

Araloside C Prevent Myocardial Cell Apoptosis Through Regulating PI3K/Akt to Relieve Heart Failure

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Abstract

Background Araloside C (AsC), a natural saponin isolated from *Aralia elata*, has a wide range of anti-inflammatory properties and has been found in recent years to have heart-protective effects. Present study aimed to determine the effects of AsC on myocardial cell apoptosis through regulating PI3K/Akt.

Methods and Results Statistical analyses were performed using GraphPadPrism7.0 software. The differences between two groups and multiple groups were analyzed using t-test and one-way ANOVA, respectively. In vivo results showed that AsC administration could improve cardiac functions and apoptotic rate in HF model through PI3K/Akt signaling pathway, including increasing left ventricular ejection fraction (LVEF) and left ventricular fraction shortening (LVFS), and decreasing left ventricular end systolic diameter (LVESD) and left ventricular end diastolic diameter (LVEDD) in detection of myocardial function, inhibiting LDH, CK, CK-MB, CK and HBDH in biochemical index level assessment, inhibiting BNP, ANG II, IL-1b, IL-4, IL-6 and TNF-a in immunological index level. ASC regulates the expression of key apoptotic molecules, including increasing the expression of Bcl-2 and Bax. ASC also regulates phosphorylation of p-PI3K and p-Akt.

Conclusion This study suggested for the first time that AsC could partially regulate the PI3K/Akt signaling pathway to prevent myocardial cell apoptosis. This study provided a basis for further research on effective substances in the treatment of HF.

Introduction

In recent years, the research on the treatment of heart failure (HF) with traditional Chinese medicine has attracted wide attention¹. In particular, salvia miltiorrhiza, berberine, ginseng, astragalus and other traditional Chinese medicines, including salvianolic acid A, salvianolic acid B, tanshinone IIA, ginsenoside Re, berberine, flavonoids, have been proved to improve cardiac hypertrophy, reduce cell apoptosis and inflammatory response, and protect heart function, has an important role²⁻⁵. Araloside C (AsC), a natural saponin isolated from *Aralia elata*, has a wide range of anti-inflammatory properties and has been found in recent years to have heart-protective effects. Luo et al studied on atherosclerotic mice, AsC can regulate macrophage polarization through sirt1-mediated autophagy, thereby reducing the formation of plaque area in atherosclerotic mice⁶. Moreover, inhibiting oxidative stress and Ca²⁺ overload by regulating HSP90 can effectively reduce hypoxia/reoxygenation of H9c2 cardiomyocyte ischemia/reperfusion (I/R) injury⁷⁻⁹.

However, the therapeutic effect of AsC in rats with HF has not been reported so far, and its internal mechanism has not been reported. Therefore, we will focus on AsC to study its effect on cardiac function in rats with HF. Sun et al. found that the HSP90/Akt pathway induces cardiac hypertrophy and cell death, affecting the course of HF¹⁰. Therefore, we will study whether AsC can inhibit cell death, improve cardiac function and delay the occurrence and development of HF through the PI3K/Akt signaling pathway regulated apoptosis.

Results

AsC repairs cardiac function in HF rats

In detection of myocardial function, we found that compared with Sham group, the heart function of rats in the HF group was significantly decreased, with LVEF (Fig. 1C) and LVFS (Fig. 1D) decreased, and LVEDD (Fig. 1A) and LVESD (Fig. 1B) increased ($P < 0.01$), indicating the success of the model establishment. Compared with the HF group, the HF + PBS group showed no significant change in the ultrasonic indexes of cardiac function, while the HF + AsC group showed increased LVEF (Fig. 1C) and LVFS (Fig. 1D), and decreased LVESD ($P < 0.01$), indicating significant improvement in cardiac function after AsC administration.

Pathological changes of cardiac muscle in HF rats inhibited by ASC

Description of Masson staining results: myocardial cell fibers in sham group were evenly and neatly arranged, with clear transverse lines, and a small amount of blue-dyed fibrous tissue was observed between the cells (Fig. 2A). In the HF and HF + PBS groups, myocardial fibers were fractured, the cells showed granular degeneration to varying degrees, and a large number of blue-stained fibrous tissues were observed in the tissues. The Model + AsC group showed a significant reduction in the blue-stained fibrous tissue of myocardial tissue.

HE staining results (Fig. 2B): compared with Sham group, HF and HF + PBS rat had disordered arrangement of cardiomyocytes, hypertrophy of cardiomyocytes, and enlarged nuclei. Compared with the HF group, the Model + AsC group significantly reduced the degree of myocardial cell hypertrophy and nuclear enlargement, while the Model + PBS group showed no significant changes.

AsC reduced plasma biochemical index level

Myocardial enzyme (CK, CK-MB, LDH, AST and HBDH) had become biomarkers for the diagnosis of myocardial injury^{10,11}. Myocardial injury caused the release of LDH, AST, CK, CK-MB, and HBDH into bloodstream. The expression of LDH (Fig. 3A), CK (Fig. 3C), CK-MB (Fig. 3D), and HBDH (Fig. 3E) decreased significantly after AsC intervention compared to HF and HF + PBS groups. However, the expression of AST (Fig. 3B) had no significantly change after AsC intervention in HF rats (Fig. 3B). Above all monitoring of biochemical indexes showed that the drug intervention had obvious improvement on HF in rats.

AsC reduced plasma Immunological index level

ELISA were used to detect the expression of inflammatory factors (IL-1 β , IL-6, IL-4 and TNF- α) in the experiments. The results showed that sham group had significant difference in expression of BNP (Fig. 4A), Ang II (Fig. 4B), IL-1 β (Fig. 4C), IL-4 (Fig. 4D), IL-6 (Fig. 4E), and TNF- α (Fig. 4F) compared to

other groups ($P < 0.05$). There was no significant change between HF group and HF + PBS group. The expression of indicators decreased significantly after AsC intervention compared to HF and HF + PBS groups. Above all these results suggested that AsC treatment might decrease the expression of inflammatory factors to improve heart function in HF rats.

ASC inhibits cardiomyocyte apoptosis in HF rats

Apoptosis of HF rat cardiomyocytes was detected by TUNEL assay to evaluate the antiapoptotic effect of AsC on cardiomyocytes (Fig. 5). The results showed that compared with sham rats, the percentage of cardiomyocyte apoptosis in HF and HF + PBS rats was significantly increased. However, percentage of apoptotic myocardial cells was significantly lower after the intervention of the AsC, which indicated that the AsC had the effect of preventing the apoptosis of heart cells.

AsC promotes PI3K/Akt signaling pathway activation

Total protein was extracted from cardiac tissue and WB was used to detect the changes in the levels of myocardial protein p/t-PI3K, p/t-Akt, Bcl-2, Bax, Caspase-3 and cytochrome C (Fig. 6A). WB analysis results (Fig. 6B) showed that compared with sham group, the expressions of P-PI3K, P-Akt and Bcl-2 were significantly reduced in the HF and HF + PBS groups and significantly increased after drug intervention. However, the expression of Bax increased compared with sham group, and decreased after AsC intervention. There were no statistically significant differences in the expression of other proteins between these groups.

Discussion

There are more than 4 million patients with HF in China, and the fatality rate is much higher than that in developed countries¹³. The research on the mechanism of HF has been in progress, and apoptosis has been proved to be helpful to the development of HF. Cardiomyocyte apoptosis occurs in a variety of cardiovascular diseases, such as myocardial infarction and I/R^{14,15}. ASC is one of the most abundant triterpenoids isolated from *A. elata*, which has been clearly shown to stimulate cardiac activity^{16,17}. However, the cardioprotective effect of AsC and its mechanism remain unclear. In this study, we demonstrated that ASC could protect HF cardiomyocytes from apoptosis and inhibit the activation of Akt in HF myocardium. The results showed that AsC had a protective effect on myocardial cell apoptosis in rats with HF by inhibiting the overexpression of PI3K/Akt signaling pathway.

It has been reported that HF is not only due to the decrease of myocardial contractility, but also to the increase of apoptotic cells¹⁹. Apoptosis plays an important role in ventricular remodeling and HF^{19,20}. Bcl-2 protein family determines the commitment of cells to apoptosis, and the activation of Caspase-3 triggers the execution of apoptosis. There are both pro apoptotic and anti-apoptotic proteins in Bcl-2 protein family. Bax promotes the formation of membrane pores in the form of oligomers, and releases pro-apoptotic substances into the cytoplasm to play a pro-apoptotic role, while Bcl-2 inhibits apoptosis by

blocking the oligomerization of pro-apoptotic proteins²¹. The expression of Bcl-2, Bax, caspase-3 and cytochrome C were detected by Western blot. The balance between Bcl-2 and Bax was disrupted, while the expression of Caspase-3 and cytochrome C was not changed, suggesting that the apoptotic pathway was activated in the HF model. AsC treatment can increase the level of Bax, inhibit the expression of Bcl-2, but not affect the expression of Caspase-3.

In this study, the rat model of HF was established to explore the effects of PI3K/Akt signaling pathway on cardiac apoptosis. PI3K/Akt, as an important signal transduction pathway, plays an important role in cell survival, apoptosis and proliferation²¹.

The expression of p-PI3K and p-Akt increased in ASC treatment group, but decreased in HF rats. It suggests that AsC may play an anti-apoptotic role by regulating P-PI3K and P-Akt, rather than directly interacting with PI3K and Akt. Some ASC components have been confirmed to have anti apoptotic effect. For example, quercetin, luteolin and tanshinone IIA have been shown to have anti-apoptotic effects on myocytes²²⁻²⁴. In future studies, we will verify the role of these potential active ingredients through in vitro and in vivo experiments. In conclusion, this study explored the protective effect of ASC in HF animal model and apoptosis model. The results showed that AsC could partially regulate the PI3K/Akt signaling pathway to inhibit myocardial cell apoptosis. This study provides a basis for further study of effective substances for the treatment of HF.

Materials And Methods

Establishment of the HF Model in Rats and Grouping

The HF model was established by abdominal aortic constriction. After feeding adult Wister male rats for 1 week, pentobarbital sodium anesthetized rats, and then the abdominal aorta was separated 1cm above the left renal artery through a median incision. Abdominal aorta was sutured with no. 22 needle 4-0 silk thread to form abdominal aortic stenosis (about 50% ~ 60%). Abdominal dissection was performed. At the same time, 1×10⁵ U penicillin was intraperitoneally injected to prevent infection. The rats were monitored daily after surgery, and after 10 weeks, AsC medication interfered with HF (2.5 mg/kg/day) for 4 weeks.

The rats were divided into four groups (sham group, HF model, HF+PBS and HF+ AsC group) and five of each group. Sham group rats received DMEM 70μl for control.

All rats were used for subsequent experiments in accordance with the Laboratory Animal Management Regulations and Animal Ethical Requirements before modeling. Animal experimental protocols were approved by the Ethics Committee of the Inner Mongolia People's Hospital and the study was carried out in compliance with the ARRIVE guidelines. Rats were sacrificed and anesthetized by intraperitoneal injection of pentobarbital sodium solution (1%) at a dose of 50mg/kg. We tried our best to reduce the number of animals that are used and reduce their suffering.

Detection of myocardial function

Ten weeks after the last administration, the rats in each group were anesthetized. After removing the chest hair and applying the coupling agent, the VisualsonicVevo 2100 imaging system was used to evaluate the preoperative and postoperative cardiac function through echocardiography. Mice were anesthetized with 2.5l /min isoflurane before evaluation. Left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), left ventricular fraction shortening (LVFS) and left ventricular ejection fraction (LVEF) were calculated with Vevo analysis software²⁵.

Histological examination in rats

The rat heart was dissected, fixed with 4% formalin for 24 hours, embedded in paraffin, and cut into 5µm-thick slices. Then the slices were stained with Masson's trichrome staining (Solarbio, Beijing, China) and hematoxylin-eosin (HE) staining to observe heart tissue morphology. Image analysis software (Image-Pro Plus v4.0, Media Cybernetics, USA) was used to calculate the area occupied by collagen

Analysis of serum biochemical indexes

The contents of creatine kinase (CK), creatine kinase isoenzyme (CK-MB), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and hydroxybutyrate dehydrogenase (HBDH) in plasma were determined by automatic chemical analyzer (Labospect-008, Hitachi High-Tech Diagnostics (Shanghai) Ltd., Japan)

ELISA Analysis

The levels of BNP, TNF-α, AngII, IL-6, IL-4, IL-1b in plasma were quantified using Glucagon Quantikine ELISA Kit (Elabscience Biotechnology Co., Ltd, Wuhan, China). Analyze according to manufacturer's instructions.

Apoptosis Assay

According to the manufacturer's instructions, cardiomyocyte apoptosis was detected by TUNEL (EMD Millipore, Billerica, MA). The apoptosis of cardiomyocytes was brown. The average percentage of apoptotic cells in 5 randomly selected fields (under magnification ×40) was calculated by Olympus microscope.

Western blotting (WB)

Total proteins were extracted from cardiac tissue and the levels of cardiac proteins P/t-PI3K, P/t-Akt, Bcl-2, Bax, Caspase-3 and cytochrome C were detected by WB.

Statistical analysis

All statistical analyses were performed using GraphPadPrism7.0 software. The differences between two groups and multiple groups were analyzed using t-test and one-way ANOVA, respectively. $P < 0.05$ was considered a statistically significant difference.

Declarations

Competing interests

The authors declare that they have no competing interests.

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2. Inner Mongolia Autonomous Region Science and Technology Project (201702118)

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Figures

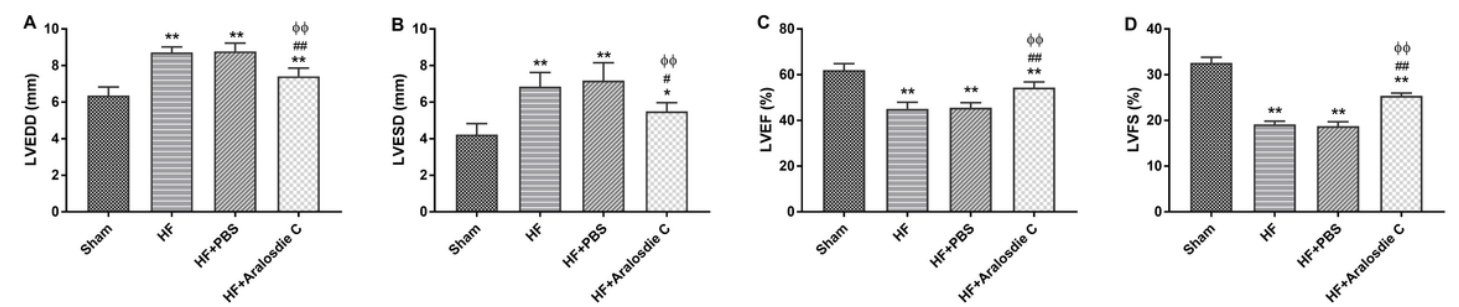


Figure 1

Detection of myocardial function.

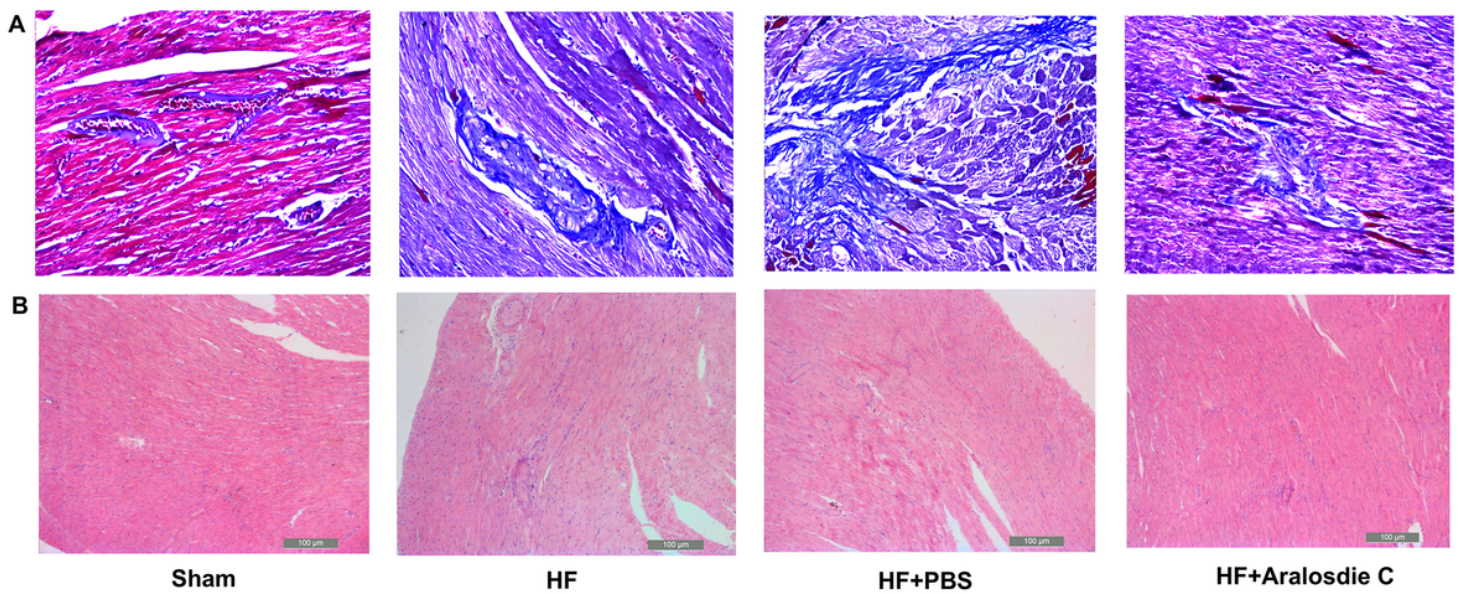


Figure 2

Histological examination in rats.

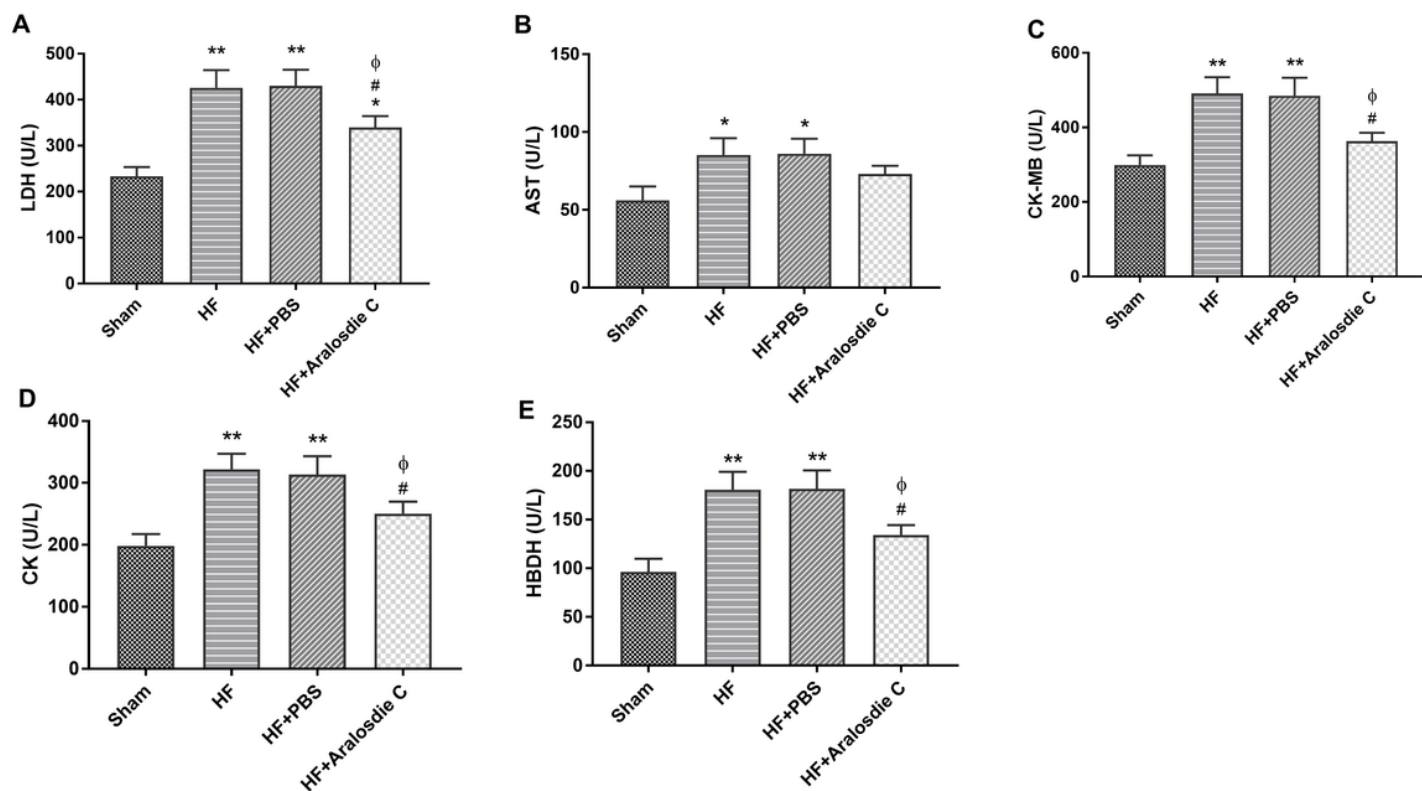


Figure 3

AsC reduced plasma biochemical index level

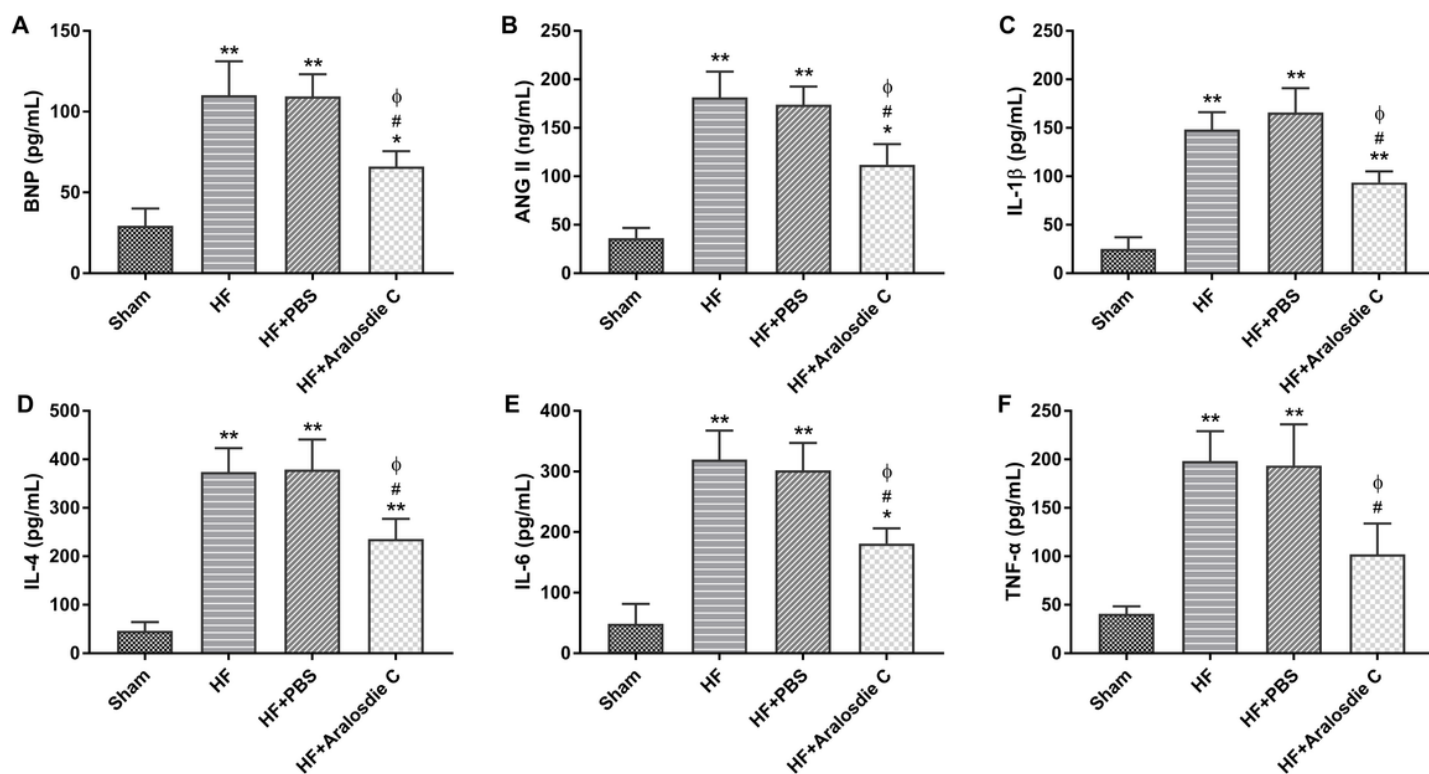


Figure 4

AsC reduced plasma Immunological index level.

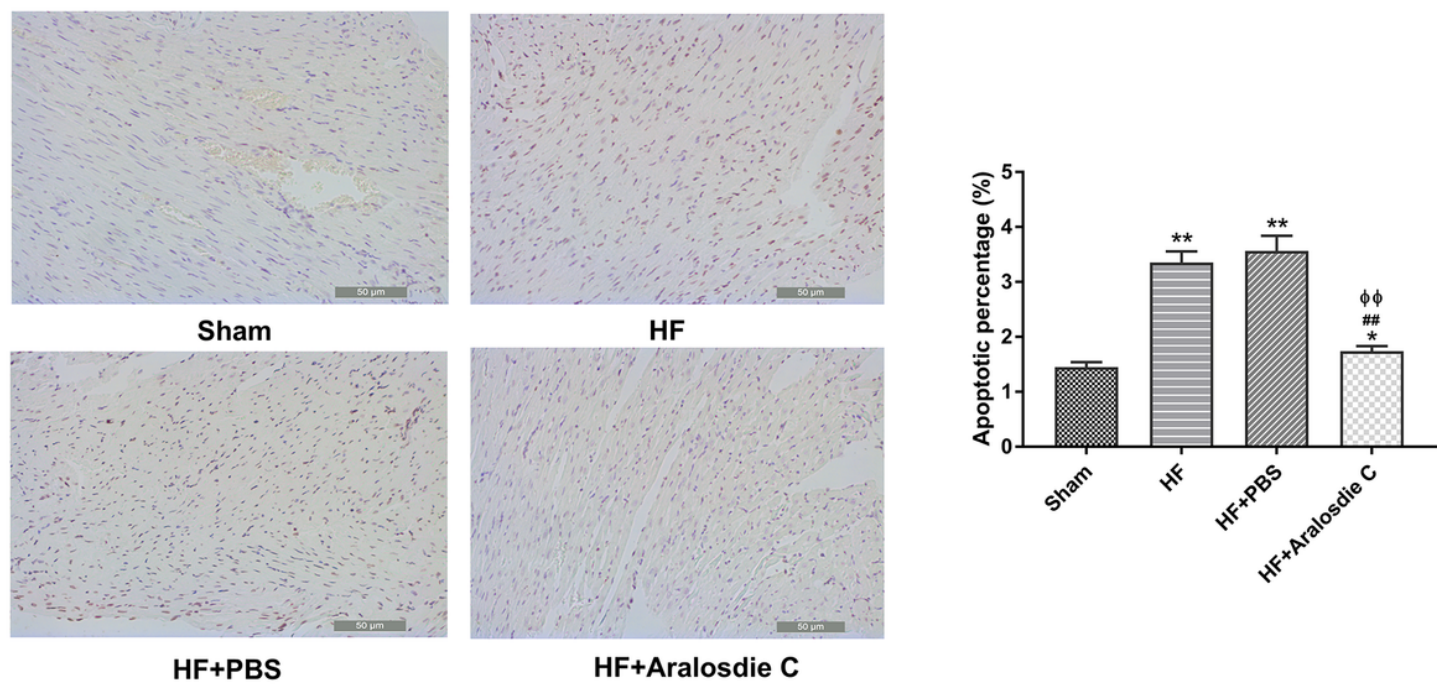


Figure 5

AsC reduced plasma immunological index level.

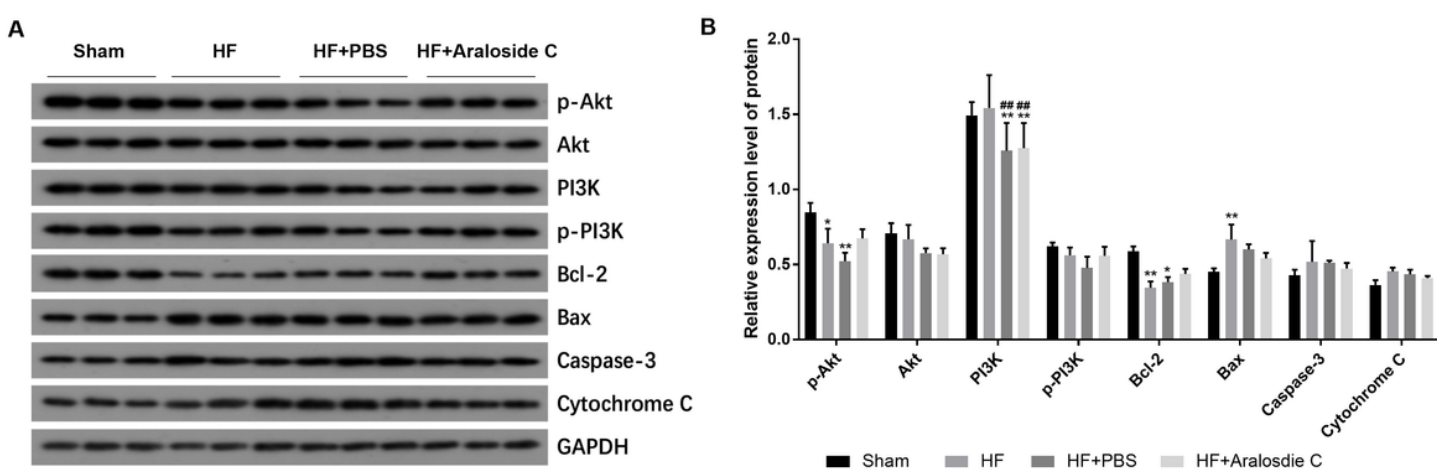


Figure 6

Total protein was extracted from cardiac tissue and WB was used to detect the changes in the levels of myocardial protein.