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YQWY decoction reverses cardiac hypertrophy induced by TAC through inhibiting GATA4 phosphorylation and MAPKs

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[ABSTRACT] To investigate the effect of *Yiqi Wenyang* (YQWY) decoction on reversing cardiac hypertrophy induced by the transverse aortic constriction (TAC). Wistar rats aged 7–8 weeks were subjected to TAC surgery and then randomly divided into 4 groups (n = 5/group): Sham group, TAC group, low-dose group and high dose group. After 16-week intragastric administration of YQWY decoction, the effect of YQWY decoction on alleviating cardiomyocyte hypertrophy was examined by transthoracic echocardiography (TTE), hematoxylin/eosin (HE), wheat germ agglutinin (WGA) staining, enzyme linked immunosorbent assay (ELISA), Western blot (WB), immunohistochemistry (IHC) and immunofluorescence (IF), respectively. The results showed significant differences in left ventricle volume-diastole/systole (LV Vol d/s), *N*-terminal pro-B-type brain natriuretic peptide (NT-proBNP) ($P < 0.01$), Ejection Fraction (EF), LV mass and fractional shortening (FS) ($P < 0.05$) between YQWY-treated group and TAC group. HE and WGA staining showed that treatment with YQWY decoction dramatically prevented TAC-induced cardiomyocyte hypertrophy. Moreover, the results of WB, IHC and IF indicated that administration of YQWY could suppress the expressions of cardiac hypertrophic markers, which included the atrial natriuretic peptide (ANP), BNP and myosin heavy chain 7 (MYH7) ($P < 0.05$) and inhibit phosphorylation of GATA binding protein 4 (P-GATA4) ($P < 0.05$), phosphorylation of extracellular signal-regulated kinase (P-ERK) ($P < 0.05$), phosphorylation of P38 mitogen activated protein kinase (P-P38) ($P < 0.05$) and phosphorylation of c-Jun *N*-terminal kinase (P-JNK) ($P < 0.05$). Thus, we concluded that YQWY decoction suppressed cardiomyocyte hypertrophy and reversed the impaired heart function, and the curative effects of YQWY decoction were associated with the decreased phosphorylation of GATA4 and mitogen activated protein kinases (MAPKs), as well as the reduced expression of the downstream targets of GATA4, including ANP, BNP, and MYH7.

[KEY WORDS] YQWY decoction; Cardiac hypertrophy; GATA4 phosphorylation; MAPKs

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Introduction

Heart failure is the terminal stages of multiple heart diseases and has a poor 5-year survival rate [1]. Pathological cardiac hypertrophy (PCH), as an adaptive response of heart to volume or pressure overload, is the major cause of heart failure [2-3]. Prevention and reversion of PCH might be the key to decrease the high mortality and morbidity of heart failure. Cardiac hypertrophy is mainly divided into the eccentric hypertrophy and concentric hypertrophy. The occurrence of concentric hypertrophy is associated with increased ventricular volume overload, as well as a coordinated growth in septal and relative wall thickness. However, the eccentric hypertrophy only shows the volume overload without the thicken in the septum and relative wall [3]. Cardiac hypertrophy is characterized by the increase in myocardial cells size, as well as related genetic changes.

The mechanism of PCH is very complex and has been reported to be associated with fibrosis, inflammation, oxidative stress, endoplasmic reticulum (ER) stress and autophagy alterations [4]. The transcriptional factor GATA4 was proved

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to play key roles in the progression of cardiac hypertrophy and heart failure [5]. Overexpression of GATA4 in the heart could result in cardiac hypertrophy, whereas the specific deletion of GATA4 markedly attenuated hypertrophy induced by pressure overload and exercise stimulation [6-7]. Previous studies have also identified a variety of drugs that could reverse the progression of cardiac hypertrophy by down-regulating GATA4 [8-9]. GATA4 acts as a key transcriptional controller of numerous cardiac genes, including ANP, BNP and MYH7 [10-11]. Among them, MYH7, also named cardiac myosin heavy chain β, MHCβ, is general considered as the the most commonly mutated genes in patients with cardiac hypertrophy [12]. The latest 2017 AHA guideline pointed out that higher level of natriuretic peptide biomarkers (NT pro-BNP and BNP) has great value in the prevention, diagnosis and prognosis of heart failure [13]. The activation of BNP and MYH7 both require to bind with the GATA elements in the promoter regions [10].

The MAPKs signaling pathway mainly includes three major branches, which are the ERK, JNKs and P38 MAPK. All these signals exhibited a remarkable enhancement in cardiac tissue of the pressure overload-induced animal models and in patients with heart failure [14]. Besides, MAPK signaling pathways are also activated by certain pathogenic stimuli, pro-inflammatory cytokines or extracellular stress [15]. It has been indicated that MAPKs were sensitive to pressure overload induced by TAC in cardiac tissue and had a strong linear correlation with the amount of pressure overload and the cardiac hypertrophy [16].

Recent years, various new drugs, including traditional Chinese medicine (TCM), have been developed to improve the curative effect of cardiac remodeling and heart failure. *Qiliqiangxin* (QLQX) capsule, the component of various herbal medicine, could decrease the rehospitalization and mortality of patients with heart disease, improve cardiac function and tolerance and attenuate the clinical symptoms [17-20]. Another TCM, YQWY decoction, has been used to treat the heart failure for almost decade years in the Jiangsu Province Hospital of TCM, and thousands of patients have benefited from it. YQWY decoction is composed of eight traditional medicinal herbs, including Astragli Radix, Rhodiolae Crenulatae Radix et Rhizoma, Aconiti Lateralis Radix Preparata, Polyporus, Descurainia Semen, Curcumae Rhizome, Paeoniae Radix Alba, Zingiberis Rhizoma Recens, have been widely proved to be effective in preventing cardiomyocyte injury. It is reported that astragaloside IV (AsIV) protects rat cardiomyocytes from hypoxia-induced injury *via* inhibiting ERK signaling pathways [21]. AsIV can also inhibit isoproterenol (ISO)-induced cardiac fibrosis by suppressing reactive oxygen species (ROS)-mediated MAPK activation [22]. Besides, salidroside can attenuate myocardial injury induced by lipopolysaccharide through the ROS-mediated phosphoinositide-3-kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR) signaling pathway *in vivo* [23]. And it also suppressed the myocardial ischemia-reperfusion injury by regulating adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK)/peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and AMPK/nuclear factor kappa B (NF-κB) [24].

Here, we aimed to explore the curative effects of YQWY decoction on the cardiomyocyte hypertrophy and the impaired heart function, as well as explain whether above effects were associated with the phosphorylation of GATA4 and MAPKs signals.

Material and Methods

Preparation of the aqueous extract

The prescription of YQWY was supplied by the division of cardiology in Jiangsu Province Hospital of TCM. It is composed of Astragli Radix (60 g), Rhodiolae Crenulatae Radix et Rhizoma (15 g), Aconiti Lateralis Radix Preparata (10 g), Polyporus (15 g), Descurainia Semen (15 g), Curcumae Rhizome (10 g), Paeoniae Radix Alba (12 g), Zingiberis Rhizome Recens (6 g). All the herbal medicine was obtained from Jiangsu Province Hospital of TCM and identified morphologically by Dr. LIU Zheng at Nanjing Hospital of TCM (Nanjing, China).

Concentrated decoction: To be more consistent with clinical practice, the traditional and standard decocting methods were adopted to get the liquid of YQWY. Then, a rotary evaporator was used to evaporate excess water to concentrate drugs to 3.575 g·mL^{-1}.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis of YQWY

Paeoniflorin (CAS number: 23180-57-6), AsIV (CAS number: 84687-43-4), calycosin 7-*O*-β-D-glucopyranoside (CAS number: 20633-67-4), salidroside (CAS number: 10338-51-9) and formononetin (CAS number: 485-72-3) were purchased Sigma-Aldrich (St. Louis, MO, USA). LC-MS/MS was performed using Type 1200 liquid phase color spectrum system (Agilent company, USA), and Q-Trap 3200 tandem quadrupole-ion trap mass spectrometer (Applied Biosystems company, USA). Conditions of LC-MS/MS are as follows: TSK Gel amide 80 chromatographic column (4. 5 mm × 150 mm, 5 μm), column temperature 35 °C, mobile phase acetonitrile-6 mmol·L^{-1} ammonium formate aqueous solution (pH 5.5) (67.5 : 32.5), flow rate 1 mL·min^{-1}, injector temperature 4 °C, mass spectrometry detector ionization by electrospray ionization, positive and negtive ion multi-reaction scanning in the detection (MRM) mode, the ion source and other relevant parameters are optimized as spray voltage 4 500 V, heating temperature 450 °C, Gas1 50 Psi, Gas2 50 Psi.

Animals

All the animal experimental procedures were under the guidelines of the use and care of laboratory animals for biomedical research published by National Institutes of Health (No. 85–23, revised 1996). The experimental protocol was reviewed and approved by the ethical committees of Nanjing University of Chinese Medicine. All rats (Male, 7–8 weeks

old and weighing 270 ± 10 g) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The rats were then raised on a 12-h-light/ dark cycle in a temperature and humidity-controlled room (temperature: 23 ± 1 °C, humidity: 60%) in the Animal Center of Nanjing University of Chinese Medicine (Nanjing, China). Free food and water were supplied in sufficient quantities and an adaptation was allowed for 1 week before experiments.

The TAC was performed as follows: Rats were anesthetized with a mixture of 2% isoflurane and 0.5–1.0 L·min^{-1} 100% O_2 in an induction chamber. After fixed in a hypsokinesis position atop a heating pad, all rats then received the partial thoracotomy to the second rib. The 27-gauge blunt needle were placed parallel to the transverse aorta, and the silk suture was then used to tie two knots before removal of the blunt needle. Sham-operated Rats underwent the same surgery without constriction.

Totally 20 Wistar rats were randomly divided into groups (n = 5/group): the sham-operated group, the TAC group with intragastric administration of purity water, the lower-dose treatment group (3.6 g·kg^{-1}·d^{-1} of YQWY, 120 days) and high dose treatment group (18 g·kg^{-1}·d^{-1} of YQWY, 120 days).

Echocardiography

The rats were fixed in a supine position after anesthesia with 1.5%–2.0% isoflurane. Echocardiography was performed in Nanjing medical University using Vevo 2100 (VisualSonics Inc, Toronto, Ontario, Canada) with a 30 MHz central frequency scan head. The indexes measured in this study included the LVFS, LVEF, LV Mass, LV Vol d and LV Vol s. The results were obtained from the M-mode and 2-D images taken from the parasternal short-axis view at papillary muscle level.

ELISA for NT-proBNP

Blood samples were collected from the abdominal artery. The serum was then separated and used for the detection of Serum NT-proBNP by ELISA kit (Catalog: E08752r, Cusabio, USA) following the manufacturer's instructions.

Histological analysis

The histological analysis was performed as previously described [25]. Briefly, heart tissues were fixed in 4% paraformaldehyde solution, embedded in paraffin, cut into about 5-μm thick sections and stained with HE staining. The WGA staining was used to exam the cell size. The slides were stained for 30 min with WGA-Alexa Fluor™ 488 labeled antibody (Catalog: W11261, Thermo Scientific, Hudson, NH, USA) at room temperature (RT). The nuclei were stained with DAPI (Catalog: C1005, Beyotime, Jiangsu, Haimen, China) for 5 min.

WB analysis

Heart tissue was minced and homogenized in ice-cold RIPA buffer containing 0.1% phenylmethylsulfonyl fluoride. After centrifugation at 12 000 r·min^{-1} for 10 min at 4 °C, the supernatant of pyrolysis products was collected and stored at –80 °C until use. The protein concentration was determined using BCA protein assay kits (Thermo Scientific). After the addition of sample loading buffer, an equal amount of protein of each sample was separated on 7.5%, 10% and 12.5% SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were then blocked with 5% skimmed milk for 1 h, followed by incubation with primary antibodies at 4 °C overnight. After being washed with TBST three times, the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse/rabbit antibodies (1 : 2000 dilution) for 90 min at 37 °C. All these immunoblots were detected via the enhanced chemiluminescence (ECL) detection regent (Millipore). Primary antibodies used in this study were listed in Table 1 and the second antibodies were purchased from Abcam (Cambridge, UK).

IHC analysis

P-GATA4 (Ser105), ANP, BNP, MYH7 expression in heart tissues were measured by IHC. Briefly, isolated heart tissues were fixed in 4% paraformaldehyde overnight, dehydrated and embedded in paraffin. Following citrate antigen retrieval, the sections (4 μm thick) were blocked with blocking solution (PBST/5% normal goat serum, Beyotime) for 1 h at RT. The slices were then incubated with primary antibodies for 12 h at 4 °C. An anti- rabbit antibody labelled with HRP was then used to incubated with the sections for another 60 min at 37 °C. Primary antibodies used in this study were listed in Table 1 and the second antibodies were purchased from Abcam. Subsequently, the sections were treated with DAB substrate (Cell Signaling Technology, Danvers, MA, USA) for 10 min, and hematoxylin staining solution was used for counter-stain in nucleus for 4 min. Five microscopic fields were randomly selected in each section using an Olympus Bx43 microscope and a DP73 camera (Olympus, Tokyo, Japan). Image J software was used to determine the average optical density of positive cells in each field.

IF

The sections fixed with 4% paraformaldehyde were rehydrated in PBS for 10 min, soaked in 0.2% TritonX-100 at 37 °C for half an hour, and washed with PBS for three times. After blocked with 5% goat serum (Beyotime) for 60 min, the frozen sections were then incubated with anti-P-GATA4, P-P38, P-ERK and P-JNK at 4 °C overnight. Primary antibodies used in this study were listed in Table 1. After washed for 3 times with PBS, the sections were then incubated with Alexa Fluor 594 goat anti-rabbit IgG secondary antibody (1 : 500 dilution; Ab150080; Abcam.) for 60 min at RT. The IF results were visualized under a light microscopy (Bx43 mi- croscope and a DP73 camera, Olympus, Tokyo, Japan).

Statistical analysis

All data were analyzed with Graph Pad Prism 6 (GraphPad Software Inc., San Diego, CA, USA). One-way ANOVA was used for the comparison among three or more groups. All data were expressed as mean ± SD. $P < 0.05$ was considered to indicate be statistically significant.

Table 1 Primary antibodies

Antibody	Company	Species	Catalog	Dilution	Application
ANP	Abcam	Rabbit	Ab180649	1 : 1000	WB
				1 : 200	IHC
BNP	Abcam	Rabbit	Ab19645	1 : 500	WB
				1 : 200	IHC
MYH7	Abcam	Rabbit	Ab172967	1 : 2000	WB
				1 : 250	IHC
GATA4		Rabbit	Ab84593	1 : 2000	WB
P-GATA4	Abcam	Rabbit	Ab5245	1 : 2000	WB
				1 : 200	IHC
				1 : 200	IF
P38	Cell Signaling Technology	Rabbit	8690	1 : 1000	WB
P-P38	Cell Signaling Technology	Rabbit	4511	1 : 1000	WB
			4511	1 : 1000	IF
ERK	Cell Signaling Technology	Rabbit	4370S	1 : 1000	WB
P-ERK	Cell Signaling Technology	Rabbit	4695S	1 : 2000	WB
				1 : 200	IF
JNK	Abcam	Rabbit	Ab179641	1 : 1000	WB
P-JNK	Abcam	Rabbit	Ab124956	1 : 2000	WB
				1 : 200	IF

Results

LC-MS/MS analysis of YQWY

As shown in Fig. 1, five compounds have been identified in YQWY. The contents of formononetin (t_R = 1.630 min), calycosin 7-*O*-*β*-D-glucopyranoside (t_R = 4.299 min), AsIV (t_R = 9.106 min), salidroside (t_R = 9.383 min), paeoniflorin (t_R = 13.175 min), and have been identified as 0.348, 0.289, 0.317, 0.258 and 0.643 μg·mg^{-1}, respectively.

YQWY improved the cardiac function

Compared with sham group, TAC group showed significant decrease of EF (%) and FS (%) ($P < 0.05$). Besides, the EF (%) and FS (%) in high-dose group was increased significantly compared with TAC group (Figs. 2A–2B). These results demonstrated that YQWY decoction alleviated the impaired cardiac contractile function induced by TAC, especially under a high dose. There was no difference in LV mass between TAC group and YQWY-treated group (Fig. 2C). A remarkable decrease in LV Vol d and LV Vol s were also observed in high dose of YQWY-treated group after 16-week intragastric administration comparing to the TAC group (Figs. 2D–2E). Both high and low dose of YQWY showed significant recovery effect on serum NT-proBNP increased by TAC ($P < 0.01$) (Fig. 2F). Generally, the results of echocardiography and ELISA have identified the curative effect of YQWY decoction in improving cardiac function.

YQWY inhibited cardiomyocyte hypertrophy

TTE has been wildly used to acquire the cardiac structure and function, but it cannot detect the early and tiny changes in cardiac structure. To further verify the effects of YQWY decoction on inhibiting cardiomyocyte hypertrophy, HE staining was conducted to visualize cardiomyocyte's morphology. Compared with sham group, TAC induced visualized cardiomyocyte hypertrophy ($P < 0.01$). However, treatment of YQWY showed remarkable reversion of cardiomyocyte hypertrophy induced by TAC both in the low and high dose group ($P < 0.01$) (Fig. 3A). The result of WGA staining was also consistent with our observation in the HE staining (Figs. 3B–3C). The above morphological changes indicated that YQWY-treated group might attenuate cardiomyocyte hypertrophy by inhibiting the increase of the dimension of the cells and maintained the cell morphology.

YQWY inhibited phosphorylation of GATA4 (Ser105) and its downstream targets

Based on the results of TTE and staining, YQWY decoction could suppress TAC induced cardiomyocyte hypertrophy. To determine the potential mechanism, we detected the expression of ANP, BNP, MYH7 and P-GATA4 by WB, IHC AND IF. After TAC surgery, TAC group showed significant increase in the expression of ANP, BNP, MYH7 and P-GATA4 compared with sham group (Figs. 4A–4B), IHC and IF staining further confirmed the alteration of above markers (Figs .5A–5C) ($P < 0.01$). Additionally, both low and high dose of YQWY decoction markedly downregulated these markers comparing to the TAC group ($P < 0.01$). These results implied that YQWY decoction can reduce the expression of P-GATA4 and its downstream genes (ANP, BNP, MYH7). Thus, we can conclude that the effects of YQWY decoction

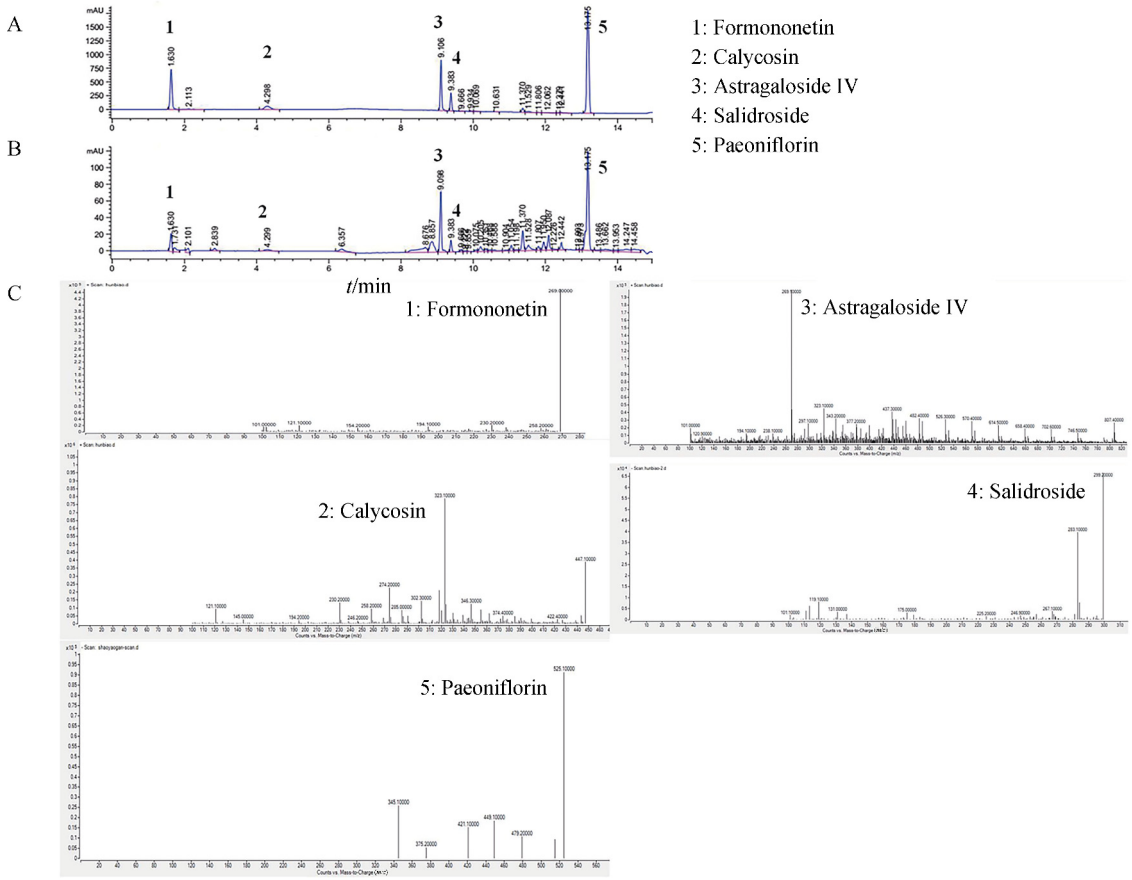

Fig. 1 LC-MS/MS analysis of YQWY. A: Elementaryparticle flow graphchromatogram of YQWY sample. 1: formononetin, 2: calycosin 7-*O*-*β*-D-glucopyranoside, 3: AsIV, 4: salidroside, 5: paeoniflorin. B: Elementaryparticle flow graphchromatogram of standards. 1: formononetin, 2: calycosin 7-*O*-*β*-D-glucopyranoside, 3: AsIV, 4: salidroside, 5: paeoniflorin. C: Representative mass spectra of the standards: 1: formononetin, 2: calycosin 7-*O*-*β*-D-glucopyranoside, 3: AsIV, 4: salidroside, 5: paeoniflorin

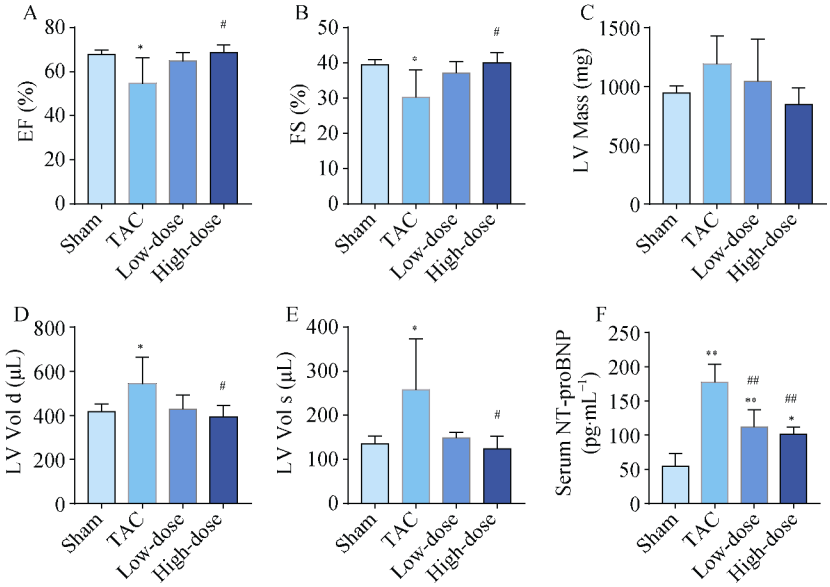

Fig. 2 YQWY improved the cardiac function. (A) EF (%); (B) FS (%); (C) LV mass (mg); (D) LV Vol d (ul); (E) LV Vol s (μl); (F) Serum NT-proBNP (pg·mL^{-1}). Values were expressed as mean ± SD, n = 5, $^{*}P < 0.05$, and $^{**}P < 0.01$ *vs* Sham group; $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ *vs* TAC group

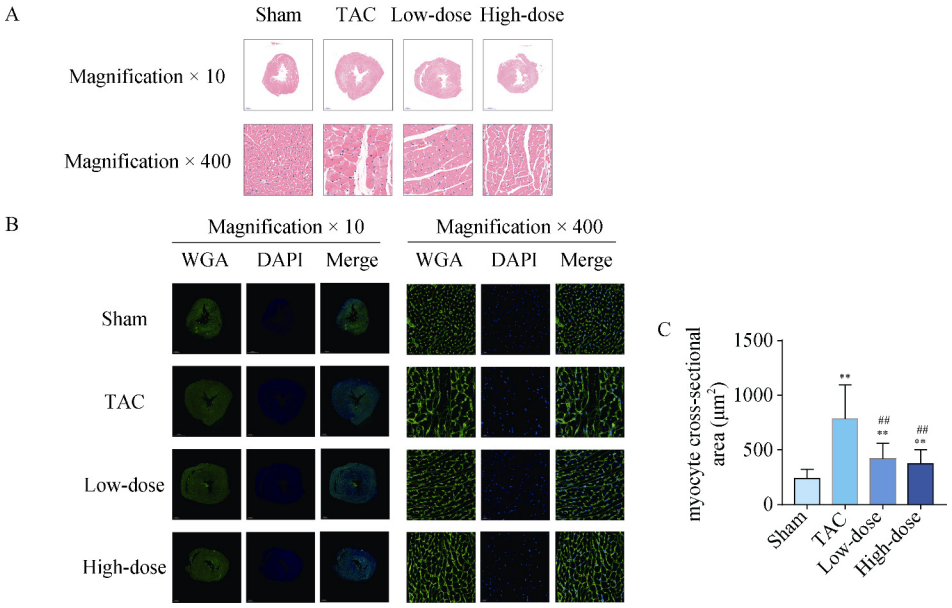

Fig. 3 YQWY inhibited TAC induced cardiomyocyte hypertrophy in *vivo*. (A) HE staining was performed to visualize global morphology. (B) WGA staining was used to examine cell size. (C) Quantitative results of WGA staining. Value was expressed as mean ± SD, $n = 5$, $^{*}P < 0.05$, and $^{**}P < 0.01$ *vs* Sham group; $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ *vs* TAC group

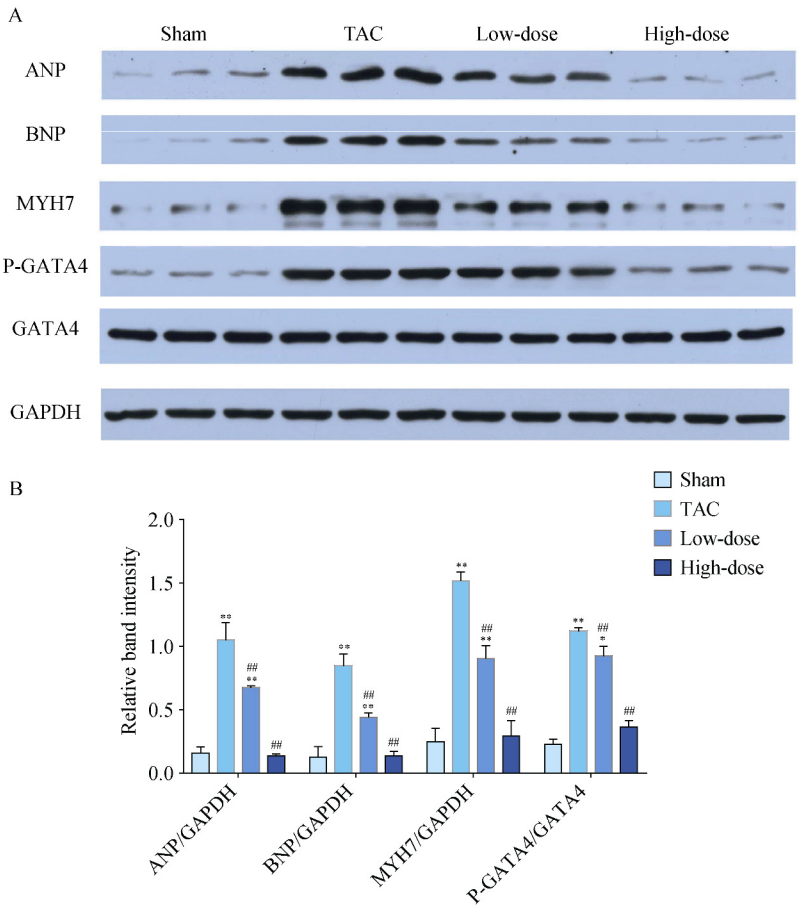

Fig. 4 The effects of YQWY decoction on suppressing the cardiomyocyte hypertrophy and reversing the impaired heart function were associated with inhibiting the phosphorylation of GATA4. and downstream targets. (A) WBs of ANP, BNP, MYH7, GATA4 and P-GATA4 from indicated groups. (B) Quantitative results of WB analysis. Value was expressed as mean ± SD, $n = 3$, $^{*}P < 0.05$, and $^{**}P < 0.01$ *vs* Sham group; $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ *vs* TAC group

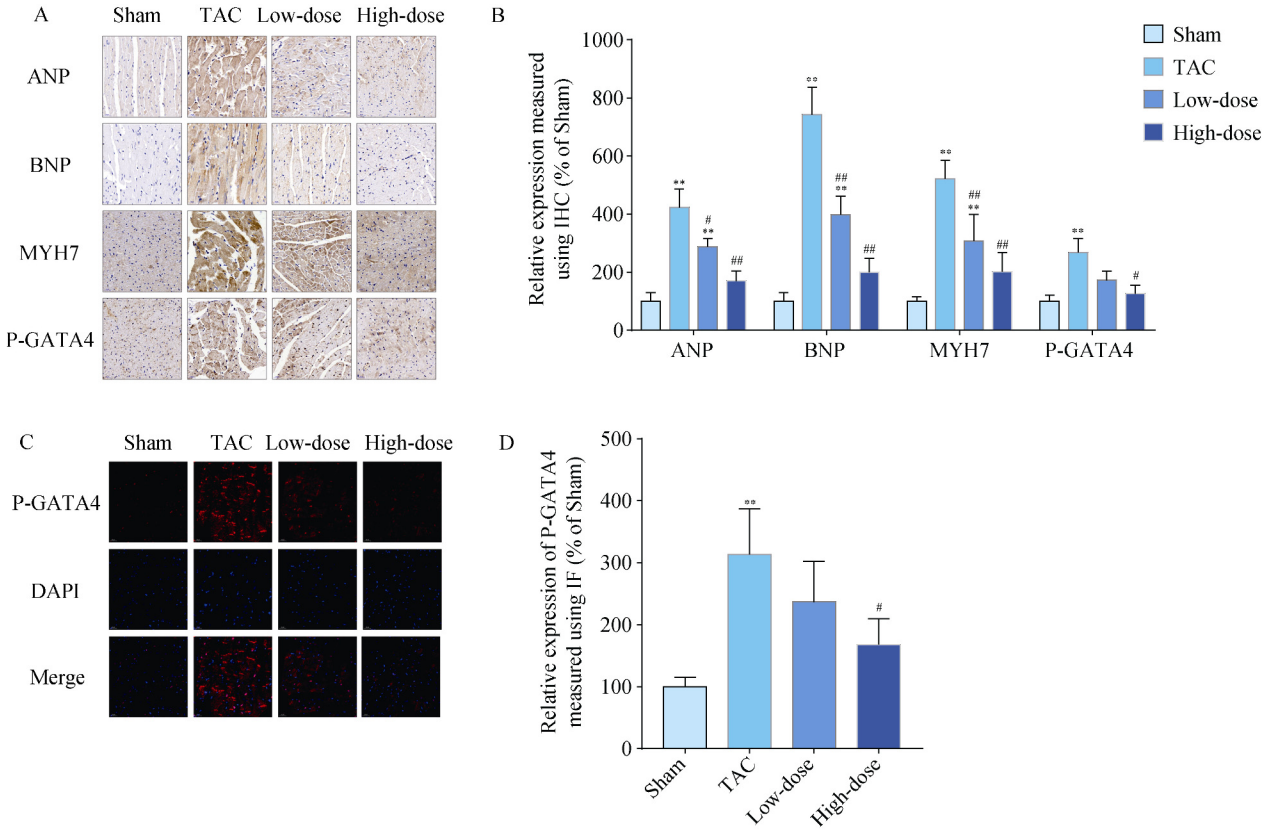

Fig. 5 The effects of YQWY decoction on suppressing the cardiomyocyte hypertrophy and reversing the impaired heart function were associated with inhibiting the phosphorylation of GATA4. and downstream targets. (A) IHC of ANP, BNP, MYH7 and P-GATA4 from indicated groups. (B) Quantification of IHC. Value was expressed as mean ± SD, n = 3, $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* Sham group; $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ *vs* TAC group. (C) IF of P-GATA4 from indicated groups. (D) Quantification of IF. Value was expressed as mean ± SD, n = 3, $^{*}P < 0.05$, and $^{**}P < 0.01$ *vs* Sham group; $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ *vs* TAC group

on suppressing the cardiomyocyte hypertrophy and reversing the impaired heart function were associated with inhibiting the phosphorylation of GATA4.

YQWY inhibited phosphorylation of ERK, P38 and JNK.

Phosphorylation of GATA4 (Ser-105) plays important role in the progression of cardiac hypertrophy and the initiation of MAPKs can specifically catalyze the Ser-105 phosphorylation of GATA4. To reveal the underlying mechanisms through which YQWY treatment protected cardiac hypertrophy induced by TAC, our study further investigated activation of phosphorylation of MAPKs signaling pathway in heart tissues of each group. The results showed that the phosphorylation levels of MAPKs ($P < 0.01$) were significantly elevated after TAC, compared with sham group (Figs. 6A–6B and Figs. 7A–7D). The YQWY treatment group significantly decreased the expression of phosphorylated ERK ($P < 0.05$), P38 ($P < 0.05$) and JNK ($P < 0.05$) induced by TAC (Fig. 6A-B and Figs. 7A–7D). These findings suggested that the effects of YQWY decoction on suppressing the cardiomyocyte hypertrophy and reversing the impaired heart function were associated with the inhibition the phosphorylation of MAPKs.

Discussion

Heart failure remains a huge burden for the social health and economy. Despite progress has been made in treatment recently, better therapies were still needed to be development. Cardiac hypertrophy is an early hallmark of the clinical course of heart failure and is also an independent risk factor for the mortality from heart disease [26]. In our study, a significant decline in EF% and FS% and remarkable increase in LV Vol d/s were observed in the TAC group, compared with sham group. However, consecutive intragastric administration of YQWY reversed above changes to the same level of sham group. The results of HE and WGA analysis further indicated that YQWY decoction attenuated the cardiomyocyte hypertrophy induced by TAC.

The pathogenesis mechanisms of cardiac hypertrophy involve a variety of complex and coordinated signaling pathways. As a key regulator of myocardial hypertrophy, GATA4 can not only activate the biomarkers (ANP, BNP and MYH7) of cardiac hypertrophy, but also interact with multiple signaling molecules including ERK, JNK and P38. The activation of BNP, ANP and MYH7 all require to bind with the GATA

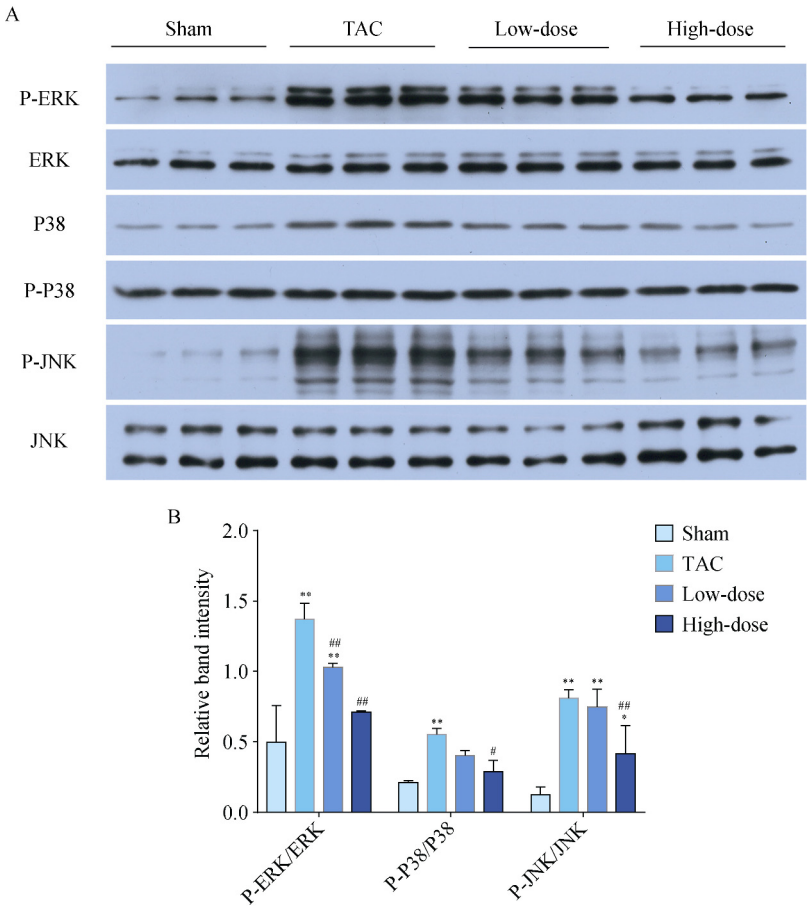

Fig. 6 YQWY suppressed TAC induced phosphorylation of ERK, P38 and JNK. (A) WBs of P-P38 P-JNK P-ERK. (B) Quantitative results of WB analysis. Value was expressed as mean ± SD, $n = 3$, $^{*}P < 0.05$, and $^{**}P < 0.01$ *vs* Sham group; $^{\#\#}P < 0.01$ *vs* TAC group

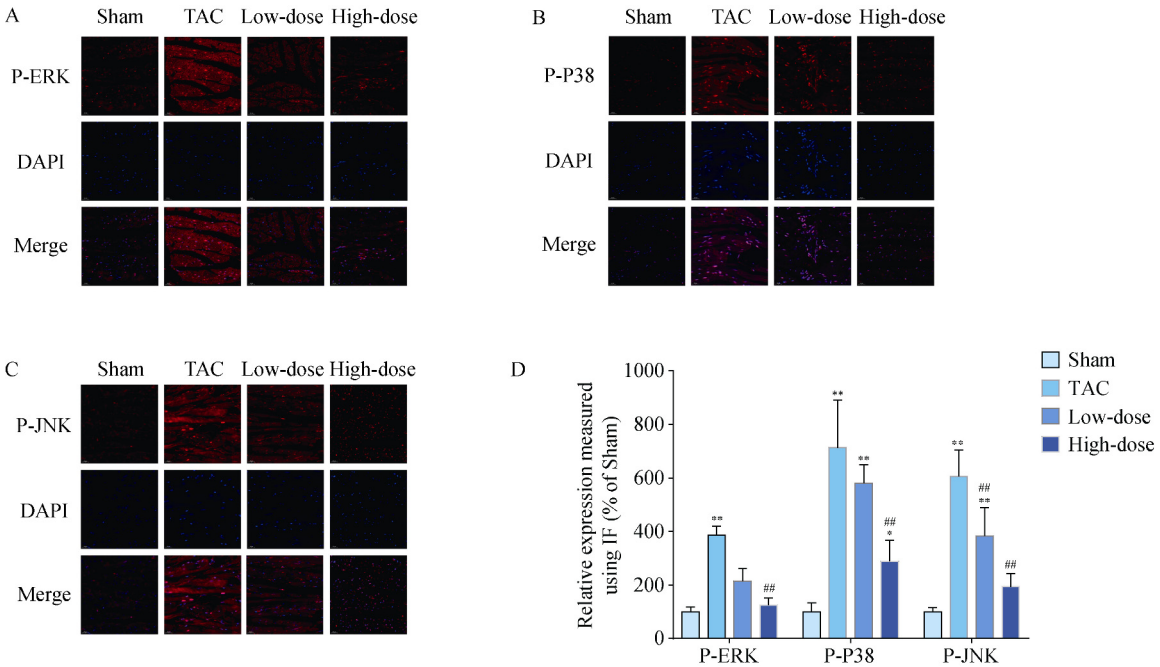

Fig. 7 YQWY suppressed TAC induced phosphorylation of ERK, P38 and JNK. (A–C) IF for P-P38, P-JNK, and P-ERK. (D) Quantification of IF. Value was expressed as mean ± SD, $n = 3$, $^{*}P < 0.05$, and $^{**}P < 0.01$ *vs* Sham group; $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ *vs* TAC group

elements in the promoter regions [10]. GATA4 phosphorylation induced by TAC, phenylephrine (PE) or ISO is increased with the accompany of GATA4 DNA binding activities in *vitro* and in *vivo* [27-31]. The key amino acid for the phosphorylation of GATA4 is Ser-105, which is necessary for GATA4 mediated transcriptional activation and improve the stability within the cell [32]. The *in vivo* experiments have found that mice with a phosphorylation defect of GATA4-Ser105 were more susceptible to PE-induced hypertrophy [33]. Besides, GATA4 can also be activated directly through serine phosphorylation (Ser-105) by ERK [5-7], P38/MAPK pathway [34] and JNK pathway [6, 35]. WB analysis of the heart lysates from wild type and ERK overexpression mice (dTg) indicated that phosphorylation of GATA4 and ERK were increased in the heart of dTg mice [36]. Briefly, GATA4 phosphorylation induced by three main branches of the MAPKs signaling pathway activated the expression of multiple target proteins (ANP, BNP and MYH7), which then resulted in the progression of cardiac hypertrophy. Our study showed that TAC induced the increase of the phosphorylation of GATA4 and the expression of ANP, BNP, MYH7, which could be reversed by the treatment with YQWY decoction ($P < 0.01$). The phosphorylation of ERK ($P < 0.05$), P38 ($P < 0.05$) and JNK ($P < 0.05$) were separately decreased to varying degrees compared with those in TAC group. Previous studies demonstrated that expression of ANP, BNP and MYH7 were commonly used as a molecular marker of hypertrophy, and their activation are partially dependent on GATA4 [37]. It has also been reported that ERK, JNK and P38 MAPK were simultaneously activated after TAC surgery [38]. Moreover, our results suggested that the activation of GATA4 and MAPKs induced by TAC was effectively reversed by administration of YQWY decoction. Thus, we proposed that the effects of YQWY decoction on suppressing the cardiomyocyte hypertrophy and reversing the impaired heart function were associated with inhibited phosphorylation of GATA4 and MAPKs.

Conclusions

The present study revealed that YQWY decoction attenuated the cardiac hypertrophy and reversed the impaired cardiac function induced by TAC. Besides, the curative effects of YQWY decoction were associated with the decreased phosphorylation of GATA4 and MAPKs, as well as the reduced expression of the downstream targets of GATA4, including ANP, BNP, MYH7.

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