Protective effects of *Polygonatum sibiricum* polysaccharide on acute heart failure in rats

Xiuying Zhu, Wei Wu, Xiyan Chen, Feiyun Yang, Jianxin Zhang, Jingyu Hou

1MD, Department of Emergency Medicine, The First Affiliated Hospital of Xinxiang Medical University, China. Design of the study, critical revision, final approval.
2MD, Department of Emergency Medicine, The First Affiliated Hospital of Xinxiang Medical University, China. Acquisition of data, critical revision, final approval.
3MD, Department of Emergency Medicine, The First Affiliated Hospital of Xinxiang Medical University, China. Statistical analysis, critical revision, final approval.
4MD, Department of Emergency Medicine, The First Affiliated Hospital of Xinxiang Medical University, China. Manuscript preparation, critical revision, final approval.

Abstract

**Purpose:** To investigate the protective effects of *Polygonatum sibiricum* polysaccharide (PSP) on acute heart failure (AHF) in rats.

**Methods:** Sixty rats were randomly divided into control, model, and low-, middle- and high-dose PSP groups, 12 rats in each group. The low-, medium- and high-dose PSP groups were intragastrically administrated with 100, 200 and 400 mg/kg PSP for 5 days, respectively. On the sixth day, the AHF model was established by intraperitoneal injection of adriamycin. After 24h, the cardiac function, serum biochemical indexes, myocardial ATPase and succinate dehydrogenase levels and apoptosis related protein expressions were determined.

**Results:** Compared with model group, in high-dose PSP group the heart rate, left ventricular systolic pressure, ±dp/dt\(_{max}\), serum superoxide dismutase level, myocardial Na\(^+-\)K\(^+-\)ATPase, Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase and succinate dehydrogenase levels and myocardial Bcl-2 and Caspase-3 protein expression levels were significantly increased (P<0.05), the left ventricular end diastolic pressure, serum cTnI, CK-MB, TNF-α, IL-6, malondialdehyde and nitric oxide levels and myocardial Bax and cleaved Caspase-3 protein expression levels were significantly decreased (P<0.05).

**Conclusions:** Polysaccharide can prevent the acute heart failure induced by adriamycin. The mechanism may be related to its anti-oxidative stress, anti-inflammatory and inhibition of cardiac myocyte apoptosis.

**Key words:** Heart Failure. Polygonatum. Polysaccharides. Oxidative Stress. Apoptosis. Rats.
Introduction

Heart failure is a common clinical disease, with incidence rising year by year. Acute heart failure (AHF) refers to the insufficient tissue and organ perfusion or acute congestion syndrome caused by obvious and sudden decrease in cardiac output due to acute heart disease\(^1\). In clinic, the acute left ventricular failure is the more common form of AHF\(^2\). AHF often occurs on the basis of original chronic heart failure, with an aggravation or sudden onset. Most of AHF patients are complicated by organic cardiovascular disease before disease onset\(^3\). AHF can be characterized by contractile heart failure or diastolic heart failure. It often endangers the life of patients, and the first aid is often needed for treating this disease\(^4\). *Rhizoma polygonati* is the dry rhizome of liliaceous plant *Polygonatum kingianum* Coll. et Hemsl. and *P. cyrtomem*. The main active ingredient of *Rhizoma polygonati* is the *Polygonatum sibiricum* polysaccharide (PSP)\(^5\). Studies show that, *Rhizoma polygonati* has the anti-tumor, antioxidant, anti-inflammatory, antibacterial, blood glucose and lipid regulating, anti-virus, immune enhancing functions\(^6\)\(^-\)\(^9\). It is found that, the bioactive polysaccharide can improve the cardiac function of model animals\(^10\)\(^-\)\(^11\). However, there is no report on the effect of PSP on AHF. In this study, the protective effects of PSP on adriamycin-induced AHF in rats and the underlying mechanisms were investigated. The objective was to provide an experimental basis for the clinical application of PSP to prevention of AHF.

Methods

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee of Xinxiang Medical University.

Animals and treatment

Sixty male Sprague Dawley rats (220-260g; Henan Experimental Animal Center, Guangzhou, China) were randomly divided into control group, model group, and low-, medium- and high-dose PSP groups, 12 rats in each group. After weighing, the rats in low-, medium- and high-dose PSP groups were intragastrically administrated with PSP (30% content; Xi’an Senran Bioengineering Co., Ltd., Xi’an, China), with dose of 100, 200 and 400 mg/kg, respectively. The control and model groups were administrated with equal volume of normal saline. The administration was performed once per day, for continuous 5 days. On the sixth day, except for the control group, the other groups received intraperitoneal injection of adriamycin (10 mg/kg) for 1 times, to establish the AHF model.

Detection of cardiac function of rats

After 24h from injection of adriamycin, the rats were anaesthetized using 20% urethane, with dose of 5 ml/kg. The rats were fixed in supine position. The cervical median incision was made, followed by tracheal intubation. The right carotid artery was isolated. After heparinization, the ventricular catheter was inserted into the left ventricle through the right carotid artery, and was connected to the biological signal recorder. The cardiac function indexes including heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP) and maximum left ventricular systolic/diastolic rate ($\pm dp/dt_{max}$) were recorded.
Determination of serum biochemical indexes

After detection of cardiac function, the blood was taken from the femoral artery aorta. After centrifuging at 2500 r/min for 10 min, the serum was obtained. The serum levels of cardiac troponin I (cTnI), creatine kinase isoenzyme (CK-MB), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), superoxide dismutase (SOD), malondialdehyde (MDA) and nitric oxide (NO) were determined according to the instructions of kits. The kits were provided by Sigma-Aldrich Corp. (MO, USA).

Determination of heart weight index and left ventricular weight index

Heart of rats was taken. After absorb the water using filter paper, the heart was weighed. Then, the left ventricle was separated and weighed. The heart weight index and left ventricular weight index were calculated by ratio of heart weight to body weight and ratio of left ventricular weight to body weight, respectively.

Determination of myocardial ATPase and succinate dehydrogenase levels

Myocardial tissue homogenate was prepared. The content of protein was determined using Coomassie bright blue method. The levels of myocardial ATPase and succinate dehydrogenase were determined according to the instructions of the kits (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China).

Determination of myocardial B-cell lymphoma-2, Bcl-2 associated X and cysteinyl aspartate specific proteinase-3 protein expressions

Myocardial tissue was homogenized. The protein was extracted using and cell lysate, and it concentration was determined. The expressions levels of B-cell lymphoma-2 (Bcl-2), Bcl-2 associated X (Bax), cysteinyl aspartate specific proteinase-3 (Caspase-3) and cleaved Caspase-3 protein in myocardial tissue were detected using western blot assays. β-actin was used as the internal reference. The ratio of integral optical density of target protein to β-actin presented the relative expression level of target protein. The experiment procedures were in accordance to the instructions of kits (Beijing Jinghongda Biotechnology Co., Ltd., Beijing, China).

Statistical analysis

Statistical analysis was performed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as mean±SD. The difference among different groups was analyzed using one-way analysis of variance with q test. The relationships among different indexes were investigated using Pearson correlation analysis. P<0.05 presented statistically significant.

Results

General situation of rats

During the experiment, no rat died in each group. After establishment of AHF model, there was no significant difference of body weight, heart weight index or left ventricular weight index among different groups (P>0.05) (Figure 1). There was no obvious side effect in each group.
Protective effects of Polygonatum sibiricum polysaccharide on acute heart failure in rats

Zhu X et al.

Acta Cir Bras. 2018;33(10):868-878

Cardiac function indexes in 5 groups

In model group the LVEDP was 11.04±3.31 mmHg, which was significantly higher than 6.23±1.68 mmHg in control group. The LVEDP in high-dose PSP group was 8.38±2.11 mmHg, which was significantly lower than model group (P<0.05). In model group the HR, LVSP, +dp/dtmax and -dp/dtmax were 281.26±29.48 beats/min, 95.27±14.45 mmHg, 4123.12±1029.42 mmHg/s and 3256.12±667.56 mmHg/s, respectively, which were significantly lower than 332.45±27.37 beats/min, 133.93±16.93 mmHg, 7056.34±1306.51 mmHg/s and 6002.31±1012.22 mmHg/s in control group, respectively. Those in high-dose PSP group were 328.71±35.03 beats/min, 130.34±22.18 mmHg, 6452.22±1023.72 mmHg/s and 5488.32±902.45 mmHg/s, respectively, which were significantly higher than model group, respectively (P<0.05) (Figure 2).

Serum cTnI and CK-MB levels in 5 groups

Figure 1 - Body weight, heart weight index and left ventricular weight index in 5 groups. PSP, Polygonatum sibiricum polysaccharide.

Figure 2 - Cardiac function indexes in 5 groups. For each index, values with different characters (a, b, c......) meant significant difference among different groups (P<0.05). PSP, Polygonatum sibiricum polysaccharide; HR, hear rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure.
In model group the serum cTnI and CK-MB levels were 2.01±0.42 ng/ml and 101.07±17.11 U/L, respectively, which were significantly higher than 0.45±0.12 ng/ml and 30.05±6.06 U/L in control group, respectively (P<0.05). The serum cTnI and CK-MB levels in high-dose PSP group were 0.67±0.13 ng/ml and 45.11±6.01 U/L, respectively, which were significantly lower than those in model group, respectively (P<0.05) (Figure 3).

![Figure 3 - Serum cTnI and CK-MB levels in 5 groups.](image)

For each index, values with different characters (a, b, c......) meant significant difference among different groups (P < 0.05). PSP, Polygonatum sibiricum polysaccharide; cTnI, cardiac troponin I; CK-MB, creatine kinase isoenzyme.

Serum TNF-α and IL-6 levels in 5 groups

As shown in Figure 4, in model group the serum TNF-α and IL-6 levels were 156.05±20.06 ng/L and 230.05±39.84 ng/L, respectively, which were significantly higher than 58.11±10.02 ng/L and 99.97±34.04 ng/L in control group, respectively (P<0.05). The serum TNF-α and IL-6 levels in high-dose PSP group were 63.07±8.09 ng/L and 102.02±22.89 ng/L, respectively, which were significantly lower than those in model group, respectively (P<0.05).

![Figure 4 - Serum TNF-α and IL-6 levels in 5 groups.](image)

For each index, values with different characters (a, b, c......) meant significant difference among different groups (P<0.05). PSP, Polygonatum sibiricum polysaccharide; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

Serum SOD, MDA and NO levels in 5 groups

As shown in Figure 5, in model group the serum SOD level was 67.84±8.55 U/ml, which was significantly lower than 122.47±18.37 U/ml in control group. The SOD level in high-dose PSP group was 102.27±18.93 U/ml, which was significantly higher than model group (P<0.05). In model group the serum MDA and NO levels were 9.07±2.15 μmol/L and 140.59±23.73 μmol/L, respectively, which were significantly higher than 5.22±0.92 μmol/L and 44.45±7.47μmol/L in control group, respectively. The serum MDA and NO levels in high-dose PSP group were 5.89±1.05 μmol/L and 55.83±9.68 μmol/L, respectively, which were significantly lower than model group, respectively (P<0.05).
Protective effects of Polygonatum sibiricum polysaccharide on acute heart failure in rats
Zhu X et al.

Acta Cir Bras. 2018;33(10):868-878

Figure 5 - Serum SOD, MDA and NO levels in 5 groups. For each index, values with different characters (a, b, c......) meant significant difference among different groups (P<0.05). PSP, Polygonatum sibiricum polysaccharide; SOD, superoxide dismutase; MDA, malondialdehyde; NO, nitric oxide.

Myocardial ATPase and succinate dehydrogenase levels in 5 groups

Figure 6 showed that, in model group the myocardial Na⁺-K⁺-ATPase, Ca²⁺-Mg²⁺-ATPase and succinate dehydrogenase levels were 2.35±0.57 μmol/mg, 1.93±0.44 μmol/mg prot and 65.26±10.03 U/mg prot, respectively, which were significantly lower than 5.24±0.77 μmol/mg prot, 4.38±0.67 μmol/mg prot and 113.84±16.63 U/mg prot in control group, respectively (P<0.05). The myocardial Na⁺-K⁺-ATPase, Ca²⁺-Mg²⁺-ATPase and succinate dehydrogenase levels in high-dose PSP group were 4.97±0.82 μmol/mg prot, 3.81±0.69 μmol/mg prot and 105.48±15.82 U/mg prot, respectively, which were significantly higher than model group, respectively (P<0.05).

Figure 6 - Myocardial ATPase and succinate dehydrogenase levels in 5 groups. For each index, values with different characters (a, b, c......) meant significant difference among different groups (P<0.05). PSP, Polygonatum sibiricum polysaccharide.

Myocardial Bcl-2, Bax and Caspase-3 protein expressions in 5 groups

As shown in Figure 7, the myocardial Bcl-2 and Caspase-3 protein expression levels in model group (Bcl-2/β-actin ratio, 0.52±0.13; Caspase-3/β-actin ratio, 0.28±0.17) were significantly lower than those in control group (Bcl-2/β-actin ratio, 0.93±0.21; Caspase-3/β-actin ratio, 0.98±0.26), respectively (P<0.05). Compared with model group, the Bcl-2 and Caspase-3 protein expression levels in high-dose PSP group (Bcl-2/β-actin ratio, 0.88±0.19; Caspase-3/β-actin ratio, 0.81±0.15) were...
significantly increased, respectively (P<0.05). The myocardial Bax and cleaved Caspase-3 protein expression levels in model group (Bax/β-actin ratio, 0.45±0.11; cleaved Caspase-3/β-actin ratio, 1.21±0.21) were significantly higher than those in control group (Bax/β-actin ratio, 0.13±0.02; cleaved Caspase-3/β-actin ratio, 0.62±0.12), respectively. Compared with model group, the Bax and cleaved Caspase-3 protein levels in high-dose PSP group (Bax/β-actin ratio, 0.14±0.03; cleaved Caspase-3/β-actin ratio, 0.73±0.13) were significantly decreased, respectively (P<0.05).

Figure 7 - Myocardial Bcl-2, Bax and Caspase-3 protein expressions in 5 groups. For each index, values with different characters (a, b, c,.....) meant significant difference among different groups (P<0.05). PSP, Polygonatum sibiricum polysaccharide; Bcl-2, B-cell lymphoma-2; Bax, B-cell lymphoma-2 associated X; Caspase-3, cysteiny1 aspartate specific proteinase-3.

Relationships among different indexes

Pearson correlation analysis showed that, the serum SOD level was positively correlated with the succinate dehydrogenase level (r = 0.701, P<0.01). The serum cTnI level was positively correlated with the myocardial Bax protein level (r = 0.474, P<0.01). The serum TNF-α level was positively correlated with the myocardial Bax protein level (r = 0.583, P<0.01). The serum cTnI level was positively correlated with the serum TNF-α level (r = 0.369, P<0.01). There was no significantly correlation between each of other indexes (P>0.05).

Discussion

Adriamycin can lead to the production of free radicals and cell oxidative damage in the body. The free radicals directly attack the membrane lipids, causing the changes in membrane structure and function12. The large dose of adriamycin can seriously inhibit the myocardial contractile function of rats, leading to the AHF. The main manifestations are the rapid decline of left ventricular systolic function, with significant decrease of HR, LVSP and ±dp/dt max in hemodynamics13. The adriamycin-induced AHF model is simple and easy to control, and is often used in the study of AHF. In this study, the adriamycin-induced AHF model of rats was established, and the protective effects of PSP on AHF were investigated. Result showed that, compared with control group, in model group the LVEDP was significantly increased, and the HR, LVSP and ±dp/dt max were significantly decreased. Compared with model group, in the high-dose PSP group, the LVEDP was significantly increased, and the HR, LVSP and ±dp/dt max were significantly decreased.
increased. In addition, the levels of serum cTnI and CK-MB were significantly decreased. This suggests that, the PSP can prevent the AHF induced by adriamycin. Our pre-experiments find that, if PSP is injected after AHF induction, the protective effects are not satisfactory. Therefore, only the pre-treatment with PSP can exert the protective effects on AHF.

Inflammatory reaction plays an important role in the occurrence, development and evolution of heart failure. TNF-α is produced by macrophages, which can regulate the immune function of body. It promoted the function of T cells and other killer cells, and its level is also significantly increased in stress state. IL-6 is a cytokine secreted by activated macrophages, and has a wide range of biological effects in the body. It can be used as a marker of clinical and experimental heart failure. In this study, the serum TNF-α and IL-6 levels in model group were significantly higher than control group. This indicates that, adriamycin can induce the inflammatory reaction in the body. Compared with model group, the serum TNF-α and IL-6 levels in high-dose PSP group were significantly decreased. This suggests that, PSP can alleviate the inflammatory reaction induced by adriamycin.

In this study, the serum MDA and NO levels in model group were significantly higher than those in control group, respectively, and the serum SOD level in model group was significantly lower than that in control group. This indicates that, the endogenous MDA and NO are excessively produced in AHF rats, and the free radical scavenging ability by SOD is reduced. NO is a highly reactive free radical that causes the damage and even denaturation and necrosis of cardiac myocytes. The serum level of MDA reflects the degree of oxidative damage in the body. SOD is the most important enzyme for scavenging oxygen free radicals in the body. It can effectively remove and terminate the chain reaction caused by superoxide anion. The activity of SOD indirectly reflects the body’s ability in removing oxygen free radicals. Joe et al. have used the lipopolysaccharide to stimulate peritoneal macrophages in rats and find that, NO can inhibit the activity of SOD in macrophages and increase the MDA production, resulting in lipid peroxidation and decreased myocardial contractile function. Results of this study showed that, compared with model group, in high-dose PSP group the serum SOD level was significantly increased, and the MDA and NO levels were significantly decreased. This indicates that, PSP can scavenge the oxygen free radicals, prevent their attack to cell membrane, and reduce lipid peroxidation damage, thus enhancing the myocardial contractility.

Adriamycin acts on the membrane of the cardiac myocytes, leading to the depolymerization the phospholipid mosaic protein in the membrane, increase of membrane permeability, and increase of the Ca²⁺ internal flow. At the same time, adriamycin can directly inhibit the activity of Na⁺-K⁺-ATPase, which decreases the Na⁺-K⁺ exchange, and increase the Na⁺-Ca²⁺ exchange. Therefore, the Ca²⁺ internal flow is increased, which induces the calcium overload, leading to the mitochondrial damage. Mitochondria are important places for energy metabolism. The production and storage of ATP are carried out in mitochondria. When mitochondria are severely damaged, the ATP production decreases, resulting in decreased myocardial contractility. Succinate dehydrogenase is a marker enzyme in the mitochondrial membrane of cardiac myocytes. It is one of the important enzymes for the electron transferring and oxidative phosphorylation in the energy metabolism of cardiac myocytes. Its activity reflects the energy metabolism of mitochondria in the cells. In this study, the myocardial Na⁺-K⁺-ATPase, Ca²⁺-Mg²⁺-ATPase and succinate dehydrogenase levels in model
group were significantly lower than control group. This indicates that, adriamycin can decrease the activity of ATPase and succinate dehydrogenase in cardiac myocytes, thus causing the decrease of cardiac function. Compared with model group, the myocardial Na\(^+\)-K\(^+\)-ATPase, Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase and succinate dehydrogenase levels in high-dose PSP group were significantly increased. This suggests that, PSP can increase the activity of ATPase and succinate dehydrogenase in cardiac myocytes, thus improving the cardiac function.

It is found that, the adriamycin-induced myocardial injury is associated with apoptosis of cardiac myocytes, with a dose-effect relationship\(^{24}\). Among the known apoptosis regulators, the Bcl-2 family plays a key role in apoptosis induced by various stimuli. The levels of Bcl-2 and Bax protein are directly related to the regulation of apoptosis. The increased Bax promotes the cell apoptosis, and the increased Bcl-2 inhibits the cell apoptosis\(^{25}\). Caspases are the promoter and executor of the cell apoptosis in mammalian, in which cleaved Caspase-3 is the most critical apoptotic protease in the downstream of Caspases cascade\(^{26}\). In addition, Bcl-2 can block the activation of Caspase-3 (cleaved Caspase-3) by interfering with the release of cytochrome C, thus inhibiting the cell apoptosis. Bax enables cytochrome C to pass through the mitochondrial membrane, which activates Caspase-9, and further activates Caspase-3, leading to the cell apoptosis\(^{27}\). Results of this study showed that, compared with control group, the myocardial Bcl-2 protein expression level in model group was significantly decreased, and the Bax and cleaved Caspase-3 protein levels were significantly increased. This has further confirmed the proapoptotic effect of adriamycin on cardiac myocytes. Compared with model group, in high-dose PSP group the myocardial Bcl-2 protein expression level was significantly increased, and the Bax and cleaved Caspase-3 protein levels were significantly decreased. This indicates that, PSP can inhibit the apoptosis of cardiac myocytes induced by adriamycin, and its mechanism may be related to the increase of Bcl-2 protein expression and decrease of Bax and cleaved Caspase-3 protein expressions.

**Conclusions**

Polysaccharide can prevent the acute heart failure induced by adriamycin. The mechanism may be related to its anti-oxidative stress, anti-inflammation and inhibition of cardiac myocyte apoptosis. This study has provided an experimental basis for the clinical application of PSP to prevention of AHF. This study still has some limitations. Firstly, the apoptosis of myocardial cells had not been investigated using TUNEL staining. Secondly, the relationship analysis showed that, there was no significantly correlation among majority of indexes. The reason may be due to the relatively small sample size. In next studies, these issues should be solved to make the results more convincing.

**References**


Protective effects of Polygonatum sibiricum polysaccharide on acute heart failure in rats

Zhu X et al.

Acta Cir Bras. 2018;33(10):868-878


Correspondence:
Dr. Xiuying Zhu
Department of Emergency Medicine, The First Affiliated Hospital of Xinxiang Medical University
88 Jiankang Road
Weihui, Xinxiang 453100 China
Phone: +86-373-4402543
zhuxiuyinghn@126.com

Received: June 06, 2018
Review: Aug 09, 2018
Accepted: Sept 04, 2018

Research performed at Central Laboratory, The First Affiliated Hospital of Xinxiang Medical University, China.

Conflicts of interest: none
Financial source: none