was exposed, and a heparin-filled polyethylene catheter attached to a pressure reducer was slowly inserted until the blood pressure column of the LabChart software indicated that the left ventricle was accessed. The left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum increase in left ventricular pressure (±dp/dtmax), maximum decrease in left ventricular pressure (±dp/dtmin), and other indicators of the ECG images were collected during a 20 min period.

**Sample collection and preparation**

After the hemodynamic parameters had been measured, the abdominal aorta was removed and placed in an anticoagulant tube for 30 min. The samples were then centrifuged, and the plasma was collected. The apical part of the heart was fixed in formaldehyde, and kept at 4 °C for 24 h. The cardiac muscle tissue was dehydrated in graded ethanols, then xylene, and then embedded in paraffin. A paraffin section microtome was used to cut 3 µm sections that were mounted on glass slides.

**Determination of plasma brain natriuretic peptide concentration**

The plasma BNP levels were determined by the double anti-sandwich enzyme-linked immunosorbent assay method, in accordance with the rat plasma BNP detection kit instructions.

**Hematoxylin-eosin staining**

Mounted sections were dewaxed in xylene, alcohol gradients, and water. Sections were then stained with hematoxylin (5 min), rinsed in water, differentiated in 1% hydrochloric acid ethanol (10 s), and neutralized in dilute ammonia (60 s). After rinsing with water back to the blue color (15 min), the sections were stained with Iriqi red (3 min) and rinsed in water. Slides were dehydrated through graded alcohol and xylene until they were dehydrated and transparent. Finally, the specimens were sealed with neutral gum and allowed to dry before being microscopically examined.

**Immunohistological detection of the expressions of Bcl-2 and Bax proteins in the myocardium**

Tissue sections were dewaxed with xylene and graded ethanol to water. The expressions of Bcl-2 and Bax monoclonal antibodies were detected using the rat Bcl-2 kit and the rat Bax kit, respectively. After application of the diaminobenzene color agent reaction and counterstaining with hematoxylin, the sections were dehydrated through graded alcohols and xylene before microscopic observation. Two slices were randomly selected from each rat sample, and the positively stained cells were observed using a digital microscope at × 5 magnification. Bax and Bcl-2 in the cytoplasm and the inner side of the membrane appeared as brown yellow particles. The number of positively-stained cells and the total number of cells in each field of view were counted, and the percentages of Bcl-2 and Bax positive cells were calculated.

**Statistical analysis**

SPSS 19.0 statistical software (SPSS Statistics for Windows, Version 19.0, SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The measurement data were expressed as the mean ± standard deviation ( ¯x ± s), and single factor analysis of variance was used in each group. P < 0.05 was considered to be statistically significant. P < 0.01 was considered to indicate extremely significant differences.

**RESULTS**

Over the course of the experiment, the CHF model groups showed varying changes in bodyweight, extensive ascites, dull hair, thin stools, and reduced movement. Deaths occurred in the control group (n = 1), model group (n = 5), Shenfu group (n = 2), high dose Qiangxin Huoli decoction group (n = 3), medium dose Qiangxin Huoli decoction group (n = 3), and low dose Qiangxin Huoli decoction group (n = 4).
Bodyweight
The mean bodyweight in the model group was significantly less than that in the control group, especially by day 30 (P < 0.01, Table 1). The treatment groups gained weight at a slower rate than the control group, but were significantly heavier than the model group; the high dose Qiangxin Huoli decoction group was significantly heavier than the model group on days 20, 30, and 40 (P < 0.01, Table 1).

Cardiac function
At the end of the experiment, the model group showed significant differences in HR, LVSP, LVEDP, ± dp/dt\textsubscript{max} compared with the control group (Table 2). The high dose Qiangxin Huoli decoction group maintained moderately strong heart function, with a significantly greater LVSP and ± dp/dt\textsubscript{max} and a significantly decreased HR and LVEDP compared with the model group (Table 2).

Electrocardiography
The ECG results indicate that the CHF modelling was successful, as that the S-T segment was significantly higher in the model group compared with the control group (Figure 1). The ECG of the Shenshu group and the high dose Qiangxin Huoli decoction was changed slightly, but to a much lesser degree than the model group. There was a downward trend of the S-T segment in the medium dose Qiangxin Huoli decoction group, while the S-T segment of the low dose Qiangxin Huoli decoction group was significantly higher than that in the high and medium dose Qiangxin Huoli decoction groups.

Plasma brain natriuretic peptide levels
The plasma BNP levels in the model group were significantly increased compared with the control group (P < 0.05, Table 3). The BNP levels in the Shenshu and high dose Qiangxin Huoli decoction groups were significantly less than that in the model group (P < 0.01), but were still significantly higher than that in the control group (P < 0.05, Table 3). The plasma BNP levels in the medium and low dose Qiangxin Huoli decoction groups were significantly lower than that in the model group (P < 0.05), but were significantly higher than that in the control group (P < 0.05, Table 3).

Table 1 Changes in bodyweight in each group (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 30</th>
<th>Day 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>266±16</td>
<td>289±16</td>
<td>309±19</td>
<td>330±18</td>
</tr>
<tr>
<td>Model</td>
<td>11</td>
<td>264±16</td>
<td>268±14\textsuperscript{a}</td>
<td>250±14\textsuperscript{a}</td>
<td>239±14\textsuperscript{a}</td>
</tr>
<tr>
<td>Shen Fu</td>
<td>14</td>
<td>266±17</td>
<td>281±16\textsuperscript{a}</td>
<td>273±17\textsuperscript{a}</td>
<td>282±16\textsuperscript{a}</td>
</tr>
<tr>
<td>High dose</td>
<td>13</td>
<td>266±15</td>
<td>274±15\textsuperscript{a}</td>
<td>270±16\textsuperscript{a}</td>
<td>272±16\textsuperscript{a}</td>
</tr>
<tr>
<td>Medium dose</td>
<td>12</td>
<td>266±16</td>
<td>269±14\textsuperscript{a}</td>
<td>261±12\textsuperscript{c}</td>
<td>265±13\textsuperscript{a}</td>
</tr>
<tr>
<td>Low dose</td>
<td>12</td>
<td>265±17</td>
<td>262±16\textsuperscript{a}</td>
<td>252±14\textsuperscript{c}</td>
<td>245±14\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Notes: control group: orally administered and injected with 0.9% physiological saline; model group: orally administered 0.9% saline and injected with ADR; Shenshu group: orally administered Shenshu injection treatment and injected with ADR; high dose group: orally administered Qiangxin Huoli decoction (3.6 mg·kg\textsuperscript{–1}·d\textsuperscript{–1}) and injected with ADR; medium dose group: orally administered Qiangxin Huoli decoction (1.8 mg·kg\textsuperscript{–1}·d\textsuperscript{–1}) and injected with ADR; low dose group: orally administered Qiangxin Huoli decoction (0.9 mg·kg\textsuperscript{–1}·d\textsuperscript{–1}) and injected with ADR. Each group was orally administered an equal volume of liquid. ADR: adriamycin. \(P < 0.05, P < 0.01\), compared with the control group; \(P < 0.05, P < 0.01\), compared with the model group.

Table 2 Cardiac function in each group on day 40 (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>HR/time</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>+dp/dt\textsubscript{max} (mm Hg/s)</th>
<th>-dp/dt\textsubscript{max} (mm Hg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>380±26.1</td>
<td>108±3±15.2</td>
<td>2.7±2.2</td>
<td>4548.4±225.2</td>
<td>4332.4±188.6</td>
</tr>
<tr>
<td>Model</td>
<td>11</td>
<td>488.7±42.3\textsuperscript{a}</td>
<td>64.5±12.6\textsuperscript{a}</td>
<td>20.3±6.5\textsuperscript{a}</td>
<td>2104.3±127.6\textsuperscript{a}</td>
<td>1876.0±196.3\textsuperscript{a}</td>
</tr>
<tr>
<td>Shen Fu</td>
<td>14</td>
<td>430.3±22.6\textsuperscript{a}</td>
<td>87.8±13.3\textsuperscript{a}</td>
<td>8.4±4.8\textsuperscript{a}</td>
<td>3980.0±169.3\textsuperscript{a}</td>
<td>3389.4±201.7\textsuperscript{a}</td>
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<tr>
<td>High dose</td>
<td>13</td>
<td>444.9±31.0\textsuperscript{a}</td>
<td>83.1±10.9\textsuperscript{a}</td>
<td>9.1±3.9\textsuperscript{a}</td>
<td>3784.8±233.2\textsuperscript{a}</td>
<td>3424.8±175.1\textsuperscript{a}</td>
</tr>
<tr>
<td>Medium dose</td>
<td>12</td>
<td>460.4±28.2\textsuperscript{a}</td>
<td>80.2±14.0\textsuperscript{a}</td>
<td>12.3±3.5\textsuperscript{a}</td>
<td>3568.7±242.4\textsuperscript{a}</td>
<td>2745.4±221.3\textsuperscript{a}</td>
</tr>
<tr>
<td>Low dose</td>
<td>12</td>
<td>474.7±25.2\textsuperscript{a}</td>
<td>76.3±7.5\textsuperscript{a}</td>
<td>16.9±4.0\textsuperscript{a}</td>
<td>3009.8±207.5\textsuperscript{a}</td>
<td>2489.4±167.7\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Notes: control group: orally administered and injected with 0.9% physiological saline; model group: orally administered 0.9% saline and injected with ADR; Shenshu group: orally administered Shenshu injection treatment and injected with ADR; high dose group: orally administered Qiangxin Huoli decoction (3.6 mg·kg\textsuperscript{–1}·d\textsuperscript{–1}) and injected with ADR; medium dose group: orally administered Qiangxin Huoli decoction (1.8 mg·kg\textsuperscript{–1}·d\textsuperscript{–1}) and injected with ADR; HR: heart rate; LVSP: left ventricular systolic pressure; LVEDP: left ventricular end-diastolic pressure; dp/dt\textsubscript{max}: maximum rate of left ventricle diastolic pressure change; dp/dt\textsubscript{max}: minimum rate of left ventricle diastolic pressure change. Each group was orally administered an equal volume of liquid. ADR: adriamycin. \(P < 0.05, P < 0.01\), compared with the control group; \(P < 0.05, P < 0.01\), compared with the model group.
Immunohistochemical analysis of the expressions of myocardium

Expressions of Bcl-2 and Bax proteins in the control group, but less than the model group (Figure 2).

Figure 1 Rat electrocardiograms using limb II leads
A: control group: orally administered and injected with 0.9% physiological saline; B: model group: orally administered 0.9% saline and injected with adriamycin (ADR); C: Shenfu group: orally administered Shenfu injection treatment and injected with ADR; D: high dose group: orally administered Qiangxin Huoli decoction (3.6 mg·kg⁻¹·d⁻¹) and injected with ADR; E: medium dose group: orally administered Qiangxin Huoli decoction (1.8 mg·kg⁻¹·d⁻¹) and injected with ADR; F: low dose group: orally administered Qiangxin Huoli decoction (0.9 mg·kg⁻¹·d⁻¹) and injected with ADR. X-axis: time; Y-axis: voltage.

Table 3 Plasma brain natriuretic peptide levels in each group on day 40 (pg/mL, ± s ±)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>44±6</td>
</tr>
<tr>
<td>Model</td>
<td>11</td>
<td>387±16²</td>
</tr>
<tr>
<td>Shen Fu</td>
<td>14</td>
<td>179±10⁻⁴</td>
</tr>
<tr>
<td>High dose</td>
<td>13</td>
<td>154±15⁻⁵</td>
</tr>
<tr>
<td>Medium dose</td>
<td>12</td>
<td>266±19⁻⁴</td>
</tr>
<tr>
<td>Low dose</td>
<td>12</td>
<td>327±13⁻⁴</td>
</tr>
</tbody>
</table>

Notes: control group: orally administered and injected with 0.9% physiological saline; model group: orally administered 0.9% saline and injected with ADR; Shenfu group: orally administered Shenfu injection treatment and injected with ADR; high dose group: orally administered Qiangxin Huoli decoction (3.6 mg·kg⁻¹·d⁻¹) and injected with ADR; medium dose group: orally administered Qiangxin Huoli decoction (1.8 mg·kg⁻¹·d⁻¹) and injected with ADR; low dose group: orally administered Qiangxin Huoli decoction (0.9 mg·kg⁻¹·d⁻¹) and injected with ADR. Each group was orally administered an equal volume of liquid. BNP: brain natriuretic peptide. ADR: adriamycin. *P < 0.01, **P < 0.05, compared with the blank group; †P < 0.01, ‡P < 0.05, compared with the model group.

Myocardial hematoxylin-eosin staining
The control group had clearly structured myocardial tissue, with parallel myocardial fibers arranged in fascicles; the myocardial cells were clear, the nuclei were discrete, and the cytoplasm was not edematous. In the model group, the myocardial cells had cavities and were edematous, and the myocardial fibers were fractured. The myocardial fibers of each treatment group showed different degrees of damage, but were not as severely damaged as those in the model group. The treated groups had a greater degree of cell edema than the control group, but less than the model group (Figure 2).

Expressions of Bcl-2 and Bax proteins in the myocardium
Immunohistochemical analysis of the expressions of Bcl-2 and Bax indicated apoptosis. Staining for the anti-apoptotic protein Bcl-2 was very light in the control group. Myocardial tissue in the model group was stained brownish yellow, whereas the stain was a deeper brown color in the myocardial cells and myocardial fibers in the treatment groups (Figure 3). The apoptotic accelerator, Bax, in myocardial cells and myocardial fibers stained cells a deep brown color, whereas the staining in the Shenfu group and the high dose Qiangxin Huoli decoction group was yellow to pale brown. In the medium and low dose Qiangxin Huoli decoction groups, the staining was pale to mid-brown (Figure 4).

The expressions of Bcl-2 and Bax proteins in each group of myocardial apoptosis. The expressions of Bcl-2 protein were significantly increased and the expressions of Bax protein were significantly decreased in the model group compared with the control group (P < 0.05 and P < 0.01, respectively). The expressions of Bcl-2 protein were significantly increased, and the expressions of Bax protein were significantly decreased in all treatment groups compared with the model group (Table 4); the Shenfu and high dose Qiangxin Huoli decoction groups showed the most significant differences (P < 0.01, Table 4).

DISCUSSION
The experimental CHF model was successfully established in the present study using the gradient dose interval administration method of Wang et al. This method allowed the hearts of the rats to gradually adapt to the drug toxicity, and hence avoided excessive damage of the heart. This technique ensures both the establishment of the CHF model and the acquisition of an adequate number of experimental samples, whilst extending the time taken before the occurrence of other adverse reactions, and delaying the disease progression and cardiac toxicity. At the end of the ADR ad-
Figure 2 Myocardium fibers stained with hematoxylin-eosin (×100)
A: control group: orally administered and injected with 0.9% physiological saline; B: model group: orally administered 0.9% saline and injected with ADR; C: Shenfu group: orally administered Shenfu injection treatment and injected with ADR; D: high dose group: orally administered Qiangxin Huoli decoction (3.6 mg·kg⁻¹·d⁻¹) and injected with ADR; E: medium dose group: orally administered Qiangxin Huoli decoction (1.8 mg·kg⁻¹·d⁻¹) and injected with ADR; F: low dose group: orally administered Qiangxin Huoli decoction (0.9 mg·kg⁻¹·d⁻¹) and injected with ADR. ADR: adriamycin.

Figure 3 Expressions of B-cell lymphoma-2 (Bcl-2) protein in rat myocardial tissue (Bcl-2 staining, ×100)
A: control group: orally administered and injected with 0.9% physiological saline; B: model group: orally administered 0.9% saline and injected with ADR; C: Shenfu group: orally administered Shenfu injection treatment and injected with ADR; D: high dose group: orally administered Qiangxin Huoli decoction (3.6 mg·kg⁻¹·d⁻¹) and injected with ADR; E: medium dose group: orally administered Qiangxin Huoli decoction (1.8 mg·kg⁻¹·d⁻¹) and injected with ADR; F: low dose group: orally administered Qiangxin Huoli decoction (0.9 mg·kg⁻¹·d⁻¹) and injected with ADR. ADR: adriamycin.

Figure 4 Expressions of B-cell lymphoma-2-associated X protein (Bax) protein in rat myocardial tissue (Bax staining, ×100)
A: control group: orally administered and injected with 0.9% physiological saline; B: model group: orally administered 0.9% saline and injected with ADR; C: Shenfu group: orally administered Shenfu injection treatment and injected with ADR; D: high dose group: orally administered Qiangxin Huoli decoction (3.6 mg·kg⁻¹·d⁻¹) and injected with ADR; E: medium dose group: orally administered Qiangxin Huoli decoction (1.8 mg·kg⁻¹·d⁻¹) and injected with ADR; F: low dose group: orally administered Qiangxin Huoli decoction (0.9 mg·kg⁻¹·d⁻¹) and injected with ADR. ADR: adriamycin.
ministration, the symptoms and signs of the rats were evaluated. Compared with the control group, the model group showed greater weight loss, decreased food and water consumption, lower body temperature, dull fur, twisted spines, and sluggish behavior. The myocardial contractility was insufficient due to the decline in cardiac muscle function. The LVSP decreased, and the dysfunction of the cardiac diastolic action and ejection of blood increased the LVEDP value. The ventricular wall tension was also insufficient, so the pressure change (dp/dtmax) decreased, and the rate of change of the ventricular wall tension significantly decreased. The CHF model caused inadequate circulatory blood volume, and increased cardiac output, ejection frequency speed, and HR, resulting in the myocardial tissue being overworked, ischemic necrosis, and elevation of the S-T segment on the ECG.

When heart failure occurs, the changes in pathology are manifested in the irregular arrangement of myocardial tissue and myocardial cell degeneration. There is a close relationship between cardiovascular pathophysiology and the cardiovascular endocrine systems, of which the natriuretic peptide family is an important component. The neurohormone, plasma BNP, was first found and isolated from porcine brain by Maekawa et al. The ventricular synthesis and secretion of BNP controls volume expansion and pressure overload of the heart, so BNP levels can reflect the change in ventricular volume load and ventricular pressure. The biological roles of BNP include acting as a diuretic in sodium excretion, dilating blood vessels, inhibiting cardiac hypertrophy, and protecting the heart. These roles reflect the changes in the left ventricular function with high sensitivity and specificity, and hence BNP assessment is considered the gold standard for CHF evaluation, and has become a key topic of research. In CHF, the left ventricular systolic function decreases and the ventricular wall tension increases, which promotes BNP secretion and increases the plasma concentration of BNP. The concentration of BNP is therefore proportional to the severity of heart failure. The plasma BNP levels results showed that the degree of CHF was significantly less in the Shenfu and Qiangxin Huoli decoction groups than that in the model group, but was still significantly higher than that in the control group. This indicates that the administration of Shenfu and Qiangxin Huoli decoction slowed the progression of CHF in rats.

The mechanism of heart failure is complex, with its occurrence and development affected by a series of neurohumoral factors. The activities of these factors affect the myocardial cells, producing toxic effects, interfering with myocardial cell energy metabolism, cell apoptosis, and myocardial remodeling, and inducing progressive development of heart failure.

Aptosis is programmed cell death, and this is tightly controlled by genes. The genes involved in apoptosis can be divided into two categories: pro-apoptotic and anti-apoptotic. The expressions of Bcl-2 and Bax genes are currently used as indicators of the regulation of apoptosis. Bax and Bcl-2 are members of the same family, but they have opposing functions. Bax has a pro-apoptotic effect, whereas Bcl-2 promotes cell survival and inhibits apoptosis. The ratio between Bcl-2 and Bax determines cell survival; reduced Bcl-2 protein expression or increased expression of Bax protein promotes cell apoptosis, while increased Bcl-2 protein expression or reduced expression of Bax inhibits cell apoptosis. The model group had a decreased number of Bcl-2 protein positive cells and an increased number of Bax positive cells compared to the control group. Immunohistochemical analysis of Bax stained the cytoplasm and membrane yellow to brown, with darker color indicating a greater degree of apoptosis. The high dose Qiangxin Huoli decoction group showed significantly greater improvements in the expressions of Bax and Bcl-2 proteins compared with the model group, indicating that high dose Qiangxin Huoli decoction inhibited apoptosis in the myocardium of rats.

In our study, the Qiangxin Huoli decoction had a warming Yang effect, and reduced the pathogenesis of the CHF. The present results show that the vitality of the Qiangxin Huoli decoction improved the hemodynamics most significantly in the high dose group, which had significantly lower plasma BNP levels, significantly increased Bcl-2 expression, and significantly decreased Bax expression compared with the model group. In conclusion, our findings suggest that the Qiangxin Huoli decoction regulates the expression of apoptosis proteins and inhibits the apoptosis of myocardial cells by decreasing the plasma concentration of BNP, and thus protects rats from the effects of CHF.

### Table 4 Expressions of Bcl-2 and Bax proteins in each group (% ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Bcl-2</th>
<th>Bax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>0.68±0.27</td>
<td>0.46±0.19</td>
</tr>
<tr>
<td>Model</td>
<td>11</td>
<td>5.13±0.45</td>
<td>18.16±8.92</td>
</tr>
<tr>
<td>Shen Fu</td>
<td>14</td>
<td>7.73±0.62</td>
<td>5.52±0.58</td>
</tr>
<tr>
<td>High dose</td>
<td>13</td>
<td>8.03±0.78</td>
<td>6.73±0.49</td>
</tr>
<tr>
<td>Medium dose</td>
<td>12</td>
<td>3.16±1.41</td>
<td>11.21±3.81</td>
</tr>
<tr>
<td>Low dose</td>
<td>12</td>
<td>3.04±0.83</td>
<td>15.93±5.72</td>
</tr>
</tbody>
</table>

Notes: control group: orally administered with 0.9% physiological saline; model group: orally administered 0.9% saline and injected with ADR; Shenfu group: orally administered Shenfu injection treatment and injected with ADR; high dose group: orally administered Qiangxin Huoli decoction (3.6 mg·kg⁻¹·d⁻¹) and injected with ADR; medium dose group: orally administered Qiangxin Huoli decoction (1.8 mg·kg⁻¹·d⁻¹) and injected with ADR; low dose group: orally administered Qiangxin Huoli decoction (0.9 mg·kg⁻¹·d⁻¹) and injected with ADR. Bcl-2: B-cell lymphoma-2; Bax: Bcl-2-associated X protein. Each group was orally administered an equal volume of liquid. ADR: adriamycin. *P < 0.01, †P < 0.05, compared with the blank group; ‡P < 0.01, §P < 0.05, compared with the model group.
REFERENCES


