

Effects of Yixintai Pills on Myocardial Cell Apoptosis in Rats With Adriamycin-Induced Heart Failure

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ABSTRACT

Objective: To study the effects of Yixintai pills on myocardial cell apoptosis in rats with adriamycin (ADR)-induced heart failure (HF) and the mechanism of action.

Methods: Sixty healthy male Wistar rats randomly were divided into Control, Model, Captopril, and Yixintai pill groups. A rat model of ADR-induced HF was constructed by intraperitoneal injection of ADR (2.5 mg/kg). The control group was given an equal volume of normal saline; the Yixintai pill and Captopril groups were given corresponding mediations (5 mg/kg) by lavage. After 4 weeks of treatment, fasting blood was collected to detect the contents of plasma rennin activity (PRA), angiotensin II (AngII), and aldosterone (ALD). B ultrasound was used to detect the heart structure, and the heart weight/body weight (HW/BW) ratio was calculated. The pathology of myocardial tissues was observed by HE staining. The apoptosis of myocardial cells was detected by TUNEL assay. The expression levels of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in serum were analyzed by ELISA, and the protein expression levels of protein kinase B (Akt), phosphorylated (p)-Akt, glycogen synthase kinase-3 β (GSK-3 β) and p-GSK-3 β in myocardial tissues were measured by Western blotting.

Results: Compared with the Control group, the PRA, AngII, ALD, left ventricular posterior wall thickness at end-systole (LVPWs), left ventricular posterior wall thickness at end-diastole (LVPWd), interventricular septal thickness at end-systole (IVSs), interventricular septal thickness at end-diastole (IVSd), HW/BW, TNF- α and IL-6 of model group increased significantly ($P < .05$). PRA, AngII, ALD, LVPWs, LVPWd, IVSs, IVSd, HW/BW, TNF- α and IL-6 of the Yixintai pill and Captopril groups were significantly lower than those of the Model group ($P < .05$). There were no significant differences in the indices between the Yixintai pill and Captopril groups ($P > .05$). In the Model group, lamellar necrosis, vacuolar degeneration, increased myocardial fibers and lamellar dissolution of myocardial cells were found in myocardial tissues. However, the myocardial cells of the Control group were neatly arranged and clearly structured, and only a few ones underwent fibrosis. There were mild myocardial fibrosis and vacuolar degeneration in the

Yixintai pill and Captopril groups, and the degeneration and hyperplasia of myocardial fibers were obviously relieved. Compared with the Control group, the apoptosis index (AI) of the Model group increased significantly ($P < .05$). The AI values of the Yixintai pill and Captopril groups were significantly lower than those of the Model group ($P < .05$). Compared with the Control group, the expression levels of p-Akt and p-GSK-3 β in the Model group decreased significantly ($P < .05$). The expression levels of p-Akt and p-GSK-3 β in the Yixintai pill and Captopril groups were significantly higher than those of the Model group ($P < .05$), whereas the former 2 groups had similar results ($P > 0.05$).

Conclusion: Yixintai pills may inhibit myocardial cell apoptosis and ventricular remodeling in rats by up-regulating PI3K/Akt/GSK-3 β signal, thus protecting the heart function.

INTRODUCTION

Heart failure (HF) is a series of syndromes caused by heart injury, during which the blood output of the heart cannot meet the metabolism requirement, and its common clinical manifestations include cough, dyspnea, burnout, and fatigue. In the United States, up to \$40 billion are employed to treat HF annually [Gentile 2016; Otto 2017]. HF is a cardiac dysfunction caused by myocardial cell necrosis, which is characterized by insufficient ejection power and cardiac insufficiency induced by hypertrophy, necrosis, and fibrosis of myocardial cells [McLellan 2018]. According to previous literature, [Aldahl 2017; Morton 2018], HF is mainly caused by inflammatory infection, myocardial cell necrosis, and myocardial energy metabolism disorder. Therefore, researchers have endeavored to explore the treatment of HF based on the above factors [Feng 2015]. Yixintai pills is mainly prepared by using Astragalus membranaceus, Salvia miltiorrhiza, Flos Carthami, Alisma plantago-aquatica and Polyporus umbellatus. This drug can improve the hemodynamics, inhibit ventricular remodeling, slow down the progression of HF, as well as suppress myocardial cell apoptosis, and inflammatory factors [Sun 2012; Guo 2013], but the mechanism of action remains elusive. In the process of cell apoptosis, the related proteins are mainly regulated by the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signal pathway that transduces membrane receptor signals into cells to maintain proliferation and inhibit apoptosis. Glycogen synthase kinase-3 β (GSK-3 β), a substrate of Akt, plays a key role in

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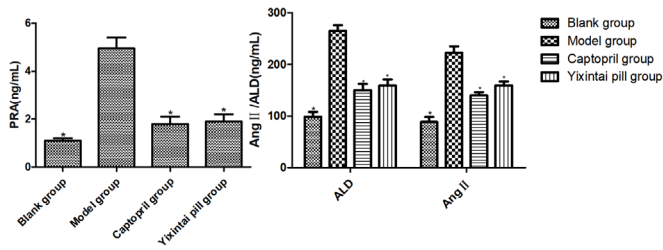


Figure 1. Effects of Yixintai pills on plasma PRA, AngII and ALD levels. **P* < .05 compared with the Control group, #*P* < .05 compared with the Model group.

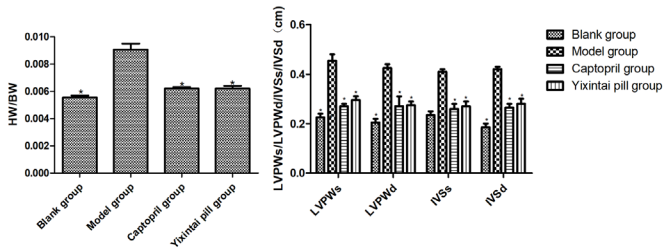


Figure 2. Effect of Yixintai pills on myocardial thickness. **P* < .05 compared with the Control group, #*P* < .05 compared with the Model group.

regulating apoptosis [Wei 2017]. This study evaluated the protective effect of Yixintai pills on the myocardial function of rats with adriamycin (ADR)-induced HF based on the PI3K/Akt/GSK-3β signal pathway, providing theoretical support for clinical application.

MATERIALS AND METHODS

Animals and reagents: SPF Wistar rats were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (License No. SCXK(Beijing)2019-0009). ADR was purchased from Sigma (USA). TUNEL assay kit was bought from Boster Biological Technology Co., Ltd. (China). Bicinchoninic acid (BCA) protein quantification kit and chemiluminescence kit were obtained from Guangzhou RiboBio Co., Ltd. (China). Rabbit anti-mouse Akt, p-Akt, GSK-3β P-GSK-3β antibodies, rabbit anti-GAPDH antibody and goat anti-rabbit IgG antibody were purchased from Abcam (USA). Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were bought from Shanghai Westang Biotechnology Co., Ltd. (China). Plasma rennin activity (PRA), angiotensin II (AngII) and aldosterone (ALD) kits were obtained from SunBio Biomedical Technology (Beijing) Co., Ltd. (China).

Modeling and grouping: Before the experiment, rats were fed for 1 week to adapt to the laboratory environment. A total of 60 rats randomly were divided into Control, Model, Captopril and Yixintai pill groups (N = 15). The rats were fasted for 24 hours before the experiment, and the model of ADR-induced HF was constructed by 2 intraperitoneal injections of ADR (2.5 mg/kg) (interval: 48 h). Color ultrasonography showed that the modeling was successful. Finally, the Control

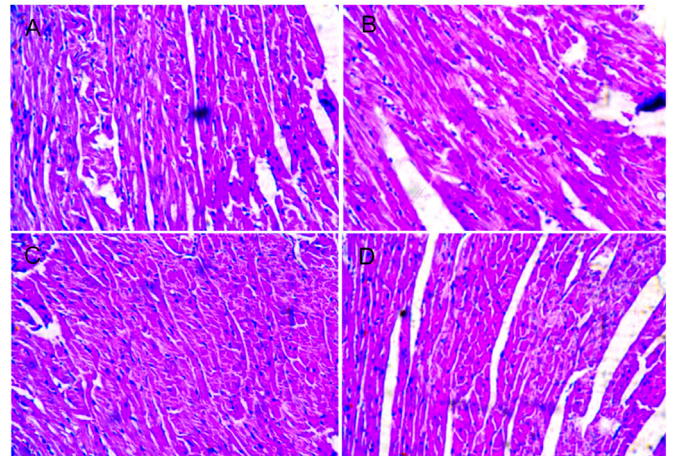


Figure 3. Effect of Yixintai pills on myocardial histopathology. A) Control group; B) Model group; C) Captopril group; D) Yixintai pill group.

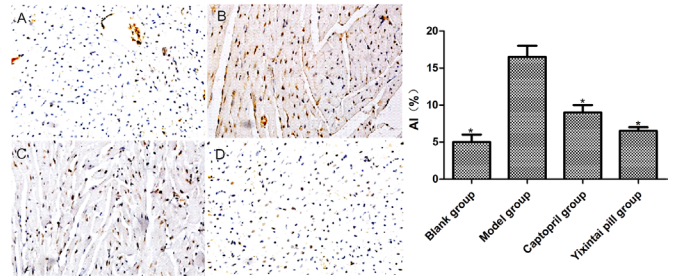


Figure 4. Effects of Yixintai pills on myocardial cell apoptosis detected by TUNEL staining. A) Control group; B) Model group; C) Captopril group; D) Yixintai pill group. **P* < .05 compared with the Control group, #*P* < .05 compared with the Model group.

and Model groups were given equal volumes of normal saline, while the Yixintai pill and Captopril groups were given corresponding mediations (5 mg/kg) by lavage for 4 weeks.

Measurement of plasma PRA, AngII and ALD contents: After treatment for 4 weeks, fasting blood was drawn from the tail, and then the contents of PRA, AngII, and ALD were detected.

Ultrasonography of cardiac structure: After treatment for 4 weeks, the rats were anesthetized and fixed on an operating table. Subsequently, the function and structure of the heart were analyzed by color ultrasonography. Left ventricular posterior wall thickness at end-systole (LVPWs) and left ventricular posterior wall thickness at end-diastole (LVPWd) were recorded.

Calculation of heart weight/body weight (HW/BW) ratio: After the rats were killed, the thoracic cavity was cut open to expose the heart, which was then removed after ligation. Then, the heart was washed with normal saline to eliminate the blood and dried by absorbent paper. The heart was weighed, and HW/BW was calculated.

Observation of myocardial tissue pathology by hematoxylin-eosin staining: Fresh myocardial tissues were collected, washed with normal saline, and fixed with 10% neutral formic

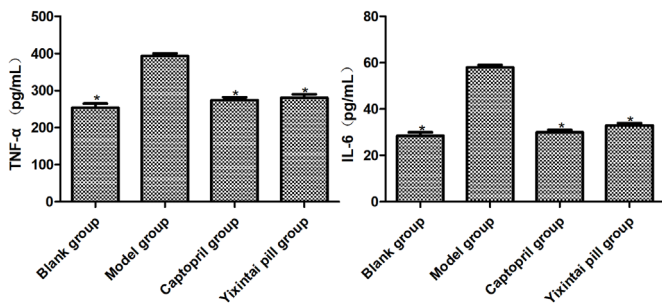


Figure 5. Effects of Yixintai pills on TNF-α and IL-6 expressions in myocardial cells. **P* < .05 compared with blank group, #*P* < .05 compared with the Model group.

acid buffer for 12 hours. The tissues then were dehydrated by gradient concentrations of ethanol solutions, permeabilized with xylene, soaked in paraffin, and embedded in a copper mould. The resulting blocks were cut into sections with a thickness of 4 μm. Subsequently, the sections were deparaffinized, hydrated with xylene, stained with hematoxylin at 65°C for 2 hours, rinsed with normal saline, differentiated with hydrochloric acid-ethanol, washed with phosphate-buffered saline (PBS), stained with eosin, dehydrated with gradient concentrations of ethanol solutions, sealed by neutral resin, and observed under a microscope.

Detection of myocardial cell apoptosis by TUNEL assay: The paraffin sections of myocardial cells were immersed in 3% H₂O₂ three times, washed with PBS three times and added 0.01 M tris-buffered saline (TBS). Then, the sections were added protease K working solution, hydrolyzed at 30°C for 15 minutes, washed with PBS three times, added PBS containing 2% hydrogen peroxide for 10 minutes of chemical reaction, and washed with PBS again. The obtained sections were added TUNEL reaction mixture, reacted in a dark wet box with parafilm for 60 minutes at 37°C, and rinsed with PBS. Afterward, the sections were put into 50 μL converter-POD, reacted in the dark wet box with parafilm for 60 minutes at 37°C, and rinsed with PBS. Finally, the sections were added 3,3'-diaminobenzidine (DAB), reacted at 20°C for 15 minutes, rinsed with PBS, counterstained with hematoxylin, dehydrated with gradient concentrations of ethanol solutions and permeabilized with xylene, followed by blocking with neutral resin and observation under the microscope. The apoptotic cells were stained brownish yellow. Apoptotic cells were counted under a light microscope. Apoptosis index (AI) = Ratio of number of apoptotic cells to that of total ones in five high-power fields.

Measurement of serum TNF-α and IL-6 expressions by ELISA: Blood was collected from the tail after treatment for 4 weeks and then centrifuged at 2000 rpm for 10 minutes. The supernatant was then collected. The expression levels of TNF-α and IL-6 in the supernatant were analyzed, according to the instructions of ELISA kit.

Detection of Akt, p-Akt, GSK-3β and P-GSK-3β expression levels by Western blotting: First, the frozen myocardial tissues were added RIPA lysis buffer, crushed using a homogenizer, and centrifuged at 4°C and 13000 rpm for 10 minutes

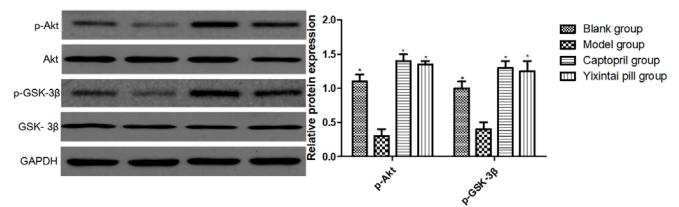


Figure 6. Effects of Yixintai pills on Akt, p-Akt, GSK-3β and P-GSK-3β expressions in myocardial tissues. **P* < .05 compared with blank group, #*P* < .05 compared with the Model group.

after 5 minutes of ice bath. The resulting supernatant was collected. The protein concentration in the supernatant was quantified by BCA protein quantification kit. The protein was isolated by 10% SDS-PAGE. Subsequently, the obtained protein was transferred to a polyvinylidene difluoride membrane by Tris/glycine buffer. The membrane was blocked in 5% TBS with Tween 20 (TBST) for 2 hours at room temperature, and then incubated with corresponding primary antibodies (1:1000 diluted) overnight at 4°C. On the next day, the membrane was washed with TBST for 10 minutes, and incubated with horseradish peroxidase-labeled goat anti-rabbit IgG secondary antibody (1:10000 diluted) for 2 hours at room temperature. DAB solution was added for color development. Gray value was detected with a gel imaging system. The relative expression level of target protein was calculated by using GAPDH as the internal reference.

Statistical analysis: All data were statistically analyzed by SPSS 16.0 software. Figures were plotted with Graphpad5.01 software. Intergroup comparisons were conducted by the t test. *P* < .05 was considered statistically significant.

RESULTS

Effects of Yixintai pills on plasma PRA, AngII and ALD levels: Compared with the Control group, the plasma PRA, AngII and ALD levels of the Model group increased significantly (*P* < .05). The levels of Yixintai pill and Captopril groups were significantly lower than those of the Model group (*P* < .05). There were no significant differences in the levels between the former groups (*P* > .05) (Figure 1).

Effects of Yixintai pills on myocardial thickness

Compared with the Control group, LVPWs, LVPWd, IVSs, IVSd and HW/BW of the Model group increased significantly (*P* < .05). The indices of the Yixintai pill and Captopril groups were significantly lower than those of the Model group (*P* < .05). There were no significant differences in the indices between the Yixintai pill and Captopril groups (*P* > .05) (Figure 2).

Effects of Yixintai pills on myocardial histopathology: In the Model group, lamellar necrosis, vacuolar degeneration, increased myocardial fibers and lamellar dissolution of myocardial cells were found in myocardial tissues. In contrast, the myocardial cells of the control group were neatly arranged and clearly structured, and only a few ones underwent fibrosis. There were mild myocardial fibrosis and vacuolar

degeneration in the Yixintai pill and Captopril groups, and the degeneration and hyperplasia of myocardial fibers were obviously relieved (Figure 3).

Effects of Yixintai pills on myocardial cell apoptosis: Compared with the Control group, AI of the Model group increased significantly ($P < .05$). The AI values of the Yixintai pill and Captopril groups were significantly lower than those of the Model group ($P < .05$) (Figure 4).

Effects of Yixintai pills on TNF- α and IL-6 expressions in myocardial cells: The Model group had significantly higher TNF- α and IL-6 expressions in myocardial cells than those of the Control group ($P < .05$). The expressions of the Yixintai pills and Captopril groups were significantly lower than those of the Model group ($P < .05$), but the former 2 groups had similar expressions ($P > .05$) (Figure 5).

Effects of Yixintai pills on Akt, p-Akt, GSK-3 β and p-GSK-3 β expressions in myocardial tissues: Compared with the Control group, the expression levels of p-Akt and p-GSK-3 β in the Model group decreased significantly ($P < .05$). The expression levels of p-Akt and p-GSK-3 β in the Yixintai pill and Captopril groups were significantly higher than those of the Model group ($P < .05$), whereas the former 2 groups had similar results ($P > .05$) (Figure 6).

DISCUSSION

Yixintai pills have excellent effects, including immune function enhancement, diuresis, hepatoprotection, depressurization, coronary artery expansion, heart function improvement, heart rhythm adjustment and microcirculation improvement [Guo 2011]. In this study, a rat model of ADR-induced HF was constructed, and Yixintai pills were employed for treatment. Yixintai pills significantly reduced the levels of PRA, AngII and ALD in the Model group, indicating that Yixintai pills inhibited the over-activation of renin-angiotensin-aldosterone system and protected myocardial cells, thus improving heart function, being consistent with a previous literature [Yang 2009].

HF is a kind of cardiac dysfunction caused by myocardial cell necrosis [Sun 2017], characterized by cardiac fiber necrosis, insufficient ejection power and cardiac insufficiency, which seriously endangers people's health. The left ventricular function is first damaged in the case of HF, and other structures and functions of myocardial tissues are affected with the development of the disease, eventually inducing irreversible HF [Pan 2015]. The ultrasonic examination in this study displayed that compared with the Control group, LVPWs, LVPWd, IVSs, IVSd and HW/BW of the Model group were significantly increased ($P < .05$), indicating that the left ventricular structure of the heart was damaged. Compared with the Model group, the indices of the Yixintai pill group significantly reduced ($P < .05$), suggesting that Yixintai pills effectively protected heart function.

At present, the pathogenesis of HF is still unclear. Cardiomyocyte apoptosis and ventricular remodeling play key roles in the onset and progression of HF [Dick 2016]. Blocking cardiomyocyte apoptosis is capable of reducing the

incidence rate of HF, slowing down the deterioration rate of HF, and improving the various functions of the heart [Su 2019]. In this study, lamellar necrosis, vacuolar degeneration, increased myocardial fibers and lamellar dissolution of myocardial cells were found in the myocardial tissues of the model group, suggesting that the cardiac cell structure of HF rats changed significantly. Yixintai pills alleviated myocardial fibrosis and suppressed the proliferation of fibrous tissues. Yixintai pills can significantly inhibit myocardial cell apoptosis in rats with myocardial infarction [Sun 2013]. TUNEL staining herein revealed that compared with the Control group, AI of myocardial cells was significantly increased in the Model group, which decreased significantly after administration with Yixintai pills.

The activated PI3K/Akt/GSK-3 β signal pathway is one of the essential signal transduction pathways in ventricular remodeling, being closely related to cardiomyocyte apoptosis [Xu 2017]. Herein, the levels of p-Akt and p-GSK-3 β in the Model group were significantly lower than those of the Control group. Compared with the Model group, the levels of Yixintai pill group and Captopril group were significantly increased ($P < .05$). Besides, there were no significant differences in the indices between the Yixintai pill group and Captopril group ($P > .05$), so the PI3K/Akt/GSK-3 β pathway predominantly participated in the onset and progression of HF. The levels of p-Akt and p-GSK-3 β increased significantly after treatment with Yixintai pills, verifying the restoring effects on heart function.

CONCLUSION

In summary, Yixintai pills may up-regulate the PI3K/Akt/GSK-3 β signal pathway and inhibit myocardial cell apoptosis and ventricular remodeling, thus protecting heart function.

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