European Review for Medical and Pharmacological Sciences 2023; 27: 7264-7275

Qiliqiangxin capsule improves cardiac remodeling in rats with DOCA-salt-induced diastolic dysfunction

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Abstract. – OBJECTIVE: The aim of this study was to investigate the protective effect and mechanism of action (MOA) of Qiliqiangxin capsule (QL) in the deoxycorticosterone acetate (DOCA) salt-induced rat heart failure with preserved ejection fraction (HFpEF) model.

MATERIALS AND METHODS: Nono-nephrectomy sixty Sprague Dawley (SD) rats received DOCA salt injection and 1% saline in drinking water for 4 weeks and were randomly divided into four groups on average: Model group (n=15), Sac/Val group (Sacubitril Valsartan 0.02 g/kg, n=15), QL-L group (Qiliqiangxin 0.25 g/kg, n=15) and QL-H group (Qiliqiangxin 1 g/kg, n=15). Another Normal group was set (n=15). Blood pressure, N-terminal pro-brain natriuretic peptide (NT-proBNP), cardiac index, echocardiography, and hemodynamics were measured to evaluate heart function. Masson and Wheat germ agglutinin (WGA) staining was performed to observe the fibrosis deposition and the cross-sectional area (CSA) of cardiomyocytes. The concentration levels of the serum cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-2, IL-6, and IL-10 inflammatory factors, were detected by ELISA; matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), transforming growth factor-β1 (TGF-β1), nuclear factor-κB (NF-κB), Smad homologue 2 (Smad2) and Smad homologue 3 (Smad3) expression were detected by Western-blot.

RESULTS: Compared with the Model group, QL treatment significantly ameliorated the heart function in DOCA salt-induced rat HFpEF model, showing a decrease in cardiac index, an increase of the EF and E/A ratio, a reduction in the left ventricular anterior/posterior wall (LVAW/LVPW), in the time contraction of isovolumic diastolic time (IVRT), -dP/dt Max, and Tau, and the decrease of serum NT-ProBNP. Masson and WGA staining indicated that QL inhibited the fibrosis deposition and the myocardial hypertrophy compared with the Model group, which was consistent in reducing the protein expression levels of cardiac remodeling such as TGF-β1, MMP2, MMP9, Smad2, and Smad3. Moreover, QL treatment inhibited the expression of NF-κB in the heart tissues and decreased the serum concentration of pro-inflammatory cytokines TNF-α and IL-2, instead, increasing the IL-10 concentration.

CONCLUSIONS: QL improved the cardiac function and inhibited the myocardial fibrosis in DOCA salt-induced rat HFpEF by improving diastolic dysfunction, preventing left ventricular hypertrophy, and ameliorating the inflammatory responses model in DOCA salt-induced rat HFpEF model.

Key Words:
EQiliqiangxin capsule, HFpEF, Myocardial fibrosis, Hypertension, TGF-β1/Smads pathway.

Introduction

Hypertension is one of the most important risk factors for cardiac disease[1]. Persistent hypertension can lead to maladaptive cardiac changes, including structural left ventricular remodeling with left ventricular hypertrophy and fibrosis, impaired left heart function (diastolic and systolic), left atrial enlargement, and atrial fibrillation. Heart failure with preserved (HFpEF) or reduced ejection fraction (HFrEF) may occur in severe cases[2-4]. Nearly half of all patients presenting with heart failure have

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HFpEF[3] manifested with diastolic dysfunction. Given that hypertension is the primary causative factor for HFpEF[5], there is a lack of efficient ways to treat the disease but blood pressure control[6].

Impairment of diastolic function caused by pathological remodeling is an important feature of HFpEF[7]. Cardiac remodeling is an adaptive cellular response to various biomechanical stresses or hemodynamic overloads involving a range of structural and functional changes. These changes include increased size and/or thickness of the left ventricular wall, perivascular and interstitial fibrosis, and various types of ventricular and atrial arrhythmias[8,9]. Inflammation and myocardial fibrosis are important pathological factors leading to ventricular remodeling in HFpEF[10-12]. Clinical studies[13] have found that anti-inflammatory and anti-fibrosis treatments were important therapies to inhibit HFpEF ventricular remodeling and improve cardiac function. Slowing down or reversing cardiac pathological hypertrophy is of great significance for the prevention and treatment of HFpEF.

Qiliqiangxin capsule (QL), a patent Traditional Chinese Medicine (TCM) formula approved by China FDA has been widely applied for the management of chronic heart failure; it is prepared by eleven TCM herbs including *astragalus radix*, ginseng radix et rhizoma, aconite root, *salvia miltiorrhiza*, *descurainiae semen lepidii semen*, *alismatis rhizome*, radix polygonati officinalis, *cinnamomum ramulus*, *carthami flos*, *periploca cortex* and *citri reticulatae pericarpium*[14]. Previous studies[15] have shown that QL improves cardiac function, 6-min walking distance, and the ratio between mitral diastolic maximum flow rate E peak and late diastolic maximum flow rate A peak (E/A) in HFpEF patients. QL was reported to improve ventricular remodeling by reducing the expression levels of inflammatory proteins such as interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), Nuclear factor-κB (NF-κB), and NOD-like receptor thermal protein domain associated protein 3 (NLRP3) in transverse aortic constriction (TAC) rats[16]. Meanwhile, QL could improve cardiac function in chronic heart failure (CHF) rats by reducing myocardial fibrosis and regulating specific miRNAs[17]. Given the cardiotonic and anti-inflammatory effects of QL, whether QL can improve cardiac function and attenuate cardiac remodeling in HFpEF is worth investigating.

In this study, deoxycorticosterone acetate (DOCA) salt-sensitive rats, a model of hypertensive heart failure, were used for the evaluation of QL in the treatment of HFpEF. Furthermore, the mechanism of action (MOA) of QL in inhibiting the cardiac remodeling caused by hypertension was revealed by inhibiting the inflammation-induced fibrosis deposition.

Materials and Methods

Animal Grouping and Modeling

Specific pathogen Free (SPF) male Sprague Dawley (SD) rats (200±10 g), obtained from Vital River Laboratory Animal Technology Co. Ltd (Beijing, China, license number SCXK2016-0006), were housed in a well-controlled environment with a 12-hour light/dark cycle at room temperature (23±2°C). All rats were given adaptive feeding for one week and a free-wading diet. All animals were approved by the animal ethics committee of Hebei Yiling Medical Research Institute (approval No. 2020141). Food and water were supplied continuously. Rats were randomly divided into five groups: Normal group (n=15), Model group (n=15), Sac/Val group (Sacubitril Valsartan 0.02 g/kg), QL-L group (Qiliqiangxin 0.25 g/kg) and QL-H group (Qiliqiangxin 1 g/kg).

Rats in the Normal group were untreated. Rats in other groups were induced HFpEF, as published in another study[18]. Briefly, firstly, to perform mono-nephrectomy, the animals were anesthetized with pentobarbital sodium (25 mg/kg, i.p.) by intraperitoneal injection. Subsequently, the left renal vessels and ureter were ligated from a longitudinal incision made on the side of the abdomen. Afterward, the incision site was sutured with a sterile suture needle. Postoperative rest and penicillin (100,000 units/day) injection were continued for 3 days. After mono-nephrectomy, rats were injected with DOCA (25 mg/kg, i.h., Aladdin Biochemical Technology Co. Ltd, Shanghai, China) and given 1% sodium chloride in drinking water for 4 weeks to induce HFpEF. Rats in the administration groups were given corresponding drugs by oral gavage daily for additional 8 weeks. Blood pressure was measured every 2 weeks after treatment through the Tail-Cuff method (BP-2000; Visitech Systems, Allen, NC, USA) until the end of 12 weeks. All animals were handled and maintained under the Animal Care and Use Committee of Hebei Yiling Chinese Medicine Research Institute (Shijiazhuang, China).

Biomarker's Assessments

The N-terminal pro-brain natriuretic peptide (NT-proBNP) was obtained using an En-

zyme-Linked Immunosorbent Assay Kits (Immunoway, Plano, TX, USA) to evaluate the cardiac function. At the end of the 12-week experiment, blood samples from the abdominal aorta were collected. The blood samples were centrifuged at 1,800 g for 10 minutes, and the supernatant samples obtained were centrifuged at 13,000 g for 2 minutes to test.

Echocardiographic Measurements

Rats were anesthetized with a 1% isoflurane oxygen mixture and monitored by echocardiography (Vevo 3100 LT, FUJIFILM Visual Sonics, Toronto, ON, Canada). Standard M-mode imaging was performed on a parasternal long-axis section to obtain the heart rate (HR), percentage fractional shortening (FS), ejection fraction (EF), and left ventricular anterior/posterior wall thickness (LVAW/LVPW), isovolumic diastolic time (IVRT), isovolumic systolic time (LVCT). Passive peak left ventricular filling velocity E (cm/s) and peak atrial systolic flow velocity A (cm/s) were obtained from the mitral valve Doppler flow images. The scanning speed of M-mode and Doppler ultrasound was 100 mm/s, and the heart rate was maintained at 250-350 beats per minute.

Hemodynamic Measurements

Invasive hemodynamic tests were performed in rats under general anesthesia: isovolumic diastolic maximum left ventricular rise and fall rates (±dP/dt Max), left ventricular end-diastolic pressure (EDP), diastolic duration (DD), and left ventricular relaxation time constant (Tau). The data were recorded continuously for 20 minutes until the hemodynamic parameters reached a stable state, and we used LabChart8 software (AD Instruments Pty Ltd, Bella Vista, NSW, Australia) to obtain conductance volume data.

Morphological Analysis and Immunofluorescence Staining

Hearts dissected from the executed animals were fixed in 10% formalin solution, dehydrated in ethanol, and then paraffin-embedded. Tissue sections (4 um) were performed parallel to the apical-basal axis. After dewaxing and rehydration, Masson trichrome staining (Life Sciences, Shanghai, China) was performed for fibrosis quantification. The cross-sectional area (CSA) of cardiomyocytes was assessed by immunofluorescence measurement of wheat germ agglutinin (WGA, Alexa Fluor 488-conjugated, Thermo Fisher Scientific, Waltham, MA, USA) and α-smooth muscle actin (α-SMA, Abcam, Waltham, MA, UK, Alexa Fluor 594-conjugated). Five randomly selected fields of view were evaluated by the blinded method, and one representative image was selected.

Enzyme-Linked Immunosorbent Assay (ELISA)

According to the reagent manufacturer's instructions, the serum inflammatory factor of rats in each group was detected by ELISA for tumor necrosis factor-α (TNF-α, Elabscience, EK382HS, Wuhan, China), Interleukin (IL)-2 (Elabscience, EK302/2, China), IL-6 (Elabscience, EK302/2, China) and IL-10 (Elabscience, EK310HS, China) levels.

Western Blot Analysis

Heart tissue was lysed for protein extraction and bicinchoninic acid (BCA) quantification. Appropriate samples from each group were separated using 4-20% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to nitrocellulose membranes for semi-dry transfer of membranes. After blocking with 5% bovine serum albumin for 1 h, primary antibodies were incubated overnight at 4°C, then washed and incubated for secondary antibody binding. The films were scanned with a Bio-Rad calibrated densitometer, and the intensity of the grayscale values of the immunoblotted bands was compared to the internal reference (GAPDH, glyceraldehyde-3-phosphate dehydrogenase).

Statistical Analysis

All data were expressed as standard error (Mean ± SEM). One-way analysis of variance (ANOVA) or repeated measurement was used for homogeneity of variance for all groups. The least significant difference (LSD) test was used for comparison between groups. The Dunnett post-hoc T3 test was used for uneven variances, and $p<0.05$ was considered statistically significant. All statistical analyses were completed with the SPSS version 26 (IBM Corp., Armonk, NY, USA).

Results

QL Protected the Heart Against Hypertensive Cardiac Remodeling in Rats with HFpEF

Marked abnormalities were observed in DOCA-salt rats, such as an increased ratio of heart weight/body weight (HW/BW), and increased

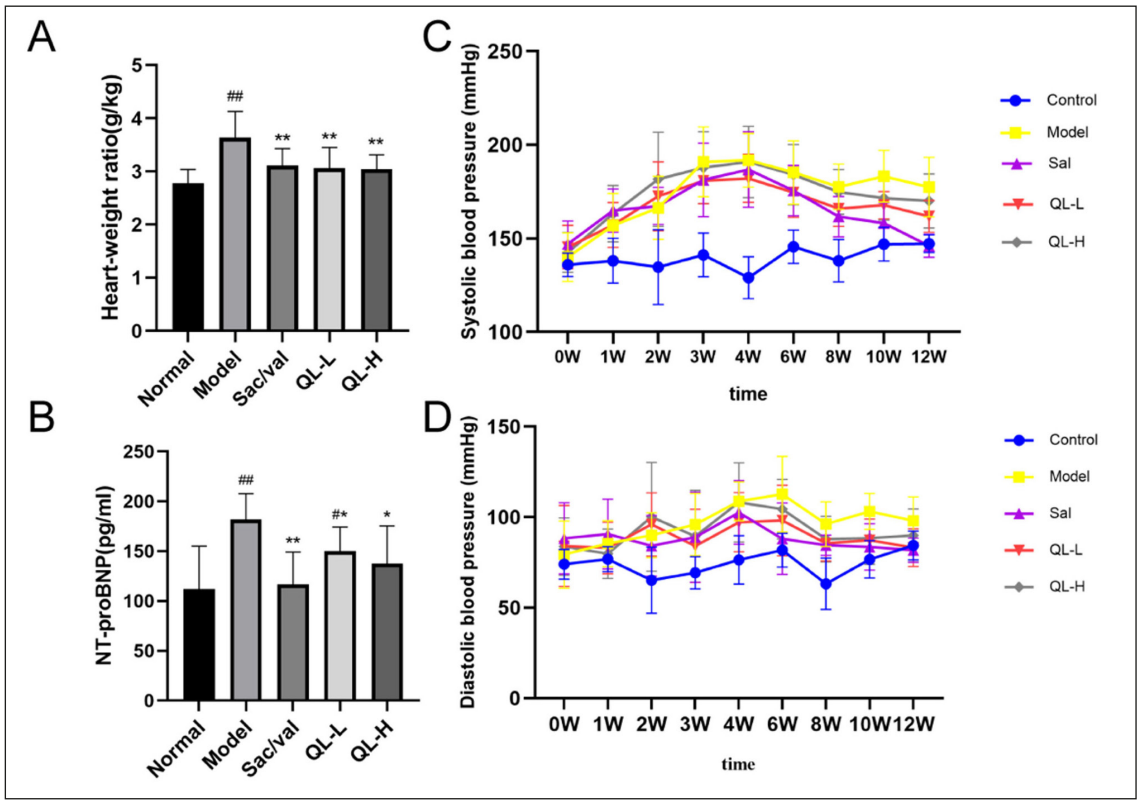

Figure 1. QL alleviated heart concentric remodeling in DOCA-rats. **A**, Quantitative results of heart weight/body weight ratio in each group. **B**, QL and Sac/Val could reduce the NT-proBNP value of model rats. **C-D**, Both systolic and diastolic blood pressure in the model, QL-L and QL-H groups remained higher than in normal, while Sac/Val group had no difference with the normal group until the end of the experiment. #p<0.05, ##p<0.01, *vs.* normal group; * p<0.05, ** p<0.01 *vs.* model group, all by analysis of variance, n=15 in each group.

concentration of NT-proBNP in the serum (Figure 1A-B), indicating hypertensive cardiac remodeling. In addition, both systolic and diastolic blood pressure were increased in a time-dependent manner during the four weeks of HFpEF modeling, as reported in a study[19]. Sac/Val, QL-L, and QL-H groups could significantly reduce the heart size, the ratios of HW/BW, and the concentration of serum NT-proBNP. Sac/Val group also brought blood pressure back to normal, while QL-H and QL-L failed to lower the blood pressure (Figure 1C-D), suggesting that a protective effect of QL against hypertensive cardiac remodeling does not depend on reducing blood pressure.

QL Alleviated Left Ventricular Diastolic Dysfunction in Rats with HFpEF

To explore the structural changes in the left heart of HFpEF, echocardiography was performed, and vital parameters were recorded. Representative results from Typical M-mode and Doppler imaging are shown in Figure 2A-B. Consistent with the cardiac structure characteristics of HFpEF, ejection fraction, E/A ratio were significantly decreased, while LVAW, LVPW, and IVRT were significantly increased in the Model group compared to the Normal group. There was no significant difference in isovolumic contraction time (IVCT)for each group. Interestingly, eight weeks of treatment of Sac/Val and QL-H resulted in remarkably rebounded cardiac structural remodeling parameters suggesting that QL could effectively alleviate concentric LV remodeling in rats with HFpEF (Figure 2C-H).

To further evaluate the left ventricular diastolic function, we measured the commonly used parameters, including left ventricular pressure (LVP), end-diastolic pressure (EDP), diastolic duration, ±dP/dt Max, and Tau by invasive left heart catheter[20] (Figure 3A-E). A significant reduction of left ventricular compliance, impaired relaxation, and increased left ventricular filling pressures were found in the Model group com-

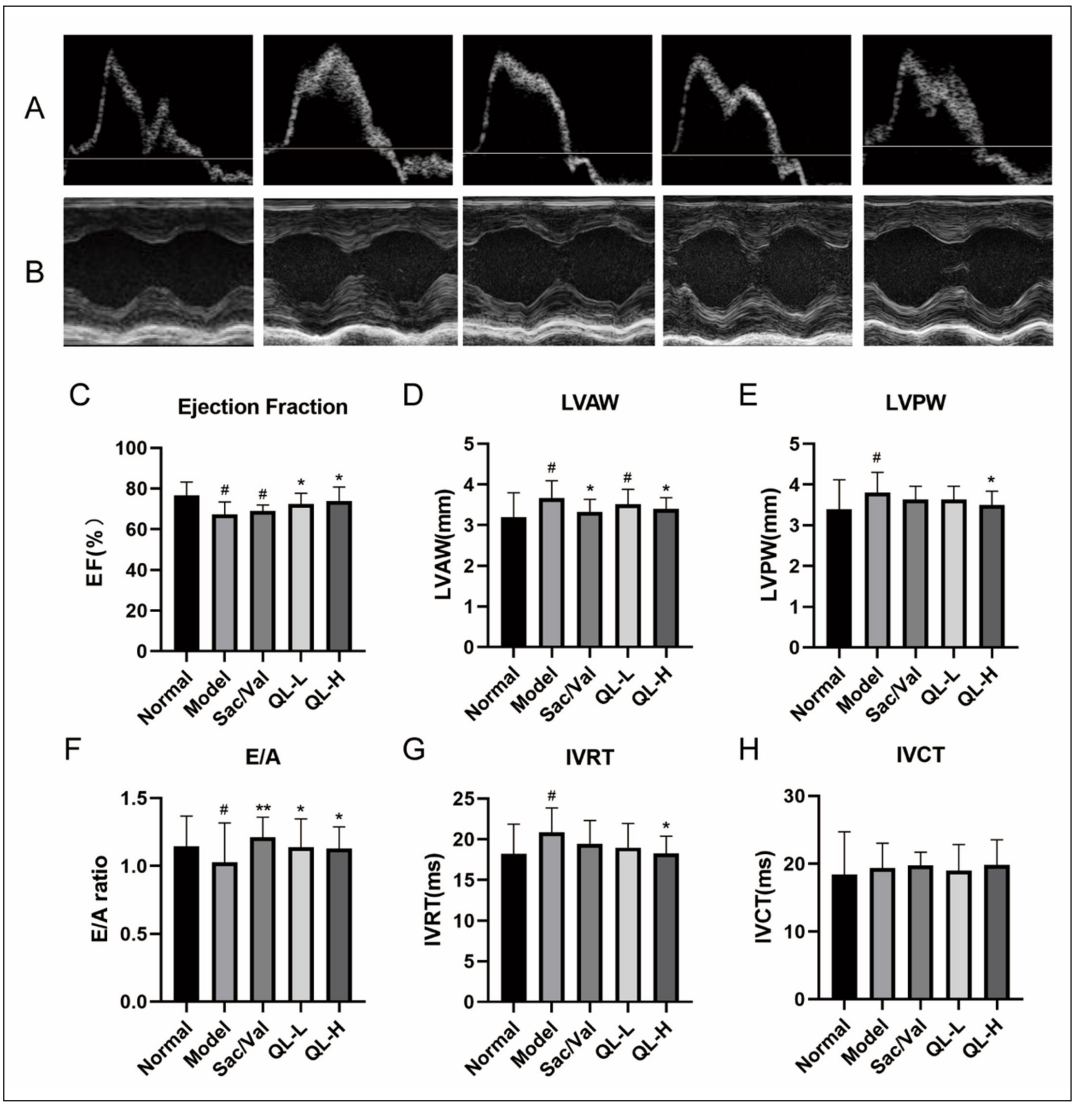

Figure 2. QL alleviated left ventricular diastolic dysfunction by echocardiography analysis. **A-B**, Representative images of transmittal flow by Doppler echocardiography (upper) and M-mode (lower) in each group. **C-E**, Measurement in M-mode of left ventricular ejection fraction, stroke volume, left ventricular anterior wall (LVAW), left ventricular posterior wall (LVPW). **F-H**, Measurement in Doppler echocardiography of E/A ratio and Isovolumic relaxation time (IVRT) (n=15).

pared to the Normal group, registered as the increasing LVP, EDP, diastolic duration, and -dP/dt Max parameter. In addition, the Tau was markedly prolonged in the Model group. As expected, the abnormal changes in the left ventricular were significantly alleviated upon QL and Sac/Val treatment. However, the LVP remained high in the QL-L and QL-H groups, indicating that QL did not have a significant hypotensive effect (Figure 3F). Normalization of -dP/dt Max, and diastolic duration in the QL-L and QL-H groups confirmed that the increase in E/A ratio over time was due to the normalization of the left ventricular diastolic function. Of note, we did not observe any significant differences in heart rate and +dP/dt Max in all groups.

QL Alleviates Cardiomyocyte Hypertrophy in HFpEF Rats

WGA staining showed that the CSA of myocardial cells in the Model group was larger than that in the Normal group (Figure 4). The existence of myocardial hypertrophy was confirmed by morphology, which was consistent with the

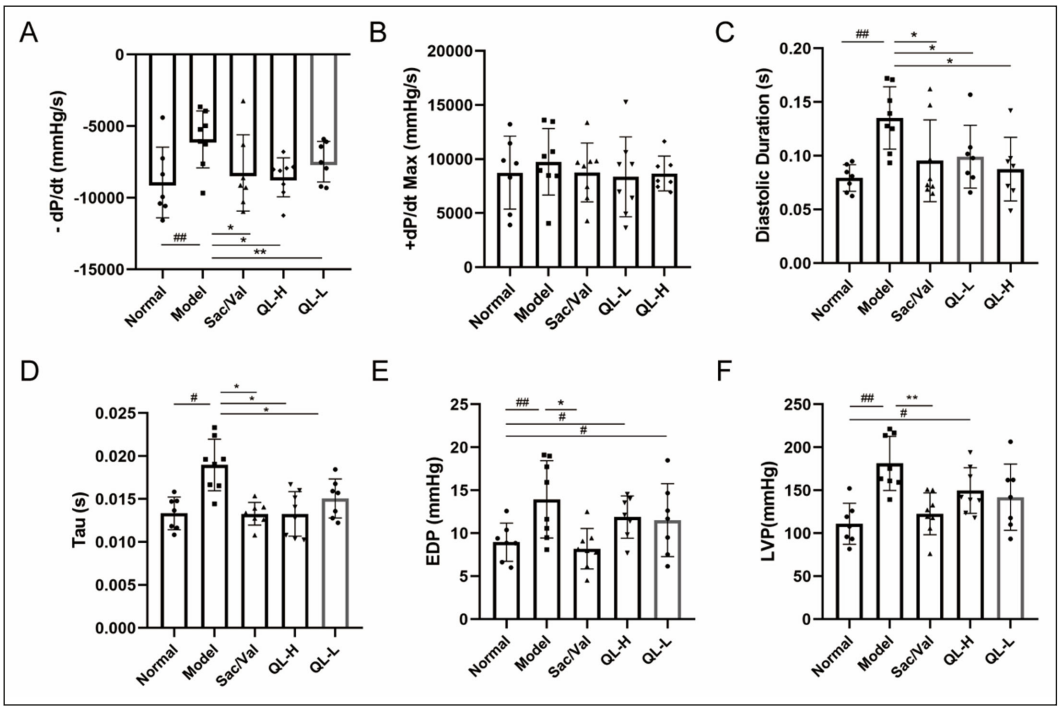

Figure 3. QL alleviated left ventricular diastolic dysfunction by hemodynamic Analysis. **A-B**, QL-L and QL-H normalize -dP/dt Max but with no change in +dP/dt Max. **C-E**, diastolic duration, Tau, and left ventricular end-diastolic pressure (EDP) were also normalized after QL treatment. **F**, However, left ventricular pressure (LVP) is no significant difference between the model and QL-L and QL-H animals, while Sac/Val group was remarkably reduced (n=8). #p<0.05, ##p<0.01, *vs.* normal group; *p<0.05, **p<0.01 *vs.* model group, all by analysis of variance.

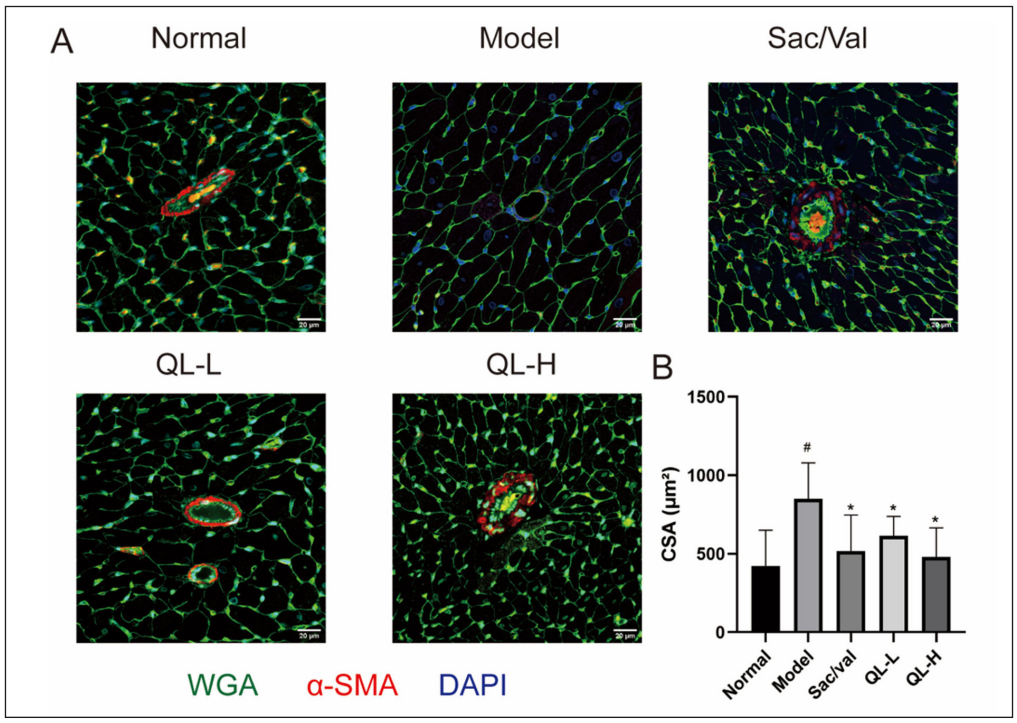

Figure 4. QL alleviates cardiomyocyte hypertrophy in HFpEF rats. **A**, Representative images of WGA-α-SMA Immunofluorescence staining (200×). **B**, the calculated myocyte cross-sectional area (CSA). #p<0.05, ##p<0.01, *vs.* normal group; *p<0.05, **p<0.01 *vs.* model group.

echocardiography data. The size of myocardial cells in Sac/Val, QL-L and QL-H groups were all significantly restored. There was no significant difference in the amount of α-SMA expression around the vessels in each group.

QL Prevented Myocardial Fibrosis

To investigate the effect of QL in relieving myocardial cell stiffness in heart failure, we subjected the myocardial tissue samples to Masson trichrome staining and Western blot protein experiment.

Masson staining showed many blue-stained collagen fibers in the Model group myocardial tissue, indicating myocardial fibrosis and proliferation of myocardial interstitial fiber tissue, which is a crucial mechanism responsible for increased myocardial stiffness in clinical HFpEF[21]. The collagen volume fraction (CVF) of Sac/Val, QL-L and QL-H groups were significantly lower than that of the Normal group, suggesting that the degree of myocardial fibrosis was alleviated (Figure 5A).

We next examined matrix metalloproteinase 2 and 9 (MMP2, MMP9) proteins, collectively known as gelatinases, which are primarily used to digest type IV collagen and gelatin, indicating the hallmarks of tissue fibrosis[22]. Western blot showed that the expression of MMP2 and MMP9 protein in the myocardium of the Model group

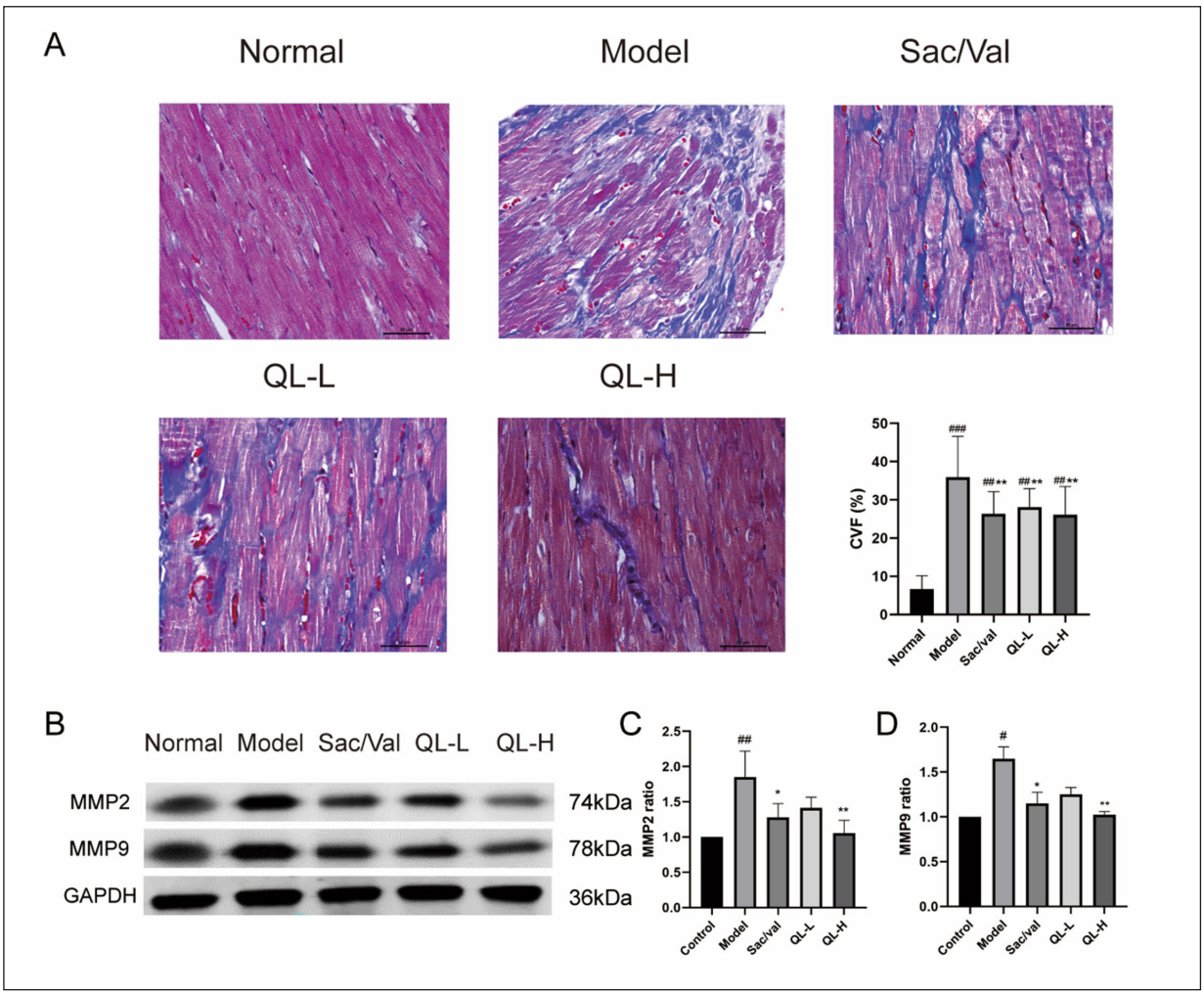

Figure 5. Changes in Masson trichrome stain and MMP2 and MMP9 proteins of myocardial tissue in each group. **A**, Representative images of Masson trichrome stain in paraffin tissue of myocardium (400×). **B-D**, MMP2 and MMP9 content were higher in the model than in the normal and QL-H group rats, indicating significant fibrosis and myocarditis. #p<0.05, ##p<0.01, *vs.* normal group; *p<0.05, ** p<0.01 *vs.* model group.

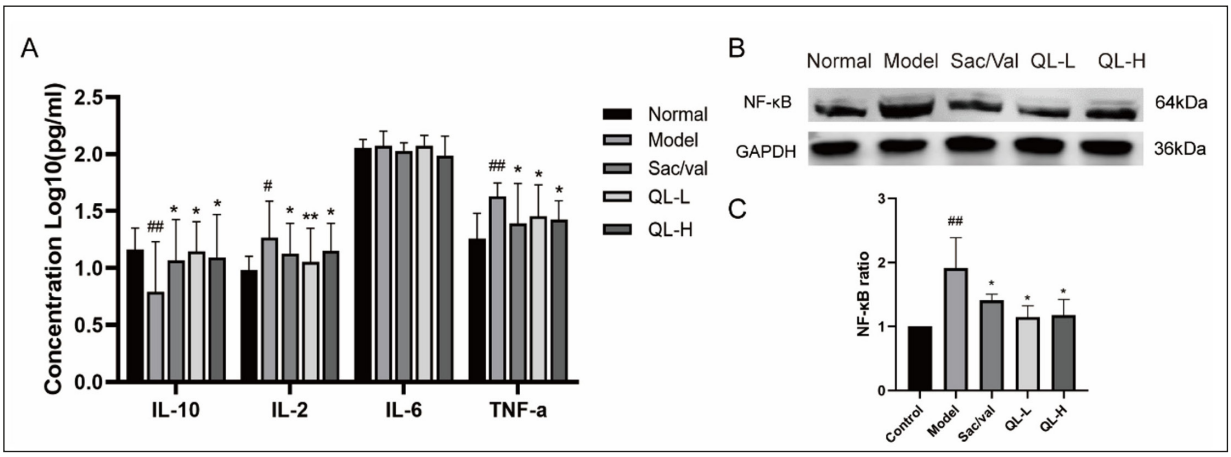

Figure 6. QL inhibits inflammation and immunocyte recruitment in HFpEF rat. **A**, QL treatment normalizes the expression of proinflammatory cytokines in serum, including IL-10, IL-2 and TNF-α, while there is no difference in IL-6 expression among all groups. **B**, the calculated NF-κB protein content in myocardial tissue. #p<0.05, ##p<0.01, *vs.* normal group; * p<0.05, ** p<0.01 *vs.* model group. All by analysis of variance.

was significantly higher than that of the Normal group. Sac/Val and QL-H groups could reduce MMP2 and MMP9 protein levels (Figure 5B-D), suggesting a reduction in fibrosis.

Effect on Serum Inflammatory Factors and Myocarditis Protein

HFpEF is associated with increased circulating cytokines, infiltration of macrophages, and other inflammatory cells in the heart[23].To determine the effect of QL in reducing cardiac inflammation and initiating an immune response to alleviate HFpEF symptoms, serum inflammatory factors detection was employed. As expected, the contents of serum TNF-α and IL-2 in the Model group were increased, whereas the IL-10 was significantly decreased compared to the Normal group. On the contrary, a significant downregulation expression of TNF-α and IL-2, while upregulation expression of IL-10 content was observed in Sac/Val and QL-L and QL-H group. There was no performance difference in IL-6 content among all groups. Furthermore, Western blot showed that the expression of inflammatory NF-κB was increased in the Model group but reversed in all the administration groups (Figure 6).

Effect of QL on the Expression of Key Proteins in Transforming Growth Factor (TGF-β1)/Smads Pathway

Activation of TGF-β1/Smads pathway plays an important role in the process of myocardial fibrosis. As shown in Figure 7, the protein expression of TGF-β1, Smad2, and Smad3 in the myocardial tissue of the Model group was significantly higher than that of the Normal group, while in each administration group, those were reduced to varying degrees (p<0.05, except Smad2 in QL-L group).

Discussion

Systemic hypertension is a causative factor in the pathogenesis and prognosis of HFpEF, which plays a key role in the occurrence and maintenance of a pro-inflammatory state, arterial stiffness, ventricular hypertrophy, myonectin-dependent stiffness, and dysfunction[24]. DOCA salt-sensitive rat model can lead to hypertension, myocardial hypertrophy, myocardial fibrosis, and diastolic dysfunction, accompanied by cardiac inflammation, hemodynamic disorder and, endothelial dysfunction[25]. This model had been considered a reliable choice for the HFpEF model with several advantages, including relatively simple preparation, a short modeling period, a high modeling rate, and low mortality. In this study, we found that QL could significantly improve cardiac function by improving the left ventricular ejection fraction and diastolic function in the DOCA salt-sensitive rat model for eight consecutive weeks. Morphological changes indicated that QL could attenuate cardiac structural remodeling by inhibiting myocardial hypotrophy and fibrosis deposition. Moreover, QL could significantly inhibit inflammatory responses by decreasing the expression of TNF-α, IL-2 and increasing the expression of IL-10.

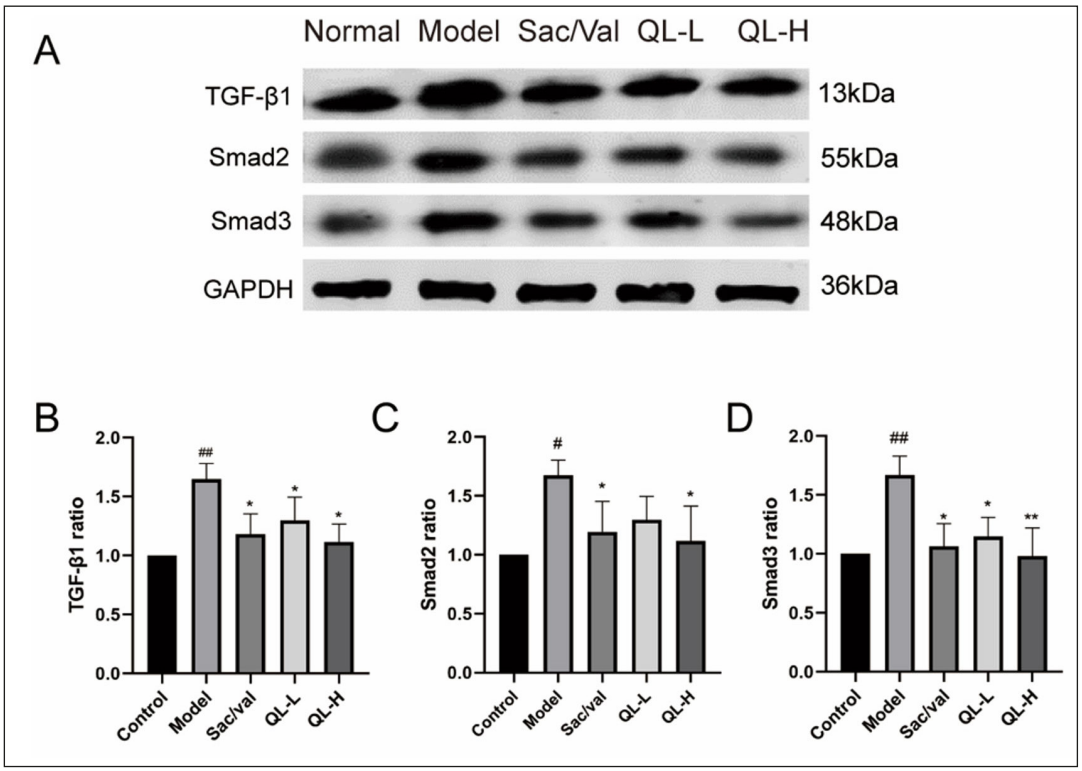

Figure 7. Expression changes of key proteins in TGF-β1/Smads pathway. **A-D**, the calculated TGF-β1, Smad2 and Smad3 protein content in myocardial tissue. #p<0.05, ##p<0.01, *vs.* normal group; * p<0.05, ** p<0.01 *vs.* model group.

To validate the therapeutic effects on HFpEF, we found that QL improved left ventricular function (especially diastolic function) and compliance using echocardiography and invasive cardiac catheterization, including the cardiac index, the ventricular remodeling indicators, and LVAW and LVPW. The level of NT-ProBNP was markedly reduced in the QL groups compared to those in the Model group (Figures 2-3). Consistently, the WGA staining results also indicated the effect of QL in attenuating cardiomyocyte hypertrophy (Figure 4). Excessive myocardial fibrosis leads to myocardial stiffness, which is the main cause of cardiac dysfunction during pressure overload[26]. Masson staining was performed to evaluate the effect of QL on myocardial fibrosis. The results showed that compared with the Normal group, the HFpEF model resulted in a significant increase in collagen deposition, and QL was effective in preventing myocardial fibrosis in DOCA rats. Myocardial collagen content is tightly regulated by the balance between collagen production and degradation. The extracellular degradation of collagen is the main limiting factor of collagen metabolism and is influenced by matrix metalloproteinases (MMPs). Collagenases (MMP 1, 8, 13) initiate collagen degradation, while gelatinases (MMP2 and MMP9) affect the digestion of collagen breakdown products (gelatin)[27], and these changes gradually impair cardiac function. We found that QL treatment reduced the expression of fibrosis indicator proteins MMP2 and MMP9 (Figure 5). All the data suggested that QL ameliorated the left ventricular diastolic function in DOCA rats by reducing ventricular stiffness and increasing left ventricular compliance.

Persistent inflammation plays a key role in the pathogenesis of chronic heart failure. Fibrogenesis is caused by too many of the same biological events occurring in the process of normal tissue repair, which is usually associated with inflammatory reactions[28]. Up-regulation of inflammatory mediators and proinflammatory cytokines leads to an activation of fibroblasts and infiltration of immune inflammatory cells, thereby triggering ventricular remodeling[29]. TNF-α is a proinflammatory cytokine released by cardiomyocytes after stress, which promotes the growth of myocardial fibroblasts and apoptosis of cardiomyocytes, leading to cardiac remodeling and dysfunction[30]. Overexpression of IL-6 and its receptor *in vivo*

can induce centripetal cardiac hypertrophy, fibrosis, and diastolic dysfunction[31]. IL-2 can enhance the activity of T lymphocytes and natural killer cells[32]. IL-10 is a pleiotropic cytokine expressed by various cells in the immune system, which can directly inhibit the expression of proinflammatory cytokines by T-helper cells and the expression of chemokines by antigen-presenting cells[33]. In this study, compared with the Model group, QL significantly decreased the expression of proinflammatory cytokines TNF-α and IL-2, and increased the expression of anti-inflammatory cytokine IL-10 in the peripheral blood of the DOCA rats (Figure 6). These results suggested that the improvement of QL in the extent of myocardial fibrosis is closely associated with a reduced inflammatory response.

NF-κB is a key inflammatory factor that can trigger the recruitment of inflammatory cells in the myocardium, thereby inducing the proliferation of myocardial fibroblasts and aggravating the deposition of myocardial collagen[34]. TGF-β1 is an important pro-fibrotic cytokine, which promotes collagen deposition by directly affecting the transformation of fibroblasts into myofibroblasts[35]. Previous studies[36] have found that QL can improve cardiac function in rats after myocardial infarction and reduce the degree of myocardial fibrosis by regulating the TGF-β1/Smad3 pathway. Meanwhile, it can reduce the myocardial immune inflammatory response and structural damage in rats with myocardial infarction through Toll-like receptor 4 (TLR4)/NF-κB signaling pathway[37], which proved QL to be the basis of anti-inflammation and anti-fibrosis. The present study confirmed that QL could significantly reduce the protein contents of NF-κB, TGF-β1, Smad2, and Smad3 in myocardial tissue, and reduce the deposition of collagen fibrin. The activation of the TGF-β1/Smad3 pathway can trigger the recruitment of inflammatory cells in the myocardium, thereby inducing the proliferation of myocardial fibroblasts, aggravating myocardial collagen deposition, inhibiting inflammation, which can delay collagen synthesis deposition and alleviate myocardial fibrosis[38]. Previous studies[17] have shown that QL could inhibit the TGF-β1/Smad3 signaling pathway and promote the TGF-β3/Smad7 signal pathway to attenuated myocardial remodeling. Therefore, it can be speculated that activating the TGF-β1/Smads pathway may be the main mechanism of QL reducing fibrosis and reducing the protective effect of myocardial hypertrophy. However, these findings are only the results of preliminary exploration, and we have not further explained how QL plays a regulatory role through this pathway, therefore, it still needs to be further explored.

Conclusions

This study investigated the effect of QL on the DOCA-induced HFpEF model and was the first attempt to explore the therapeutic effect of QL on heart disease with diastolic dysfunction. QL improved the cardiac function and inhibited the myocardial fibrosis in DOCA salt-induced rat HFpEF by improving diastolic dysfunction, preventing left ventricular hypertrophy, and ameliorating the inflammatory responses model in DOCA salt-induced rat HFpEF model. Thus, QL may serve as a new clinical therapeutic management strategy for HFpEF.

Conflict of Interest
The authors declare that they have no conflict of interest.

Authors' Contributions
Y.L. Hou designed the study. J.M. Hao and P. Sun collected the data, performed the analysis, and wrote the manuscript. Y. Zeng and H. Zhang project administration. Z.D. Zhang experiment and interpretation of data. L.P. Chang review and edit the original draft. All authors approved the final manuscript.

Ethics Approval
The study was approved by the Animal Ethics Committee of Hebei Yiling Medical Research Institute (approval No. 2020141).

Informed Consent
Not applicable.

Availability of Data and Materials
The combined datasets and materials were available upon reasonable request.

Funding
This study was supported by the Scientific Research Plan Project of Hebei Administration of Traditional Chinese Medicine (No. Z2022020), and the National Key Research and Development Program (grant No. 2017YFC1700500).

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