

Original Article

Cirsimaritin ameliorates cardiac remodeling and dysfunction through promoting myocardial autophagy in rats with heart failure

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Abstract: Cirsimaritin, a natural flavone, has been reported to exert various activities including antibacterial, anti-inflammation, anti-tumor, antioxidant, renal protection and so on. However, despite these pharmacological studies, whether cirsimaritin alleviates heart failure is still unknown. Administration of isoproterenol led to a serious heart failure, as evidenced by the up-regulation of heart rate, weight index and end diastolic pressure of left ventricular, while by the down-regulation of left ventricular systolic pressure, maximal rate of pressure rise or decline of left ventricular. Pretreatment of cirsimaritin significantly ameliorated these cardiac parameters in a dose-dependent manner. In addition, cirsimaritin remarkably inhibited serum levels of Ang II, NE, TNF- α and BNP in rats with heart failure and attenuated the cardiac histological changes. Moreover, matrix metalloproteinase-2&9 activities were also suppressed by cirsimaritin. Furthermore, myocardial autophagy was significantly promoted by cirsimaritin *in vivo* and *in vitro* through inhibiting AKT1-RPS6KB1 signaling. These findings reveal that cirsimaritin mitigates cardiac remodeling and left ventricular dysfunction through augmenting myocardial autophagy and decreasing matrix metalloproteinase-2&9 activities, suggesting its potential use in patients with congestive heart failure.

Keywords: Cirsimaritin, myocardial autophagy, cardiomyocytes, heart failure, matrix metalloproteinase

Introduction

Congestive heart failure is a resistant and severe cardiovascular disease with increasing morbidity and mortality worldwide [1, 2]. Therefore, new drug candidates and therapeutic approaches are urgently required to reduce prevalence and incidence in patients with heart failure. Usually, congestive heart failure is related to an inability of the heart to evacuate itself sufficiently, with the result that there is an ineffective work done by the heart muscle. Noteworthy, cardiac remodeling is a dynamic response of the heart to injury and contributes to the development of cardiac hypertrophy and heart failure [3]. Increasing evidence show that blockage of cardiac remodeling may be a useful therapeutic strategy for the prevention and treatment of heart failure [3-5]. Previous reports have indicated that impairment of myocardial autophagy is responsible for cardiac

remodeling [6-10]. For example, Wu *et al.* demonstrated that sustained myocardial ischemia impaired autophagy in cardiomyocyte, which was an essential mechanism against cardiac remodeling. This study also suggested that promoting autophagy might be a therapeutic approach for acute myocardial infarction [7]. In addition, Zhang *et al.* showed that berberine was effective in enhancing autophagy, and subsequently subdued cardiac remodeling and dysfunction after myocardial infarction [8]. All these studies reveal that myocardial autophagy is an excellent target for the improved drug therapy on heart failure. Therefore, inhibition of cardiac remodeling via regulating myocardial autophagy would contribute to the amelioration of heart failure.

Cirsimaritin, a natural small-compound, has been reported to exert various pharmacological activities including antibacterial, anti-inflamma-

tion, anti-tumor, antioxidant, renal protection and so on [11-16]. For instance, cirsimaritin was reported to exhibit potent antibacterial activity against *Helicobacter pylori* [11]. In addition, cirsimaritin exerted anti-inflammatory effect through inhibiting LPS-induced NF- κ B signaling in macrophages [12]. Moreover, Wang *et al.* demonstrated that cirsimaritin inhibited formyl-methionyl-leucyl-phenylalanine-induced respiratory burst in rat neutrophils mainly through the blockade of phospholipase D signaling pathway [13]. Furthermore, cirsimaritin induced apoptosis in human gallbladder carcinoma GBC-SD cells through reactive oxygen species-mediated endoplasmic reticulum stress and mitochondrial dysfunction [14]. However, the heart-protective effect and mechanism of cirsimaritin is still poorly understood. In the present study, we tried to examine whether cirsimaritin could be benefit for isoproterenol-induced heart failure in rats and explore its possible mechanism.

Materials and methods

Animal model, echocardiography and hemodynamic measurements

Male Sprague-Dawley rats (230-280 g) were selected for preparation of heart failure model using a modified isoproterenol administration method [17]. All rats that have an ejection fraction above 45% were excluded after the echocardiography examination. The remaining rats that have an ejection fraction less than 45% were randomly divided into 5 groups: control group (n = 10), metoprolol 8 mg/kg/day group (n = 8), cirsimaritin 25 mg/kg/day group (n = 8), cirsimaritin 50 mg/kg/day group (n = 8), cirsimaritin 100 mg/kg/day group (n = 8). These drugs were given *i.g.* at the specified doses for 8 weeks. Then, the animals were anesthetized for measuring hemodynamic parameters as previously reported [16]. The animals were then killed with intravenous pentobarbital sodium (100 mg/kg). The hearts were removed, weighed, and rinsed in ice-cold normal saline. Ventricles were separated from septum and weighed. Transmural samples from left ventricular were processed immediately or stored in liquid nitrogen for later analysis. All the animal experiments were approved by the Zhengzhou University Animal Care and Use Committee and made to minimize suffering and to reduce the number of animals used.

Chemicals, reagents and antibodies

Cirsimaritin (PubChem CID: 188323, purity > 98%) was purchased from Chengdu Herbpurify Co., Ltd (Chengdu, China). Isoproterenol, monodansylcadaverine (MDC) and 3-methyladenine (3-MA) were purchased from Sigma-Aldrich. Metoprolol tartarate was purchased from Astra-Zeneca. Kit for determining serum tumor necrosis factor (TNF- α) was purchased from Dakewei Co., Ltd (Beijing, China). Brain natriuretic peptide (Enzyme immunoassay goat anti-rat BNP) was purchased from Adlitteram Diagnostic Laboratories. Kit for rat norepinephrine (NE) and angiotensin II (Ang II) were purchased from RapidBio (Calabasas, California, USA). 96-well culture plates (Corning); RPMI 1640 (Invitrogen); fetal bovine serum (FBS, Invitrogen, USA). Anti-RPS6KB1 (ribosomal protein S6 kinase), anti-phospho-RPS6KB1 (Thr389), anti-RPS6/S6 ribosomal protein, anti-phospho-RPS6/S6 ribosomal protein (Ser235/236), anti-phospho-AKT1 (Ser473), anti-phospho-AKT1 (Thr308) and anti-AKT1 were purchased from Cell Signaling Technology. Anti-p62, anti-LC3 and anti-GAPDH were purchased from Santa Cruz Biotechnology. All other chemicals were purchased from Sigma-Aldrich.

Isolation of neonatal rat cardiomyocytes

Neonatal rat cardiomyocytes were prepared according to Louch *et al.* [17] using 1-day-old Sprague-Dawley rat pups. Briefly, rats are sacrificed, hearts removed, atria excised, and the ventricles then minced in Dulbecco's modified Eagle's medium (DMEM). Neonatal cardiomyocytes were isolated from ventricular tissue by 0.06% Trypsin enzymatic dissociation as previously described. After isolation cardiomyocytes were preplated for 10 min on laminin-coated 60-mm culture dishes to deplete fibroblasts and then plated at a final density of 50-500 cells/mm² on collagen-coated 6-well slides. Cardiomyocytes were cultured overnight in a 4:1 mixture of DMEM and M199 supplemented with 15% fetal bovine serum.

Real-time quantitative PCR

Real-time quantitative PCR was performed in the ABI 7500 Sequence Detection System (Applied Biosystems Inc.) as previously described [18]. The amplification was performed using the following primers (Genscript, Nanjing,

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Table 1. Cardiac morphometric and hemodynamic parameters in rats 12 weeks after isoproterenol injection treated either with normal saline, metoprolol and various doses of cirsimaritin

Parameter	Normal (n = 8)	Isoproterenol injection				
		Control (n = 10)	Metoprolol (n = 8)	CIRS-L (n = 8)	CIRS-M (n = 8)	CIRS-H (n = 8)
BW (g)	326.5 ± 19.6	306.3 ± 28.7	295.2 ± 15.4	283.7 ± 17.2	298.1 ± 19.3	296.5 ± 18.9
LVM (g)	0.71 ± 0.05	0.85 ± 0.07	0.65 ± 0.04**	0.76 ± 0.03**	0.71 ± 0.04	0.67 ± 0.04**
LVMI	2.17 ± 0.07###	2.77 ± 0.22	2.20 ± 0.16*	2.67 ± 0.15	2.38 ± 0.13*	2.26 ± 0.11**
HR (bpm)	406.7 ± 14.8###	461.2 ± 12.9	359.2 ± 10.3**	451.7 ± 13.4	417.0 ± 16.4	375.6 ± 13.5**
LVSP (mmHg)	122.4 ± 2.5###	103.6 ± 1.9	116.1 ± 2.5	107.3 ± 2.7	111.5 ± 3.2	116.8 ± 2.9**
LVEDP (mmHg)	1.8 ± 0.7##	10.4 ± 0.9	5.4 ± 0.3**	8.3 ± 0.4**	7.3 ± 0.18**	5.2 ± 0.5**
+dp/dt _{max} (mmHg/s)	3.84 ± 0.06##	2.42 ± 0.07	3.45 ± 0.05**	2.68 ± 0.06**	3.14 ± 0.08**	3.52 ± 0.07**
-dp/dt _{max} (mmHg/s)	-3.77 ± 0.04##	-1.75 ± 0.05	-2.97 ± 0.06**	-2.46 ± 0.03**	-2.79 ± 0.1**	-3.06 ± 0.05**

BW = body weight; LVM = left ventricular weight; LVMI = left ventricular weight index; HR = heart rate; bpm = beats per minute; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end diastolic pressure; dp/dt_{max} = maximal rate of LV pressure rise; dp/dt_{min} = maximal rate of LV pressure decline. Normal: normal rats without isoproterenol injection; Control: model rats treated with normal saline after isoproterenol injection; Metoprolol: model rats treated with 8 mg/kg of metoprolol after isoproterenol injection; CIRS-L, CIRS-M and CIRS-H: model rats treated with 25 mg/kg, 50 mg/kg and 100 mg/kg of cirsimaritin (CIRS) after isoproterenol injection, respectively. Values are mean ± SEM. ##P < 0.01 vs. control (Student's t test); *P < 0.05, **P < 0.01 vs. control (Dunnett's test).

China): GAPDH 5'-TATCGGACGCCTGGTTAC and 3'-CGTTCAAGTTGCCGTGTC, MMP-2 5'-GGAG-GCACGATTGGTCTG and 3'-TCTGGTACGCCTTT-GGTT, MMP-9 5'-CTGCGTATTTCCATTCATC and 3'-GAGATTTGGACTGGGTTCC.

Gelatin zymography assay

Gelatin zymography assay for matrix metalloproteinase (MMP)-2&9 was performed as previously described [18].

Echocardiography

Echocardiography was performed as previously reported [19]. Ultrasound examinations were performed with GE System Five equipment with a 3.5 Hz sector transducer. American Society of Echocardiography guidelines were applied for registering all 2-D and conventional Doppler variables. Analyses were performed off-line using Echopac. The average of three consecutive heartbeats was used.

Western blot

Protein used in western blot was isolated from frozen left ventricular tissue. About 30 µg of protein was loaded on 10% SDS-PAGE. Proteins were transferred to a polyvinylidene difluoride membrane. The membrane was then incubated in 5% dry milk with PBS/0.05% Tween 20 for 1 h at room temperature and subsequently exposed to the indicated antibodies overnight at 4°C. The next day the membrane was wash-

ed and incubated with peroxidase conjugated secondary antibody for 2 h at room temperature. Then the membrane was washed and incubated with LumiGLO® Reagent and Peroxide (Cell Signaling Technology), and was visualized with X-ray films.

Statistical analysis

Results are shown as means ± SEM. The data were statistically evaluated using one-way ANOVA followed by Student's t test when only two value sets were compared, and Dunnett's test when the data involved three or more groups. P < 0.05 was considered to be significant.

Results

Cirsimaritin ameliorated cardiac remodeling, dysfunction and inflammation in rat model of heart failure

Administration of isoproterenol led to a severe heart failure, as shown by the increased levels of left ventricular weight index (LVWI), heart rate (HR), as well as LV end diastolic pressure (LVEDP), while by the decreased levels of LV systolic pressure (LVSP), maximal rate of LV pressure rise (dp/dt_{max}), and maximal rate of LV pressure decline (dp/dt_{min}) (Table 1). Against these, cirsimaritin significantly reversed the changes of these cardiac morphometric parameters and hemodynamic parameters in a dose-dependent manner.

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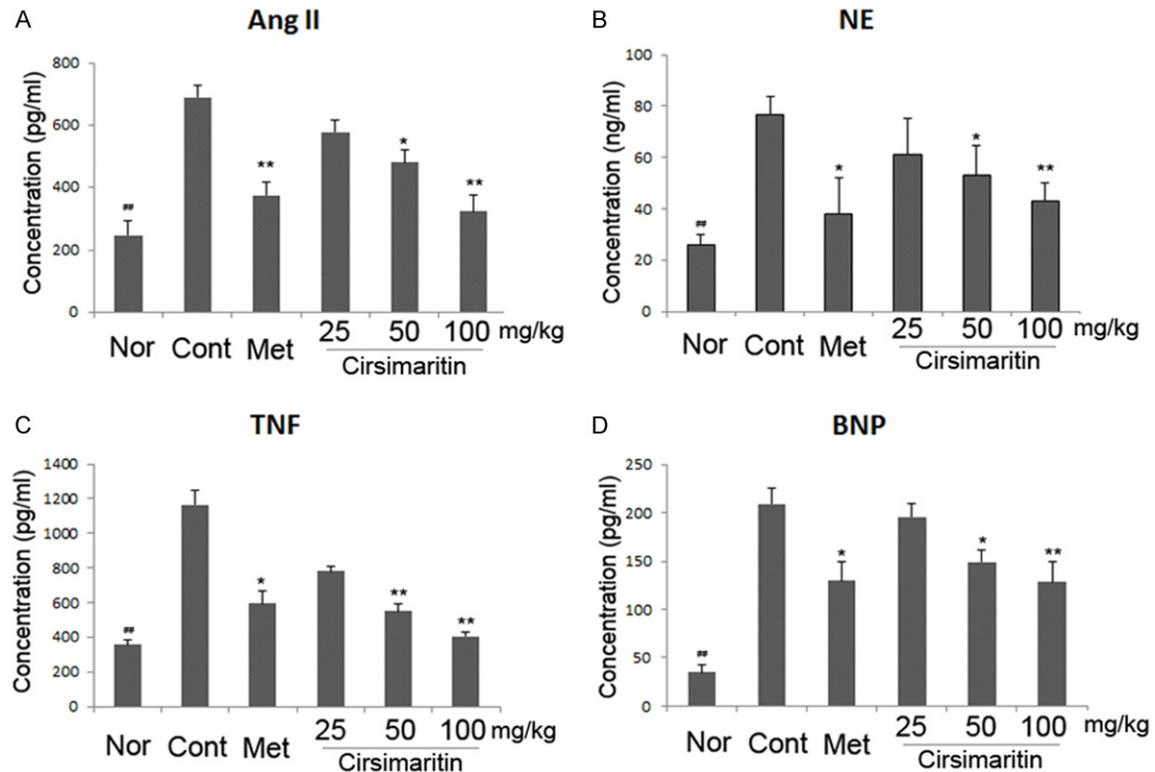


Figure 1. Plasma cytokines parameters in rats 12 weeks after isoproterenol injection treated either with normal saline, metoprolol and various doses of cirsimaritin. Nor group: normal rats without isoproterenol injection; Cont group: model rats treated with normal saline after isoproterenol injection; Met group: model rats treated with 8 mg/kg of metoprolol after isoproterenol injection; Cirsimaritin-group: model rats treated with 25 mg/kg, 50 mg/kg and 100 mg/kg of cirsimaritin after isoproterenol injection, respectively. Values are mean \pm SEM. $^{**}P < 0.01$ vs. control (Student's *t* test); $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control (Dunnett's test). Ang II = angiotensin II; NE = norepinephrine; TNF- α = tumor necrosis factor α ; BNP = brain natriuretic peptide.

Pro-inflammatory cytokines contributes to the development of cardiac remodeling and dysfunction. Thus we examined the effect of cirsimaritin on the levels of pro-inflammatory cytokines in rats with heart failure. Cirsimaritin significantly inhibited the serum levels of Ang II, NE, BNP and TNF- α in a dose-dependent manner (**Figure 1**). In addition, cirsimaritin remarkably ameliorated the histological changes including cardiocyte degeneration, cardiocyte hypertrophy, cardiac desmoplasia and inflammatory infiltration (**Figure 2**). Using echocardiography analysis, we found that the ejection fraction (EF, %) of rats with isoproterenol-treated group significantly decreased compared with that of normal rats. Against this, cirsimaritin remarkably improved these echocardiography changes (**Figure 3**). This finding suggests that cirsimaritin alleviates cardiac remodeling and dysfunction.

Cirsimaritin inhibited the expressions and activities of MMP-2&9 in rat model of heart failure

As shown in **Figure 4A**, the mRNA expressions of MMP-2&9 were markedly increased in rat model of heart failure induced by isoproterenol. Against this, cirsimaritin dose-dependently suppressed MMP-2&9 mRNA expressions as revealed by real-time quantitative PCR (**Figure 4A**). In addition, using gelatin zymography assay we confirmed that the activities of MMP-2&9 were also inhibited by cirsimaritin (**Figure 4B**).

Cirsimaritin promoted myocardial autophagy in rat model of heart failure

To find out whether autophagy was associated with the cirsimaritin-mediated protection from rat model of heart failure, we examined the

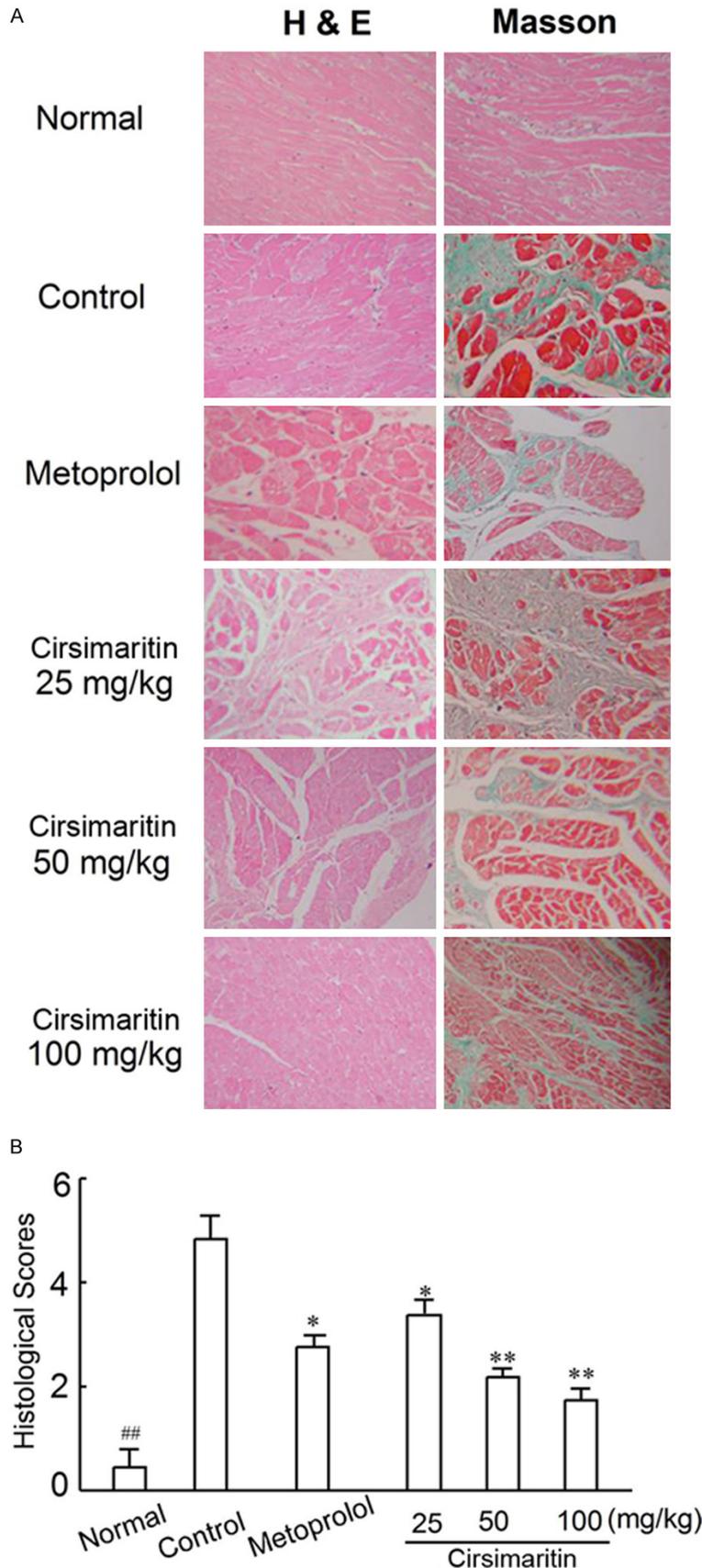


Figure 2. Effect of cirsimaritin on histopathological changes of the left ventricular remodeling in rats with heart failure caused by isoproterenol. A. Hematoxylin & Eosin (H&E) and masson staining (original magnification $\times 100$). Normal: normal rats; Control: model rats treated with normal saline after isoproterenol injection; Metoprolol: model rats treated with metoprolol after isoproterenol injection; Cirsimaritin: model rats treated with cirsimaritin after isoproterenol injection. B. Histologic scoring of left ventricular. Each column indicates the mean \pm SEM of 8 rats. ## $P < 0.01$ vs. control (Student's t test); * $P < 0.05$, ** $P < 0.01$ vs. control (Dunnett's test).

level of myocardial autophagy *in vivo*. We first examined the level of LC3-II, which is a general autophagy marker, in the left ventricular in rats with heart failure. Compared with control group, there was evident increase in LC3-conversion in cirsimaritin-treated group (Figure 5A, 5B). These results suggest myocardial autophagy can be triggered by cirsimaritin treatment in rats with heart failure.

Cirsimaritin enhanced autophagy in neonatal cardiomyocytes through inhibiting AKT1-RPS6KB1 signaling

To further determine the mechanism of cirsimaritin on autophagy, primary neonatal rat cardiomyocytes were used in this study. As shown in Figure 6A, LC3-I to LC3-II-conversion markedly down-regulated in isoproterenol-treated cardiomyocytes, compared with vehicle group. Against this, cirsimaritin significantly increased LC3-I to LC3-II-conversion in a dose-dependent

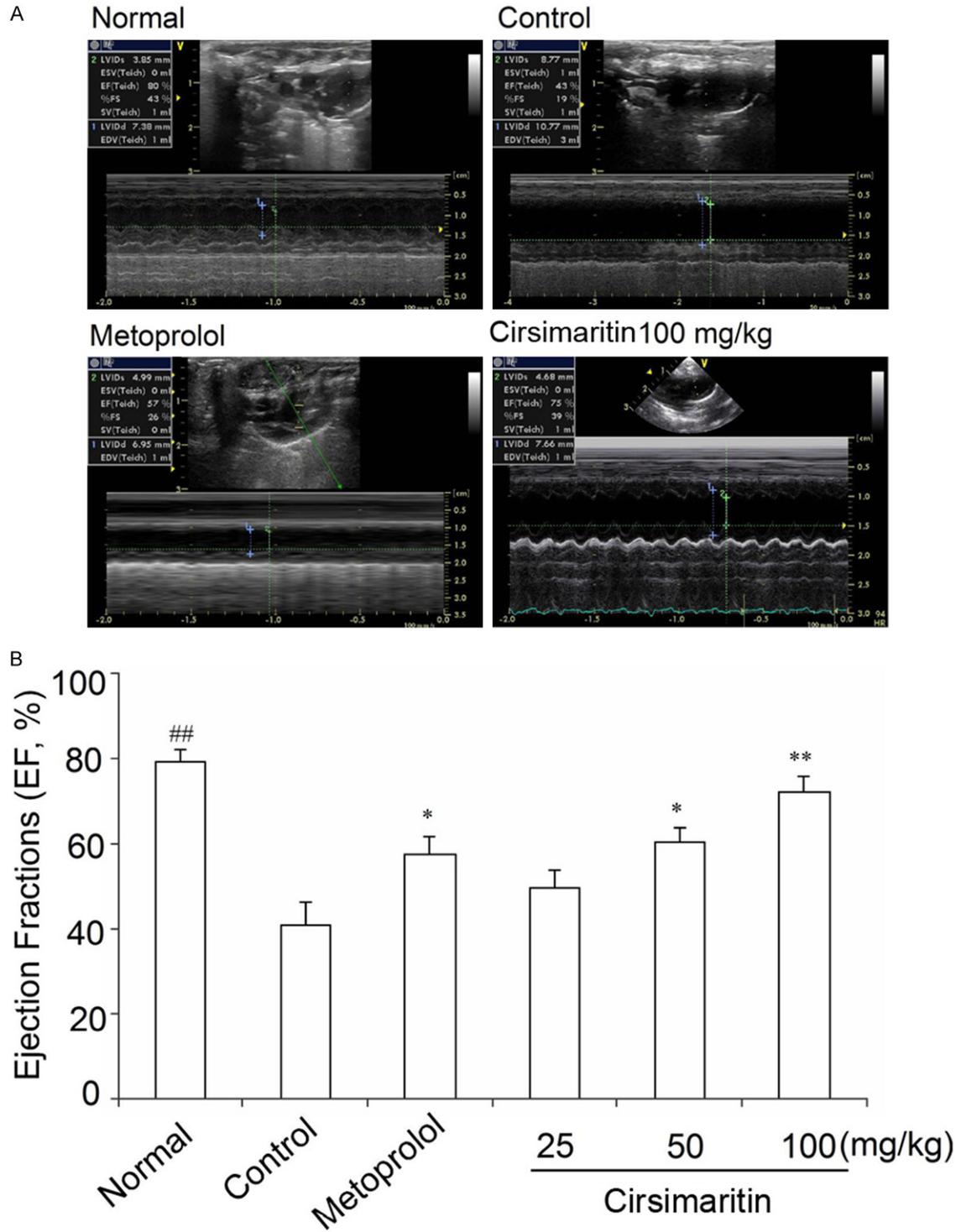


Figure 3. Effect of cirsimaritin on echocardiography parameters of the left ventricular in rats with heart failure. A. A representative of echocardiography photo. B. Ejection fraction (EF) scoring. Each column indicates the mean \pm SEM of 8 rats. $##P < 0.01$ vs. control (Student's *t* test); $*P < 0.05$, $**P < 0.01$ vs. control (Dunnett's test).

manner. Moreover, we found that the expression level of p62 was remarkably decreased by cirsimaritin (Figure 6A).

MDC is another specific marker for autolysosome formation. We found that cells treated with cirsimaritin showed an increase of MDC

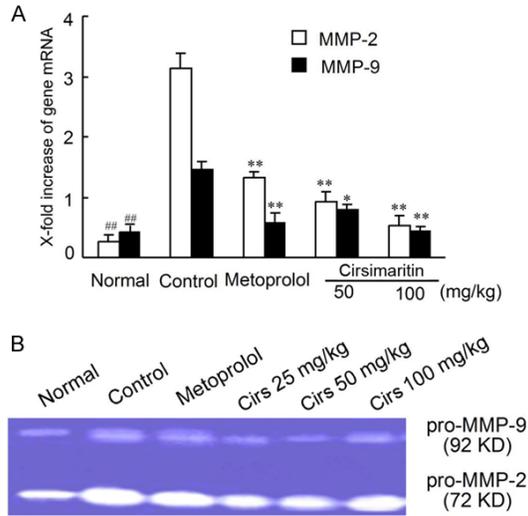


Figure 4. Effect of cirsimaritin on the mRNA expression and activities of MMP-2&9 of the left ventricular in rats with heart failure. A. MMP-2&9 mRNA expressions of the left ventricular in rats with heart failure were examined by real-time PCR. Each column represents the mean \pm SEM of three independent experiments and each experiment includes triplicate sets. $###P < 0.01$ vs. control (Student's *t* test); $*P < 0.05$, $**P < 0.01$ vs. control (Dunnett's test). B. MMP-2&9 activities were examined by gelatin zymography assay. The graph shown here is one of three different experiments.

accumulation, suggesting autophagy is promoted by cirsimaritin (Figure 6B). Correspondingly, MDC incorporation was also inhibited by autophagy inhibitor 3-MA (Figure 6B). In addition, transmission electron microscopy was also used to confirm our findings. We observed a significant accumulation of numerous lamellar structures and double-membraned cytosolic autophagic vacuoles in cardiomyocytes after cirsimaritin treatment (Figure 6C).

It is well-known that AKT1-ribosomal protein S6 kinase, 70 kDa, polypeptide 1 (RPS6KB1) signaling pathway is involved in the regulation of autophagy. Then we examined the related protein expression levels in cardiomyocytes using western blotting. As shown in Figure 7A, cirsimaritin also inhibited AKT1 phosphorylation at Ser473 and Thr308 in a dose-dependent manner. Moreover, after the treatment with cirsimaritin, there was a dose-dependent decrease in the expressions of phosphorylated RPS6KB1 and RPS6 (ribosomal protein S6) (Figure 7B). These results suggest that cirsimaritin induces myocardial autophagy in car-

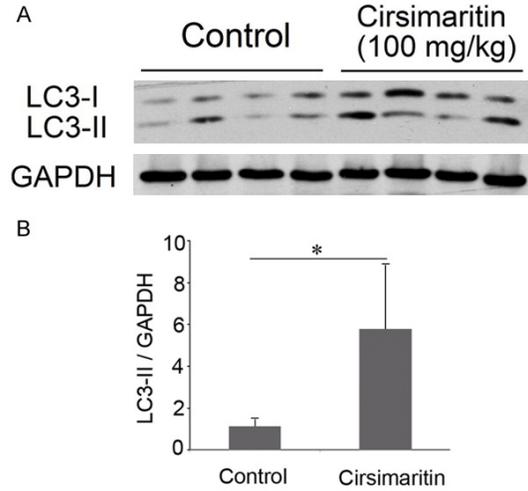


Figure 5. Effect of cirsimaritin on myocardial autophagy of the left ventricular in rats with heart failure. A. The expression of LC3 in the left ventricular in rats with heart failure was examined by western blotting. The results shown are representative of three experiments. B. Each column represents the mean \pm SEM of six rats. $*P < 0.05$.

diomyocytes through inhibiting AKT1-RPS6KB1 signaling pathway.

Discussion

Cardiac remodeling is basically considered as a critical factor for the development of heart failure, which is a dynamic transformation of impaired heart to the damaged signals. Previous report shows that targeting cardiac remodeling may be a useful therapeutic strategy for the prevention of heart failure [20]. Cirsimaritin, a natural flavonoid, was reported to exert various pharmacological activities including antibacterial, anti-inflammation, anti-tumor, antioxidant, renal protection and so on. However, how it exerts protective role in heart failure remains unclear. Herein, we first examined the effect of cirsimaritin in rat model with heart failure. The results showed that cirsimaritin significantly ameliorated cardiac remodeling and dysfunction through enhancing myocardial autophagy and decreasing matrix metalloproteinase-2&9 activities (Figure 8).

Rat model with heart failure caused by isoproterenol is considered as an ideal model for heart failure. Both cardiac remodeling and dysfunction exert an important role in the development of heart failure. It should be noted that

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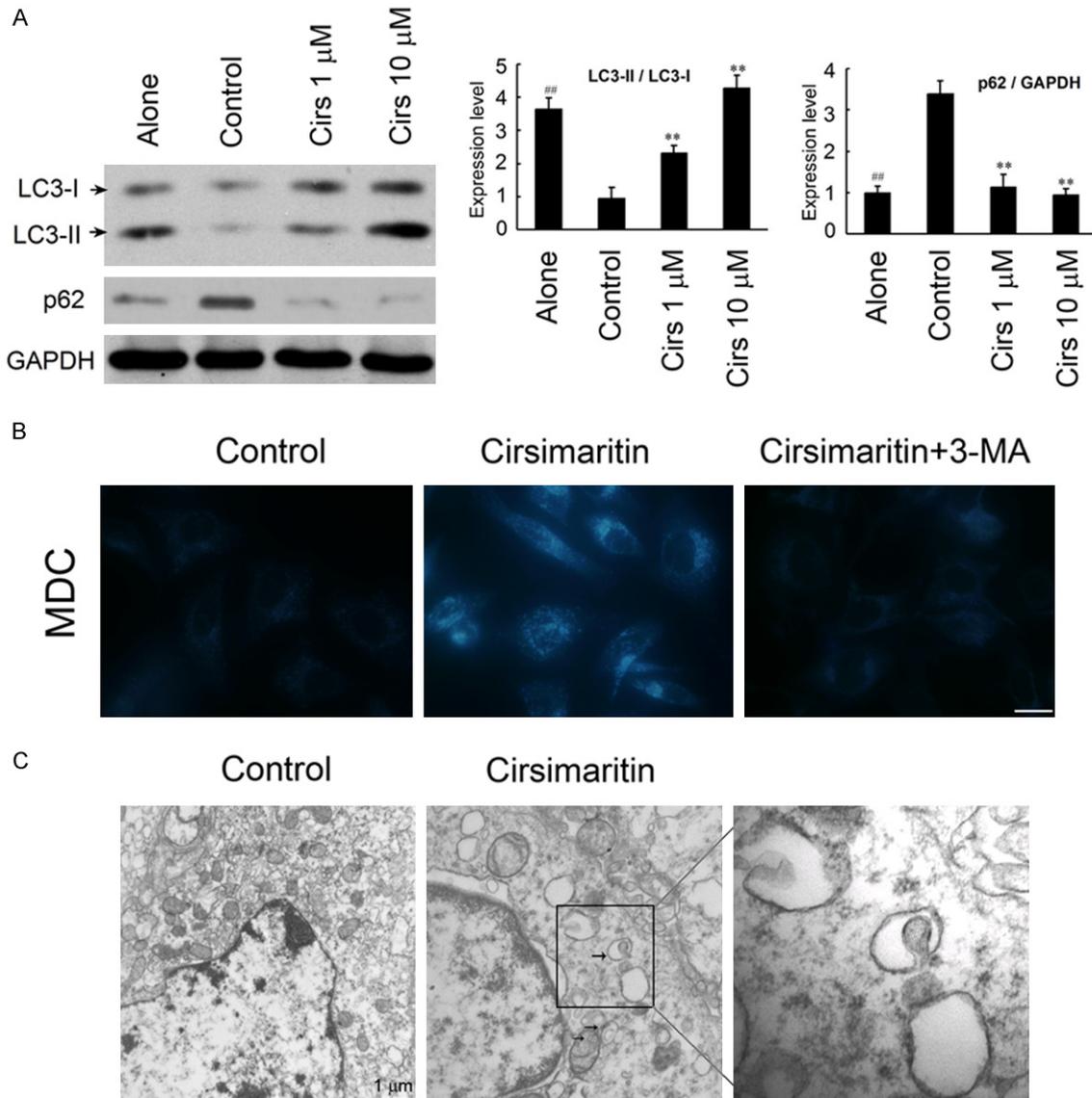


Figure 6. Effect of cirsimaritin on autophagy of neonatal cardiomyocytes. **A.** Neonatal cardiomyocytes were isolated and treated with 1 μ M isoproterenol in the presence or absence of different concentrations of cirsimaritin for 24 h. Then the expressions of LC3 and p62 were examined by western blotting. Each column represents the mean \pm SEM of three independent experiments. ^{##}P < 0.01 vs. control (Student's t test); *P < 0.05, **P < 0.01 vs. control (Dunnett's test). **B.** Neonatal cardiomyocytes were treated with 1 μ M isoproterenol in the presence or absence of 10 μ M cirsimaritin or 2 mM 3-MA for 24 h, and then incubated with 0.05 mM monodansylcadaverine (MDC) for 10 min. Cells were then analyzed by fluorescence microscopy. Scale bar: 5 μ m. **C.** The transmission electron microscopy imaging of cells showing numerous double-membraned cytoplasmic vacuolation (arrows) in 10 μ M cirsimaritin-treated neonatal cardiomyocytes for 24 h. The results shown are representative of three different experiments.

isoproterenol-triggered heart failure led to the remarkable elevation of many pro-inflammatory cytokines including Ang II, NE, BNP and TNF- α , which account for the process of cardiac remodeling [21-24]. Consistent with the results from hemodynamic and histological assay, cirsimaritin markedly reduced all levels of Ang II, NE, BNP and TNF- α in a dose-dependent man-

ner. These findings suggest that cirsimaritin can attenuate inflammation in cardiac dysfunction.

Autophagy misregulation has been causally related to the pathomechanism of cardiac remodeling [25, 26]. Autophagy is impaired during left ventricular hypertrophy and linked to

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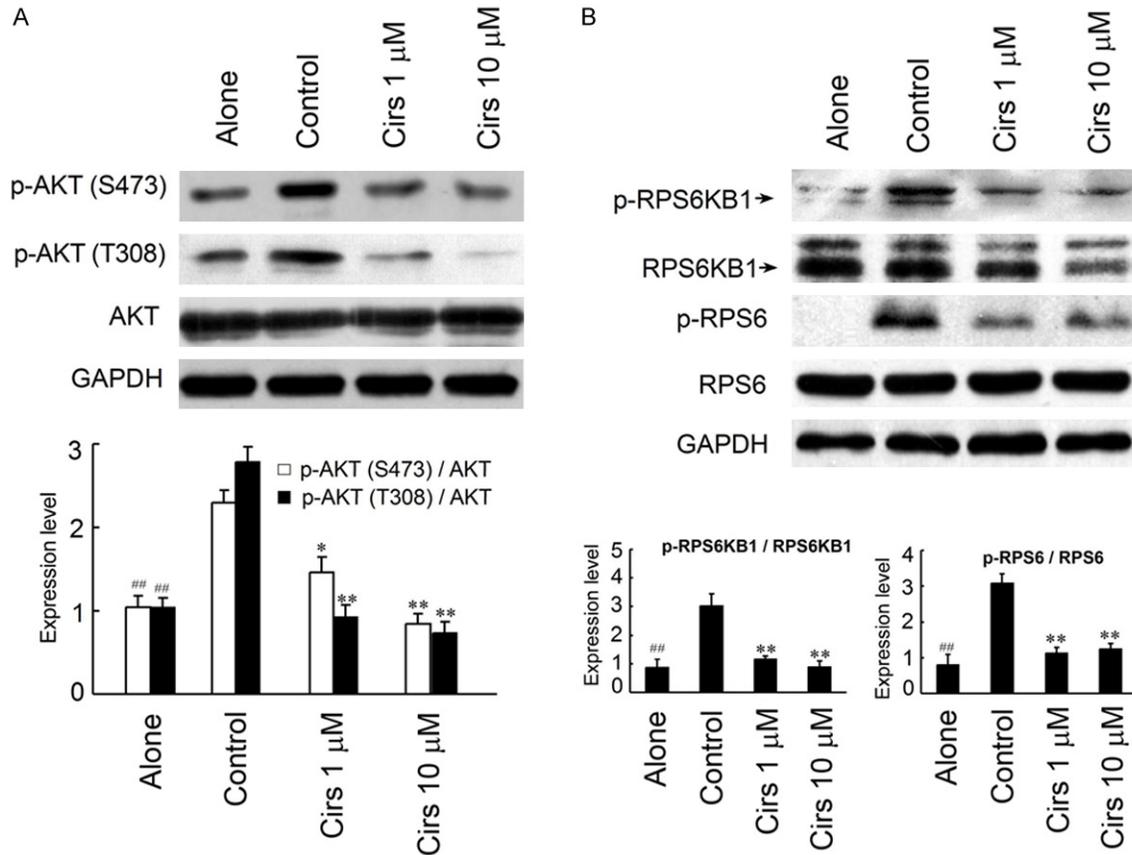


Figure 7. Effect of cirsimaritin on AKT1-RPS6KB1 signaling in neonatal cardiomyocytes. Neonatal cardiomyocytes were isolated and treated with 1 μ M isoproterenol in the presence or absence of different concentrations of cirsimaritin for 24 h. Then the expressions of AKT1, phosphor-AKT1 (A), RPS6KB1, phosphor-RPS6KB1, RPS6, phosphor-RPS6 (B) in neonatal cardiomyocytes were examined by western blotting. The results shown are representative of three independent experiments. Each column represents the mean \pm SEM of three independent experiments. ^{##} $P < 0.01$ vs. control (Student's *t* test); ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs. control (Dunnett's test).

the pathological issue of cardiac function. Previous reports have demonstrated that dysfunction of myocardial autophagy plays a critical role in cardiac remodeling and heart failure [25-29]. Our findings suggest that cirsimaritin ameliorates cardiac remodeling and left ventricular dysfunction, but whether it modulates autophagy in the myocardium is unclear. The result from **Figures 5** and **6** showed that cirsimaritin remarkably increased LC3-I to LC3-II-conversion, as well as decreased p62 expression *in vivo* and *in vitro*, suggesting impaired myocardial autophagy is reversed by the treatment of cirsimaritin. AKT1-RPS6KB1 signaling pathway has been reported to play critical role in the regulation of autophagy [30]. In this study, we found that phosphorylated RPS6KB1, phosphorylated RPS6 as well as phosphorylated AKT1 were all down-regulated by the treatment of cirsimaritin in a dose-dependent man-

ner (**Figure 7**), compared with the total expressions of the three proteins in neonatal cardiomyocytes. These findings suggested that the mechanism of cirsimaritin's action on autophagy was related to the inhibition of AKT1-RPS6KB1 signaling pathway. Still the detail mechanism for how cirsimaritin reinforces autophagy need to be explored in the further study.

Matrix metalloproteinases (MMPs) play an essential role in regulating extracellular matrix turnover and have recently been associated with myocardial injury [31]. Increasing evidence reveal that MMPs were significantly up-regulated in hypertensive rats, in rats with progressive heart failure [32], most importantly, in patients with heart failure [33]. Some scholars reported that pharmacological inhibition of MMPs led to a good readout in animal models of heart

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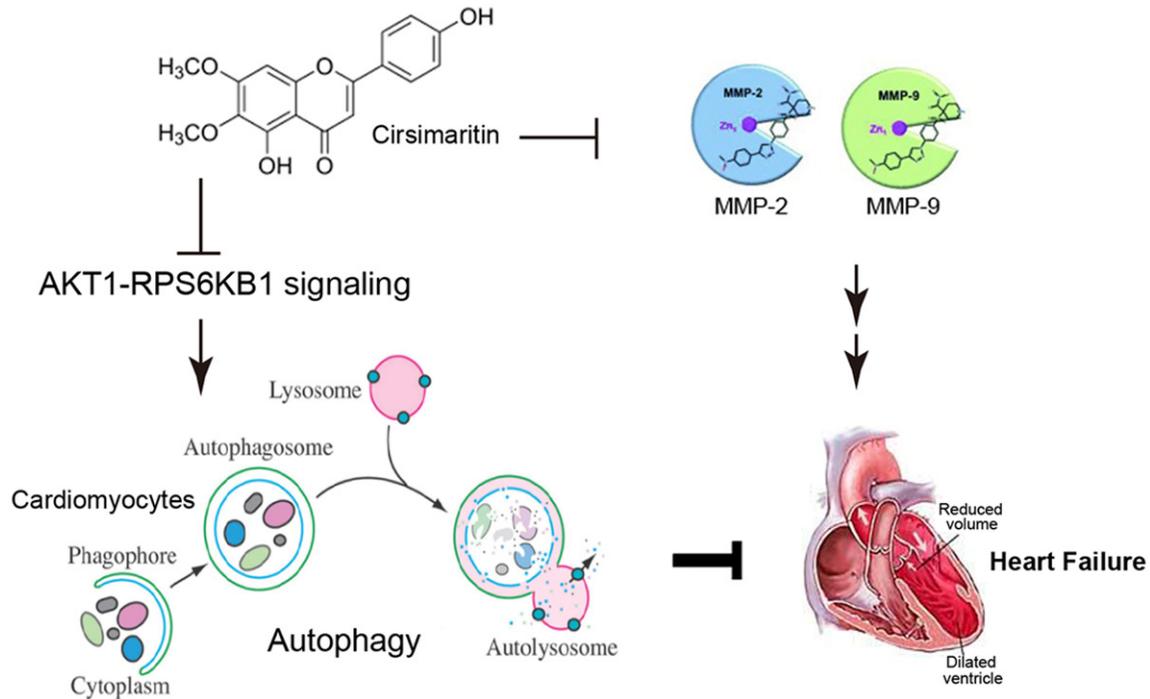


Figure 8. The graphic illustration for the mechanism of cirsimaritin ameliorating heart failure in rats. Cirsimaritin mitigates cardiac remodeling and dysfunction via augmenting myocardial autophagy through inhibiting AKT1-RPS6KB1 signaling pathway. In addition, cirsimaritin also represses the expressions and activities of MMP-2&9, which contributes to the amelioration of heart failure in rats.

failure [34]. These findings collectively support that MMPs positively regulate cardiac remodeling. Taken together, considering the expressions and activities of MMP-2&9 were also inhibited by cirsimaritin in heart tissue (**Figure 4**), therefore, it is conceivable to assume that cirsimaritin exerts inhibitory effect on MMP-2&9, leading to the prevention of cardiac remodeling.

Conclusion

Cirsimaritin significantly ameliorates cardiac remodeling and left ventricular dysfunction in rat model with heart failure. The mechanism of cirsimaritin may be mediated at least in part by enhancement of myocardial autophagy as well as inhibition of elevated MMP-2&9 activities. Our findings suggest cirsimaritin is a potential drug candidate in patients with congestive heart failure.

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Disclosure of conflict of interest

None.

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