doi.org/10.36721/PJPS.2026.39.9.251.1

Jianxin granules enhance autophagic flux and reduce apoptosis in heart failure *via* mTOR pathway: A preclinical investigation

Jun Chen*, Yong Wang, Feixiang Huang, Yirong Chen and Jinjian Guo*
The Second Affiliated Hospital of Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian, China

Abstract: **Background**: Heart failure (HF) is a leading cardiovascular disease worldwide. Jianxin Granule, a traditional Chinese medicine formula, has been clinically shown to improve cardiac function, yet its molecular mechanism remains unclear. Autophagy is crucial for maintaining cardiac homeostasis, and the mTOR pathway is a central negative regulator of autophagy. **Objectives**: To investigate whether Jianxin Granule confers cardioprotection in HF by modulating mTOR signaling to enhance autophagy and suppress apoptosis. Both *in vivo* and in vitro experiments were performed. **Methods**: In male Sprague-Dawley rats HF model and in H9C2 cardiomyocytes exposed to H_2O_2, autophagy-related proteins and apoptotic markers were assessed by Western blotting and qRT-PCR. The specific role of mTOR signaling was further examined in cardiomyocytes transfected with mTOR-siRNA. **Results**: Jianxin Granule markedly increased autophagic flux and reduced apoptosis in HF rats and these effects were attenuated by the autophagy inhibitor 3-methyladenine (3-MA). Consistent findings were observed in H_2O_2-injured H9C2 cells, where Jianxin Granule promoted autophagy and decreased apoptosis. In mTOR-siRNA–transfected cells, Jianxin Granule further enhanced autophagic flux and diminished apoptosis, supporting the involvement of mTOR signaling in its protective mechanism. **Conclusion**: Jianxin Granule protects against HF by regulating the mTOR pathway, thereby boosting autophagic flux and reducing cardiomyocyte apoptosis. These results provide mechanistic support for its clinical application in heart failure.

Keywords: Autophagic flux; Heart failure; Jianxin granules; mTOR

Submitted on 10-09-2025 – Revised on 09-01-2026 – Accepted on 26-01-2026

INTRODUCTION

Heart failure (HF) stands as one of the most severe cardiovascular diseases of the 21st century, characterized by impaired ventricular filling or ejection function, leading to high morbidity and significantly increased hospitalization and mortality rates (Savarese *et al*., 2023). With an aging population and the rising prevalence of risk factors such as hypertension, diabetes, dyslipidemia and obesity, the incidence of HF continues to climb (Khan *et al*., 2024). While early targeted treatments can delay HF progression, the molecular mechanisms underlying HF remain incompletely understood and effective therapeutic strategies are still limited. Investigating the therapeutic pathways associated with HF is essential for advancing clinical treatment.

Traditional Chinese Medicine (TCM) has accumulated substantial clinical experience in treating HF, particularly in alleviating symptoms and improving quality of life. Jianxin Granules, developed by renowned TCM practitioners, are a proprietary formulation at The Second Affiliated Hospital of Fujian University of Traditional Chinese Medicine institution. Extensive clinical studies have demonstrated that Jianxin Granules significantly improve cardiac function (Ouyang *et al*., 2025; Yongzhong *et al*., 2024). Due to the multi-target nature of TCM formulations, elucidating the mechanisms of Jianxin Granules can provide a more comprehensive theoretical basis for their development and clinical application.

The etiology of HF is complex, with myocardial hypertrophy often preceding HF—a hallmark of cardiac remodeling. Left ventricular remodeling leads to systolic and diastolic dysfunction and under sustained pressure, cardiac function gradually deteriorates, ultimately resulting in HF (Tham *et al*., 2015). Recent studies suggest that autophagy plays a crucial role in maintaining cardiac homeostasis (Miyamoto 2019; Sciarretta *et al*., 2018). Autophagy, an intracellular degradation process, delivers cytoplasmic components to lysosomes for degradation and recycling, promoting organelle turnover. Under normal conditions, there is a basal level of autophagy; under stress conditions, autophagy is induced to degrade dysfunctional organelles and proteins, preventing cell death and protecting cells from oxidative stress damage (Ajoolabady *et al.*, 2022).

Autophagy regulation involves multiple mechanisms, with the serine/threonine protein kinase mTOR (Mammalian Target of Rapamycin) being a pivotal node. mTOR, a highly conserved protein, is considered a negative regulator of autophagy. It inhibits autophagy initiation by phosphorylating Atg13, thereby preventing the formation of the ULK (Unc-51-like kinase) complex (Y. Wang & Zhang, 2019). Rapamycin, an inhibitor of mTOR complex

**Corresponding author:* e-mail: eguojj@163.com; cjun202505@163.com
#These authors contributed equally and are the co-first authors.

1 (mTORC1), can promote autophagy, thereby enhancing cell survival and providing cardioprotective effects (Gao *et al*., 2020). The PI3K (PhosphoInositide-3 Kinase) complex (comprising Beclin-1, Atg14L, Vps34, and Vps15) is also critical for autophagy initiation and is regulated by the ULK complex. Inhibitors of mTOR and its downstream target p70S6K (70 kDa ribosomal protein S6 kinase) can reduce myocardial apoptosis post-infarction, mitigating ischemic injur (Dai *et al.*, 2021; Jin *et al.*, 2020). Moreover, there is extensive crosstalk between autophagy and apoptosis as they share certain signaling pathways in adult cardiomyocytes. Physiological activation of normal autophagy can protect cells from apoptotic death (Dong *et al*., 2019).

Recent years have seen significant advances in pharmacological research on TCM-based autophagy pathways. An increasing number of herbal components have been found to regulate autophagy, each with distinct mechanisms of action. Given the multi-component nature of TCM formulations, each typically containing numerous bioactive constituents, the downstream molecular networks they regulate are highly complex, necessitating precise research into their mechanisms of influencing autophagy (Chen *et al*., 2023; Wang *et al*., 2023).

This study investigates the effects of Jianxin Granules on myocardial autophagy using *in-vivo* and *in-vitro* experiments, aiming to elucidate their regulatory role and mechanism via the mTOR pathway. This will contribute to a deeper understanding of the molecular mechanisms underlying Jianxin Granules' treatment of HF, providing new insights into their pharmacological mechanisms and providing theoretical support for their clinical application. In summary, Jianxin Granules, a traditional Chinese medicine compound, has shown significant efficacy in treating HF. As multi-component formulations, they exhibit a "multi-component, multi-target" action, though their precise mechanisms remain somewhat unclear and require further investigation. Clarifying their specific targets in heart failure treatment can provide a molecular-level scientific explanation for their cardioprotective effects, thereby promoting the modernization and internationalization of traditional Chinese medicine.

Autophagy, a crucial process for maintaining cellular homeostasis, is closely linked to the development of heart failure when dysfunctional, yet its specific regulatory network remains poorly understood. This study focuses on the mTOR pathway, a core negative regulatory node of autophagy. The mTOR pathway is considered to hold promise for deepening the understanding of the pathological mechanisms of heart failure and providing a basis for discovering new drug intervention targets. The TAC-induced heart failure rat model mimics pressure overload-induced cardiac remodeling and dysfunction observed in human heart failure and is therefore widely used for mechanistic and therapeutic studies.

MATERIALS AND METHODS

Animal experimentation

Experimental subjects and anesthesia

Adult male Sprague-Dawley (SD, SPF grade, healthy, immunocompetent, wild-type, not genetically modified, no prior procedures) rats (SD, Shanghai Slek Laboratory Animal Co., LTD, Shanghai, China), each weighing between 250-300 grams, aged 4-6 weeks, were selected as the experimental subjects. Before surgery, the animals were acclimated for 1 week under standard laboratory conditions with a 12-hour light/dark cycle, a controlled temperature (22-24°C), and free access to food and water. Anesthesia was administered via intraperitoneal injection of 1% sodium pentobarbital (P11011, Beijing Equation Biotechnology Co., Ltd, Beijing, China) at a dose of 50 mg/kg to ensure deep anesthesia, confirmed by lack of response to paw pinch (Xia *et al.*, 2023).

Induction of heart failure

HF was induced through transverse aortic constriction (TAC) surgery. Under sterile conditions, a horizontal skin incision was made at the level of the suprasternal notch, and the thoracic cavity was accessed by blunt dissection. The transverse aorta was isolated, and a 27-gauge needle was placed adjacent to the aorta. A 5-0 silk suture was tied around the aorta and the needle, which was then quickly removed to create a constriction. Sham-operated rats underwent the same procedure without aortic constriction (Farag *et al*., 2023).

Post-surgical care and grouping

Post-surgery, all animals received analgesia and were monitored until recovery. The rats were then divided into five groups: sham-operated, model, positive drug (an established efficacy comparator), Jianxin granule intervention and Jianxin granule (Z20100010, The Second Affiliated Hospital of Fujian University of Traditional Chinese Medicine, Fujian, China) with 3-Methyladenine (3-MA, an autophagy inhibitor) (M9281-100MG, Sigma, Michigan, USA). There were 8 rats in each group with 5 groups totaling 40 rats. Each group received daily oral gavage treatment for 28 days, while control and model groups received equivalent volumes of saline (Yongzhong *et al*., 2024). The formulation of Jianxin Granules includes Astragalus membranaceus, Typhae pollen, Salvia miltiorrhiza, Atractylodes macrocephala, Cinnamomum cassia twig, Descurainia semen, Red ginseng and Polyporus umbellatus."

Serum biochemical analysis

After the treatment period, blood samples (n=8) were collected from the abdominal aorta under anesthesia and centrifuged at 3000 rpm for 10 minutes to separate serum. Serum levels of brain natriuretic peptide (BNP) (E-EL-R0126, ELABS, Wuhan, China), high-sensitivity C-reactive protein (CRP) (ml002999, MlBio, Shanghai,

China), renin, angiotensin II (Ang II) (mlswE2093, MlBio, Shanghai, China), aldosterone (ALD) (mlsw_E3597, MlBio, Shanghai, China), angiotensin II receptor 1 (AT1R) (ml106845, MlBio, Shanghai, China), angiotensin II receptor 2 (AT2R) (mlE1942, MlBio, Shanghai, China) and angiotensin-converting enzyme 2 (ACE2) (ER0609, FineTest, Wuhan, China) were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Bellagambi *et al*., 2021).

Protein extraction and quantification

Myocardial tissues or H9C2 cardiomyocytes (n=3) (YB-73089RC, YBio, Shanghai, China) were lysed in RIPA buffer (89900, Thermo Scientific, Massachusetts, USA) containing protease and phosphatase inhibitors on ice for 30 minutes with vortexing every 5 minutes. The lysates were centrifuged at 12,000 rpm for 10 minutes at 4°C to remove debris. Protein concentration was quantified using a BCA protein assay kit (23227, Thermo Scientific, Massachusetts, USA) according to the manufacturer's protocol.

Electrophoresis and transfer

Equal amounts of protein (30 μg) from each sample were separated by 10% SDS-PAGE and transferred onto PVDF membranes (IPVH00010, Millipore, Massachusetts, USA). The membranes were blocked with 5% non-fat milk in TBST (60145ES76, Yeasen, Shanghai, China) for 1 hour at room temperature and then incubated overnight at 4°C with primary antibodies (e.g., LC3, p62, AMPK, etc.) (EPR18709/EPR4844/Y365, abcam, Cambridge, United Kingdom) diluted in blocking buffer.

Detection and imaging

Following primary antibody incubation, the membranes were washed three times with TBST and incubated with HRP-conjugated secondary antibodies at room temperature for 1 hour. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection reagent and detected with a chemiluminescence imaging system (1708280, BIO-RAD, California, USA). Band intensities were quantified using ImageJ software (v2.1).

RNA extraction and cDNA synthesis

Total RNA was extracted from myocardial tissues or H9C2 cardiomyocytes using Trizol reagent (15596-018, Invitrogen, California, USA), following the manufacturer's instructions. RNA purity and concentration were assessed using a UV spectrophotometer (1702525, BIO-RAD, California, USA) by measuring the absorbance at 260 and 280 nm. RNA integrity was verified by agarose gel electrophoresis.

Reverse transcription and qPCR

One microgram of total RNA was reverse-transcribed into cDNA using a reverse transcription kit (F0202A, LABLEAD, Beijing, China). The qPCR reaction system was prepared by mixing cDNA, SYBR Green qPCR Master Mix (CW3008H, CWBIO, Jiangsu, China) and specific primers. The reactions were performed in a real-time fluorescence quantitative PCR machine (A28136, Thermo Fisher Scientific, Massachusetts, USA) with the following cycling conditions: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Relative gene expression levels were calculated using the ΔΔCt method and normalized to internal reference genes such as GAPDH. Details of the primer sequences used for qPCR are provided in Table 1.

H9C2 cardiomyocyte treatment

H9C2 cardiomyocytes were cultured in high-glucose DMEM (L110KJ, Basalmedia, Shanghai, China) supplemented with 10% fetal bovine serum (FBS) (FCS500, ExCell Bio, Jiangsu, China) and 1% penicillin-streptomycin (60162ES76, YEASEN, Shanghai, China) at 37°C in a humidified atmosphere containing 5% CO2 (51032876, Thermo Scientific, Massachusetts, USA). Cells were divided into the following groups: control, H_2O_2-induced injury, Jianxin granule with H_2O_2, rapamycin with H_2O_2, rapamycin and Jianxin granule with H_2O_2, MHY1485 (mTOR agonist) (orb1307145, Biorbyt, Cambridge, United Kingdom) with H_2O_2 and MHY1485 and Jianxin granule with H_2O_2 (n=3) (Zhao *et al*., 2024) .

Apoptosis analysis

After the respective treatments, cells were harvested and stained with annexin V-FITC and propidium iodide (PI) (KGA1104-20, Keygen, Jiangsu, China) for flow cytometric analysis of apoptosis (n=3). The percentage of apoptotic cells was determined by analyzing the stained cells using a flow cytometer.

Protein analysis

Proteins were extracted from treated H9C2 cells, and levels of apoptosis-related proteins, including caspase-3, Bax, and Bcl-2, were analyzed by Western blot and immunofluorescence. The Bcl-2/Bax ratio was calculated to reflect the extent of cardiomyocyte apoptosis.

siRNA synthesis and vector construction

siRNA targeting AMPK, mTOR and AKT were synthesized and cloned into appropriate vectors using standard molecular biology techniques. The constructs were verified by sequencing.

Lentiviral production and transfection

293T cells were transfected with 3 μg of lentiviral plasmid, 9 μg of packaging plasmid (psPAX2) and 3 μg of envelope plasmid (pMD2.G) using a calcium phosphate transfection method. The medium was changed after 16 hours and the lentiviral particles were collected 48 hours post-transfection. The viral supernatant was filtered through a 0.45 μm filter (SLHU033RB, Millipore, Massachusetts, USA) and concentrated by ultracentrifugation.

Cell transduction

H9C2 cells were transduced with concentrated lentiviral particles in the presence of 8 μg/mL polybrene (AH8761-500ul, Acmec, Shanghai, China). Transduction efficiency was assessed by fluorescence microscopy or flow cytometry when using a GFP marker. Successfully transduced cells were selected with puromycin (2 μg/mL) (P8230, Solarbio, Beijing, China) for stable knockdown of target genes, facilitating the investigation of their roles in heart failure pathophysiology.

RNA extraction

Total RNA was extracted from myocardial tissues or H9C2 cells using the aforementioned Trizol reagent method. RNA purity and concentration were assessed, and RNA integrity was confirmed.

Reverse transcription and qPCR

For CCT8 (C0038, Beyotime, Shanghai, China) expression analysis, 1 μg of total RNA was reverse-transcribed into cDNA. Specific primers for CCT8 were designed and validated. qPCR was performed using the same cycling conditions described earlier. Relative CCT8 expression levels were calculated using the ΔΔCt method and normalized to GAPDH.

Statistical analysis

The experimental data were analyzed using SPSS 29.0 statistical software and are presented as mean ± standard deviation (± s). For categorical data, the chi-square (χ^2) test was used when both variables were unordered or only the grouping variable was ordered; when only the response variable was ordered, the rank-sum test was employed. For continuous data, if the data followed a normal distribution, the independent-samples t-test was used for comparisons between two groups, and the one-way analysis of variance (ANOVA) was used for comparisons among multiple groups. If the data did not meet the normality assumption, nonparametric tests were applied. A p-value of less than 0.05 was considered statistically significant. Experiments were carried out in triplicate.

RESULTS

Jianxin granules reduce hs-CRP and hs-BNP levels in heart failure rats via an autophagy-dependent mechanism

A rat model of heart failure was established, followed by group-based administration of Jianxin granules, the autophagy inhibitor 3-MA, and positive control drugs. The concentrations of hs-CRP (high-sensitivity C-reactive protein) and hs-BNP (high-sensitivity B-type natriuretic peptide) in the blood of rats from each group were measured. The results showed that the hs-CRP concentration in the model group was significantly higher than that in the control group (**$p < 0.001$), indicating successful modeling and the induction of an inflammatory response. The concentration in the Jianxin granules treatment group was lower than that in the model group, suggesting that Jianxin granules might reduce hs-CRP by inhibiting the inflammatory pathway. However, hs-CRP levels increased in the 3-MA group, suggesting that the anti-inflammatory effect of Jianxin granules may partially depend on autophagy regulation. The hs-CRP concentration in the positive control group was similar to that in the Jianxin granules group, validating the intervention's effectiveness (Fig. 1a). The hs-BNP concentration in the model group was significantly higher than that in the control group (**$p < 0.001$), reflecting cardiac dysfunction. The hs-BNP concentration in the Jianxin granules treatment group was significantly lower than in the model group (*$p < 0.05$), suggesting that Jianxin granules may improve cardiac load. However, the addition of 3-MA weakened the effect of Jianxin granules, further supporting the involvement of autophagy in its protective mechanism. The positive control group showed effects similar to those of the Jianxin granules group, indicating that both may act through similar pathways (Fig. 1b).

Jianxin granules ameliorate heart failure in rats by upregulating LC3B and downregulating P62 expression to activate autophagy

The protein and mRNA levels of LC3B (an autophagy marker) and P62 (an autophagy substrate) in heart tissue from rats in different treatment groups were assessed. The results showed that LC3B protein expression in the model group was significantly lower than in the control group, indicating reduced autophagic activity in the heart. In the Jianxin granules treatment group, LC3B expression was further increased (*$p < 0.05$). In contrast, treatment with the autophagy inhibitor 3-MA decreased LC3B expression, suggesting that Jianxin granules may exert their effects by activating autophagy. The positive drug group showed results similar to those of the Jianxin granules group (Figs. 2a-b). P62 protein expression in the model group was significantly higher than in the control group (**$p < 0.01$), consistent with P62 degradation following autophagy activation. The Jianxin granules treatment group further reduced P62 expression and treatment with 3-MA resulted in a rebound in P62 levels, further supporting the conclusion that Jianxin granules promote autophagy (Fig. 2c). Changes in the mRNA levels of LC3B and P62 in the different treatment groups were consistent with the protein levels (Figs. 2d-e).

Jianxin granules alleviate H_2O_2-induced cardiomyocyte injury by activating autophagy and inhibiting apoptosis

Cardiomyocyte apoptosis was induced using H_2O_2, followed by treatment with Jianxin granules, MHY (an autophagy inhibitor), and Ra inhibitor. The levels of apoptosis and proliferation in the cells were subsequently assessed. The results showed that the apoptosis rate in the H_2O_2 model group significantly increased to 7.43% (compared to 2.40% in the control group, *$p < 0.001$), indicating that H_2O_2 successfully induced cardiomyocyte apoptosis.

Table 1: Primer Sequences

Primer	Sequence (5'->3')
GAPDH	F: CTAGCTGGCCCGATTTCTCC; R: GCGCCCAATACGACCAAATC
mTOR	F: CCGCGCGAATATTAAAGGAAAC; R:GCAGGACGCTCACATTGCTA
p62	F: CCCAGGGCTCCCTAAAGAGG; R: GGAAGATTTCTCTTCTCCAACTGC
LC3B	F: CAGGTTCACAAAACCCGCC; R: CGTTTACCCTGCGTTTGTGC
Beclin-1	F: CTGTGAGCCTGTGGACCAG; R: GGCCTCCAGAACTACCATCG
ATG5	F: ATCCAAGGATGCGGTTGAGG; R: GTCATTCTGCAGTCCCATCCA
ULK1	F: ACGCAGGTGCAGAACTACC; R: CAGAGGACCGAGGTGTTTGG
Bax	F: GGAGCAGCCCAGAGGC; R: TGCTCGATCCTGGATGAAACC
Caspase-3	F: GGAGCTTGGAACGGTACGC; R: CCACTGACTTGCTCCCATGTA

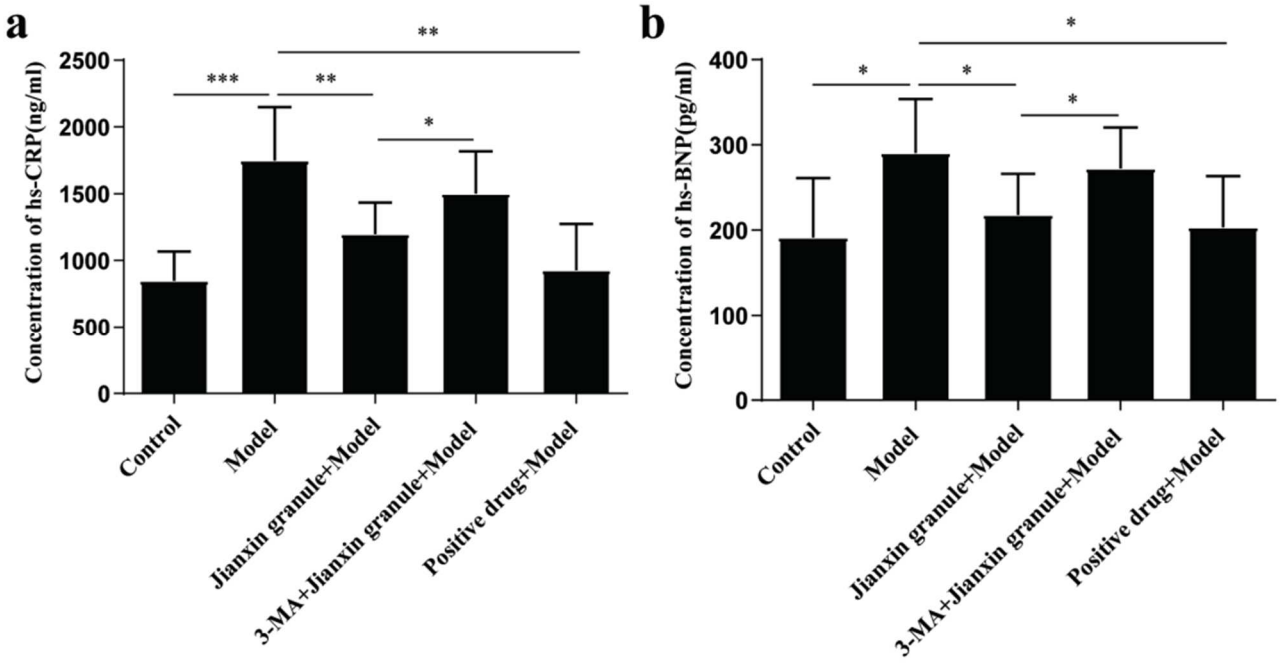

Fig 1: Effects of different treatments on IL-CRP and IL-BNP concentrations. (a) IL-CRP (interleukin-C-reactive protein) levels across experimental groups: Control, Model, Jianxin granule-Model, 3-MA-Jianxin granule-Model, and Positive drug-Model; (b) IL-BNP (interleukin-B-type natriuretic peptide) concentrations in the same groups. Control: sham operated rats; Model: heart failure rats; 3-MA: autophagy inhibitor. Significance markers: *p < 0.05, **p < 0.01, ***p < 0.001.

Treatment with Jianxin granules reduced the apoptosis rate to 4.06% ($p < 0.01$ vs. H_2O_2 group), suggesting a significant inhibition of H_2O_2-induced apoptosis. The effect of the Ra inhibitor group was similar to that of the Jianxin granules group. When both Ra and Jianxin granules were administered together, the level of apoptosis was suppressed to a greater extent. The addition of the autophagy activator MHY significantly promoted apoptosis, but this effect was reversed by the subsequent addition of Jianxin granules (Figs. 3a-b). Furthermore, cell proliferation in the Jianxin granules and Ra intervention groups was significantly higher than in the H_2O_2 group, and the combined treatment of Ra and Jianxin granules resulted in an even more pronounced increase in cell proliferation. In contrast, the MHY intervention group showed a significant decrease in cell activity, which was restored upon addition of Jianxin granules (Fig. 3c). These findings suggest that Jianxin granules may protect cardiomyocytes by promoting autophagy and inhibiting apoptosis.

Jianxin granules protect H_2O_2-damaged cardiomyocytes by inhibiting the mTOR pathway and ULK1 phosphorylation to restore autophagic flux and suppress apoptosis

In a cell experiment, qRT-PCR analysis of the H9C2 myocardial cell damage model induced by H_2O_2 revealed that mRNA levels of autophagy-related genes (ULK1, ATG5, Beclin-1, LC3B) were significantly reduced, while p62 mRNA levels were significantly increased, indicating impaired autophagic flux. The mRNA level of mTOR was significantly elevated, indicating activation of the mTOR pathway.

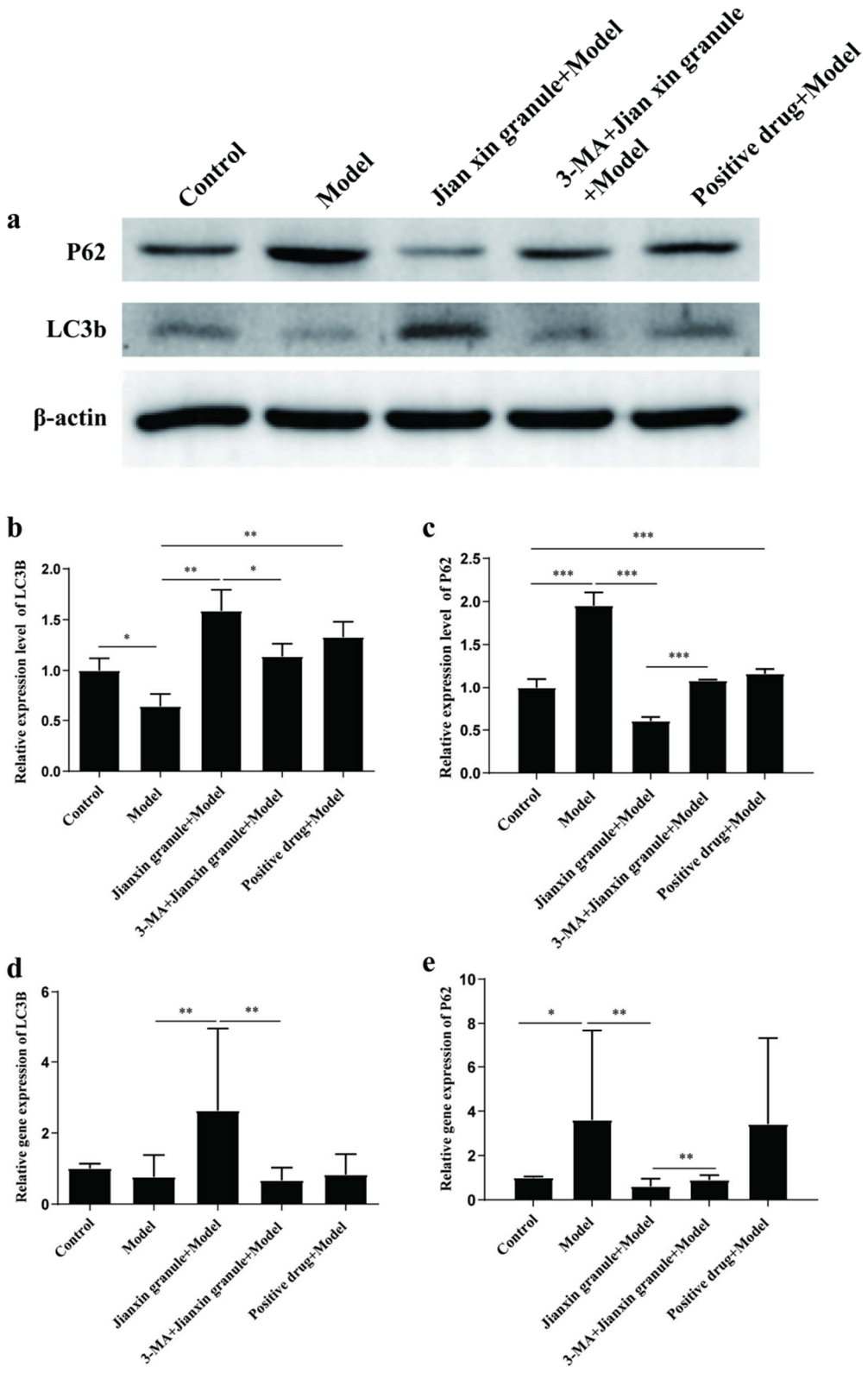

Fig 2: Western blot and quantitative analysis of autophagy markers (LC3b and P62) across experimental treatment groups. (a) Quantitative bar graphs of WB; (b, c) Showing the expression levels of autophagy-related proteins LC3b and P62 in different treatment groups; (d, e) Showing the expression levels of LC3b mRNA and P62 mRNA in different treatment groups. Control: sham-operated rats; Model: heart failure rats; 3-MA: autophagy inhibitor. Significance markers: *p < 0.05, **p < 0.01, ***p < 0.001.

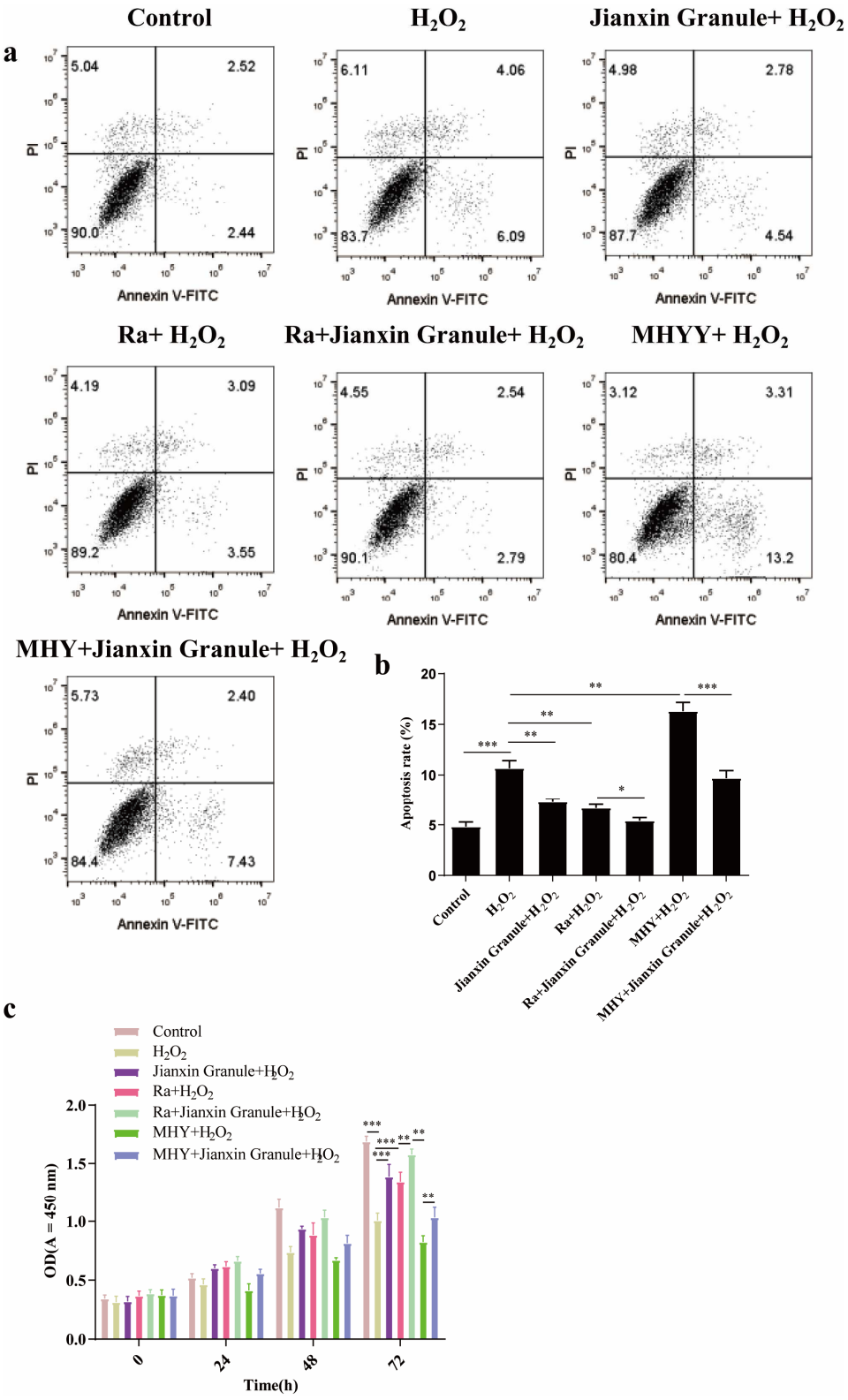

Fig 3: Flow cytometry analysis of apoptosis and cell viability under different treatment conditions. (a) Representative flow cytometry scatter plots of Annexin V-FITC staining (apoptosis marker) across treatment groups; (b) Quantification of Annexin V-FITC-positive cells (%) across groups; (c) Time-dependent cell viability measurement (OD 450 nm) under H_2O_2 stress and interventions. Control:H9C2 cardiomyocytes; Ra: Rapamycin; MHY: autophagy inhibitor. Significance markers: *p < 0.05, **p < 0.01, ***p < 0.001.

Additionally, the mRNA levels of apoptosis-related genes Bax and caspase-3 were significantly increased, suggesting enhanced apoptosis. In the intervention group with Jianxin Granules, the results showed enhanced autophagic flux, inhibition of the mTOR pathway and reduced apoptosis.

The results in the Ra intervention group were consistent with those of the Jianxin Granules group. Compared with the Ra and Jianxin Granules single-intervention groups, the combined intervention group showed more significant results. The MHY intervention group showed impaired autophagic flux, significant activation of the mTOR pathway, and elevated apoptosis. After the addition of Jianxin Granules, autophagic flux increased, the mTOR pathway was inhibited, and apoptosis levels decreased (Fig. 4). The expression levels of autophagy-related proteins, mTOR, and apoptosis-related proteins were also assessed, and the results were consistent with the mRNA levels. In cells from all groups, p-ULK1 levels correlated with mTOR, suggesting that Jianxin Granules may inhibit myocardial cell autophagy by promoting ULK1 phosphorylation through the mTOR pathway (Fig. 5).

Jianxin granules target the mTOR pathway to synergistically inhibit cardiomyocyte apoptosis and promote proliferation

The effects of Jianxin Granules on myocardial cell apoptosis and proliferation through the mTOR pathway were investigated using flow cytometry and cell proliferation assays. The results showed that the apoptosis rate in the H_2O_2 treatment group was significantly higher compared to the control group. However, specific inhibition of the mTOR pathway resulted in a significant reduction in apoptosis, suggesting that the mTOR signaling pathway plays a promotive role in H_2O_2-induced apoptosis. Notably, in the combined Jianxin Granules intervention group, the apoptosis rate was further decreased and significantly lower than in the mTOR inhibition group alone, indicating that Jianxin Granules may exert a synergistic anti-apoptotic effect by enhancing the inhibitory action of the mTOR pathway (Figs. 6a-b). Cell viability in the H_2O_2 treatment group was significantly lower than in the control group, indicating that H_2O_2 inhibits cell proliferation. After mTOR pathway inhibition, cell viability increased and in the combined Jianxin Granules treatment group, cell viability was significantly further enhanced. This result suggests that H_2O_2 may inhibit proliferation by activating the mTOR pathway, while Jianxin Granules effectively reverse the proliferation inhibition induced by H_2O_2 through modulation of this pathway (Fig. 6c).

Jianxin Granules reverse autophagy inhibition and apoptosis in cardiomyocytes by inhibiting the mTOR/ULK1 signaling axis

The regulatory effects of Jianxin Granules on the mTOR signaling axis, autophagy, and apoptosis-related genes in H_2O_2-induced myocardial cells were analyzed using qPCR. The results showed that, compared with the control group, the mTOR mRNA level was upregulated in the H_2O_2 treatment group, whereas the autophagy-related gene p62 was significantly increased, and autophagy marker genes such as LC3B, Beclin-1, ATG5, and ULK1 were significantly inhibited, suggesting that H_2O_2 inhibits the autophagic process by activating the mTOR pathway. Notably, after specific inhibition of the mTOR pathway, the expression of these autophagy-related genes was reversed, confirming that the inhibitory effect of mTOR signaling on autophagy is targeted. In the Jianxin Granules intervention group, a similar regulatory pattern to the mTOR inhibition group was observed, indicating that Jianxin Granules may reverse the inhibition of autophagy by suppressing the mTOR signaling pathway (Figs. 7a-c). Compared to the control group, the H_2O_2 treatment group showed significant increases in the pro-apoptotic genes Bax and Caspase-3, while the mTOR inhibition group significantly reduced their expression. After the addition of Jianxin Granules, the suppression was more pronounced, suggesting that Jianxin Granules may regulate apoptosis through both mTOR-dependent and independent pathways in a synergistic manner (Fig. 7d). The expression levels of autophagy-related proteins, mTOR and apoptosis-related proteins were also assessed, and the results were consistent with the mRNA levels. In all cell groups, the p-ULK1 protein level correlated with mTOR, suggesting that Jianxin granules may promote ULK1 phosphorylation via mTOR, thereby inhibiting autophagy in cardiomyocytes (Fig. 8).

DISCUSSION

HF remains a global healthcare challenge with a complex pathophysiology driven by various factors, such as myocardial hypertrophy, ventricular remodeling and impaired autophagic flux. The introduction and findings of the present study regarding Jianxin Granules' protective effects against HF via the autophagy pathway offer promising insights into traditional Chinese medicine (TCM) as an adjunctive or alternative therapeutic approach. This discussion will contextualize these findings within the existing literature, explore the mechanisms involved, and suggest implications for further clinical applications and research.

Autophagy, a highly regulated intracellular degradation mechanism, is crucial for maintaining cardiac homeostasis. Under normal physiological conditions, it acts as a quality control system, clearing damaged organelles and proteins to sustain cellular integrity. Recent studies, consistent with this study's findings, underscore the pivotal role of autophagy in cardioprotection. For example, Nakai *et al*. reported that autophagy's adaptive role is critical during stress conditions, such as ischemia or pressure overload, to prevent myocardial injury and apoptosis (Nakai *et al*., 2007).

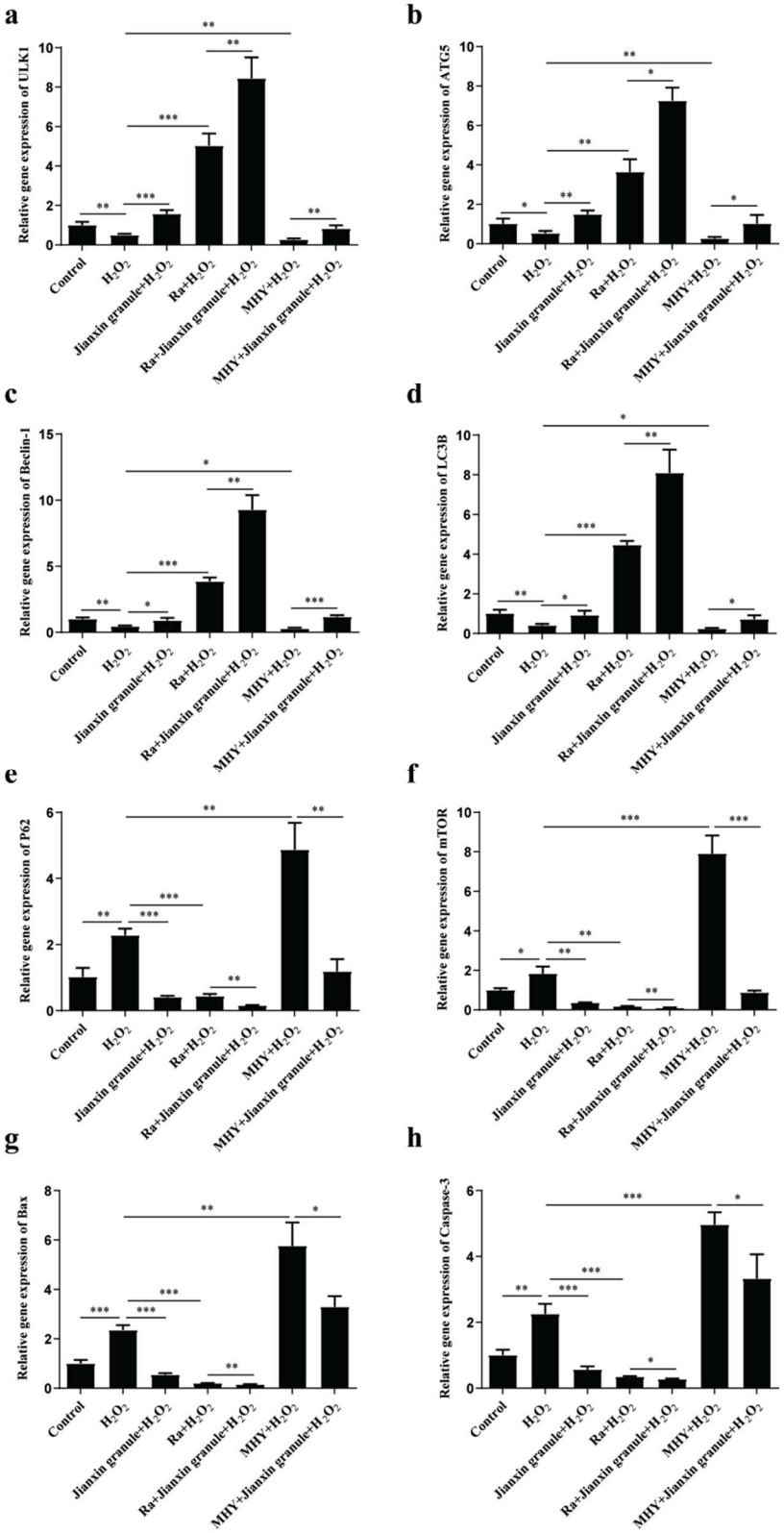

Fig 4: Quantitative analysis of autophagy and apoptosis markers' mrna under oxidative stress and therapeutic interventions. The gene expression levels in different treatment groups of (a) UKLK1; (b) ATG5; (c) Beclin-1; (d) LC3B; (e) P62; (f) mTOR; (g) Bax; (h) Caspase-3. Control:H9C2 cardiomyocytes; Ra: Rapamycin; MHY: autophagy inhibitor.. Significance markers: *p < 0.05, **p < 0.01, ***p < 0.001.

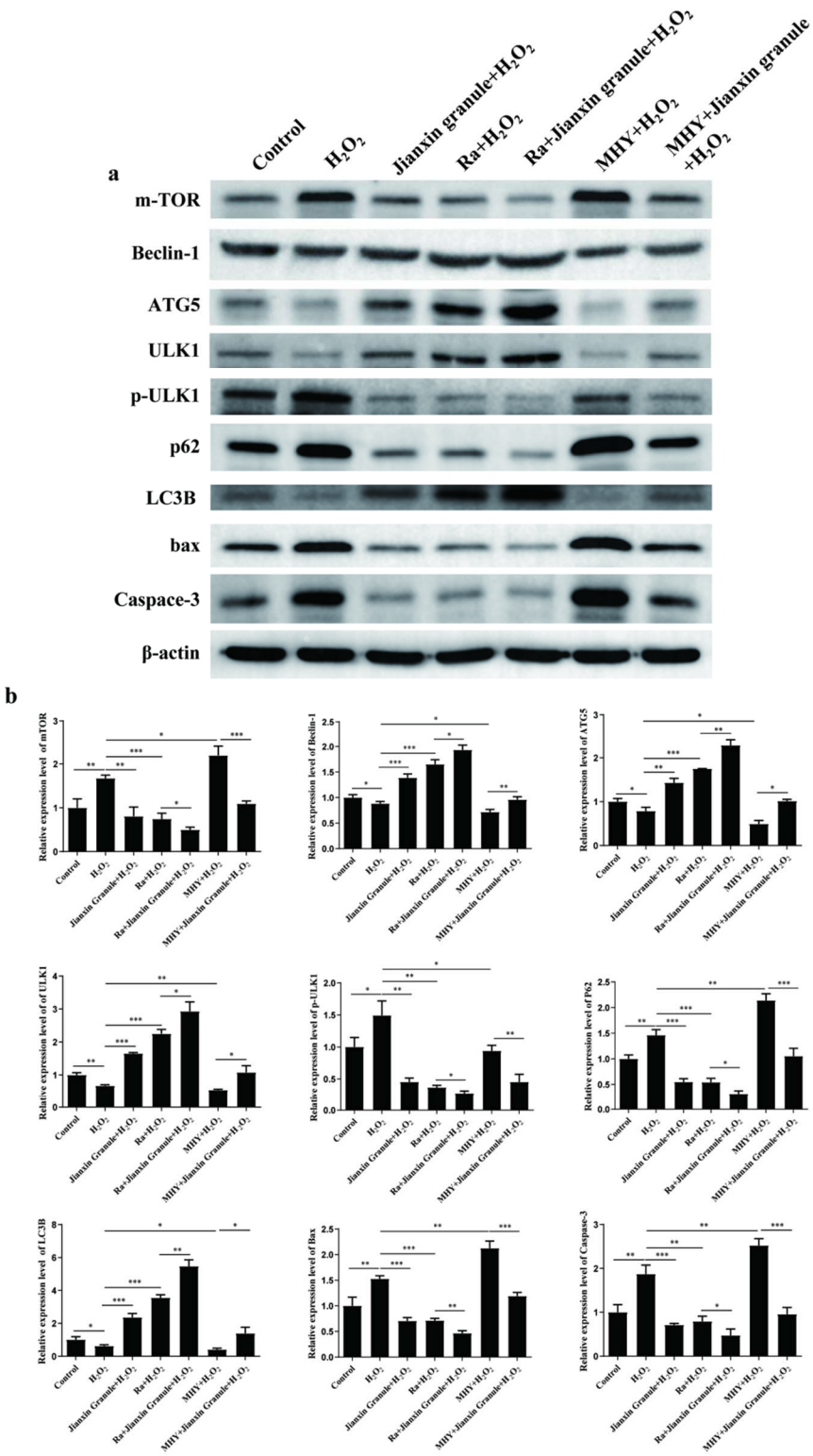

Fig 5: Western blot and quantitative analysis of autophagy and apoptosis markers under oxidative stress and therapeutic interventions. (a) Western blot images showing the expression levels of autophagy-related proteins (m-TOR, Beclin-1, ATG5, ULK1, p-ULK1, p62, LC3B-II) and apoptosis-related markers (bax, Caspase-3) across experimental groups under oxidative stress (H_2O_2) with or without therapeutic interventions; (b) Quantitative statistical analysis of protein expression levels normalized to β-actin. Control:H9C2 cardiomyocytes; Ra: Rapamycin. MHY: autophagy inhibitor. Significance markers: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

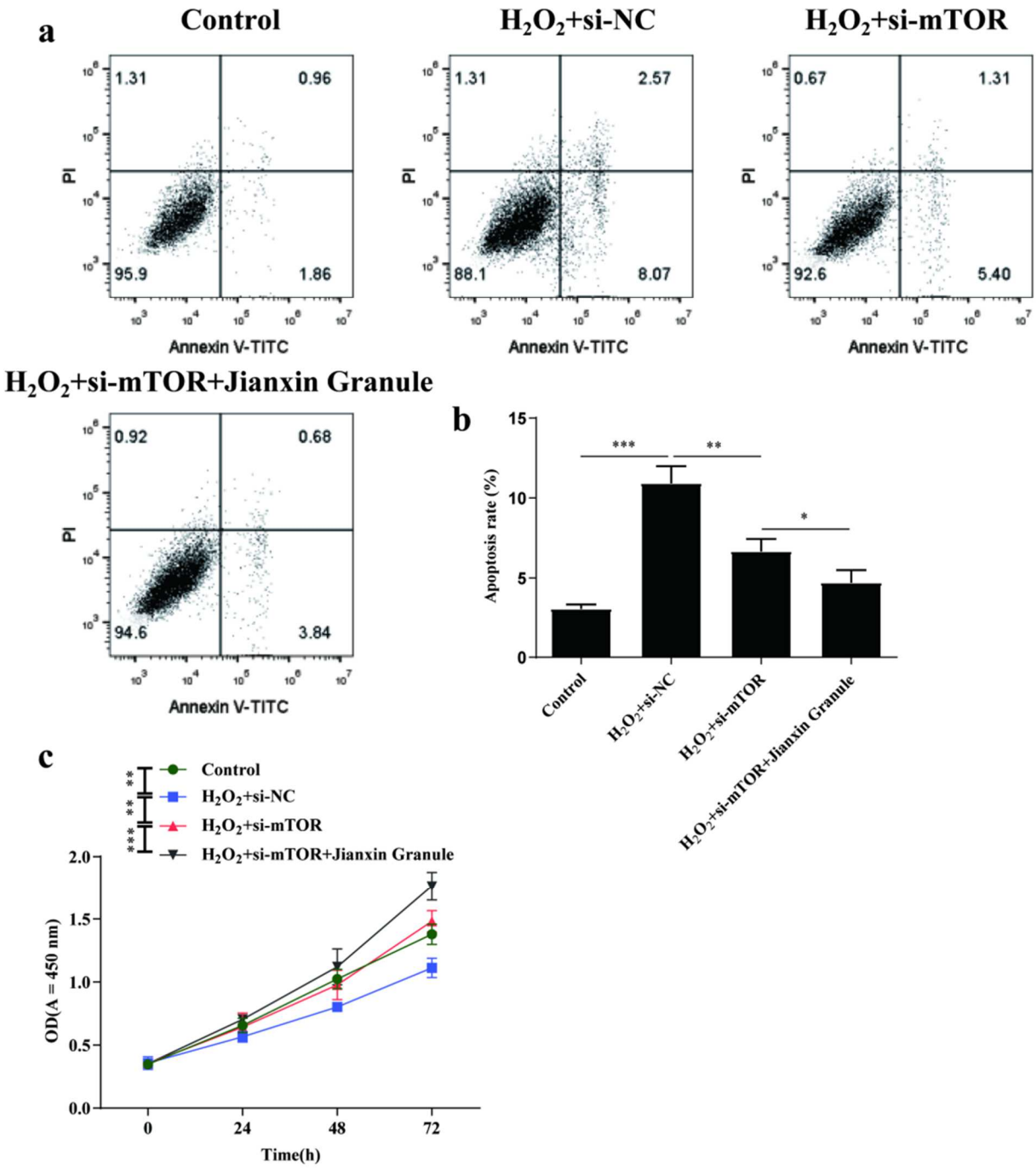

Fig 6: Effects of mTOR silencing and Jianxin Granule on H_2O_2-induced apoptosis and cell proliferation. (a) Annexin V-FITC/PI dot plots for apoptotic cells in different treatment groups; (b) Bar graph showing apoptosis rates (%) across treatment groups; (c) Cell proliferation in different treatment groups. Control:H9C2 cardiomyocytes; si-NC: small interfering RNA negative control; si-mTOR: small interfering RNA targeting mTOR. Significance markers: *p < 0.05, **p < 0.01, ***p < 0.001.

The results in the present study demonstrate the restoration of autophagic flux through Jianxin Granules administration, indicating their role in modulating this essential cellular process.

The autophagy process is regulated by a cascade of signaling pathways, with the mTOR pathway among the central regulators. Jianxin Granules' influence on the mTOR pathway, leading to enhanced autophagic flux, aligns with previous pharmacological studies showing similar effects of TCM formulations.

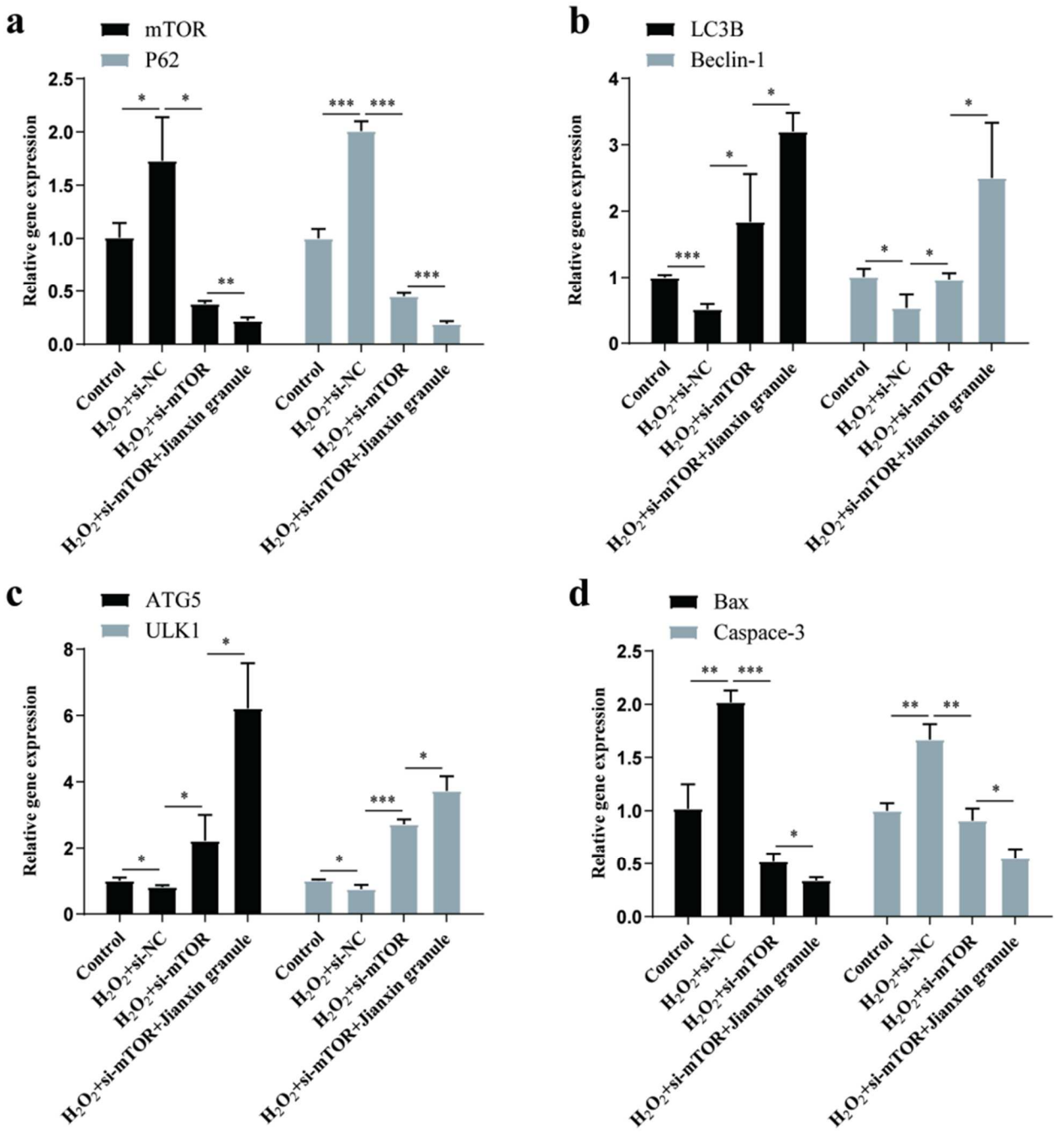

Fig 7: Expression of autophagy and apoptosis-related genes in different treatment groups. (a) Relative expression of mTOR and P62 across treatment groups; (b) Relative expression of LC3B and Beclin-1 across treatment groups; (c) Relative expression of Bax and Caspase-3 across treatment groups; (d) Relative expression of ATG5 and ULK1 across treatment groups. Control: H9C2 cardiomyocytes; si-NC: small interfering RNA negative control; si-mTOR: small interfering RNA targeting mTOR. Significance markers: *p < 0.05, **p < 0.01, ***p < 0.001.

For instance, Minru Liao *et al*. found that certain TCM compounds can downregulate mTOR, thereby enhancing autophagy and reducing apoptosis in cardiac cells (Liao *et al*., 2022). This regulation appears to be consistent across various TCM formulations, suggesting a shared underlying mechanism attributable to the multi-target nature of herbal components.

The study's findings also highlight the complex interplay between autophagy and apoptosis. Enhanced autophagic flux observed in the Jianxin Granules intervention groups corresponded with decreased expression of pro-apoptotic markers (e.g., Bax, caspase-3).

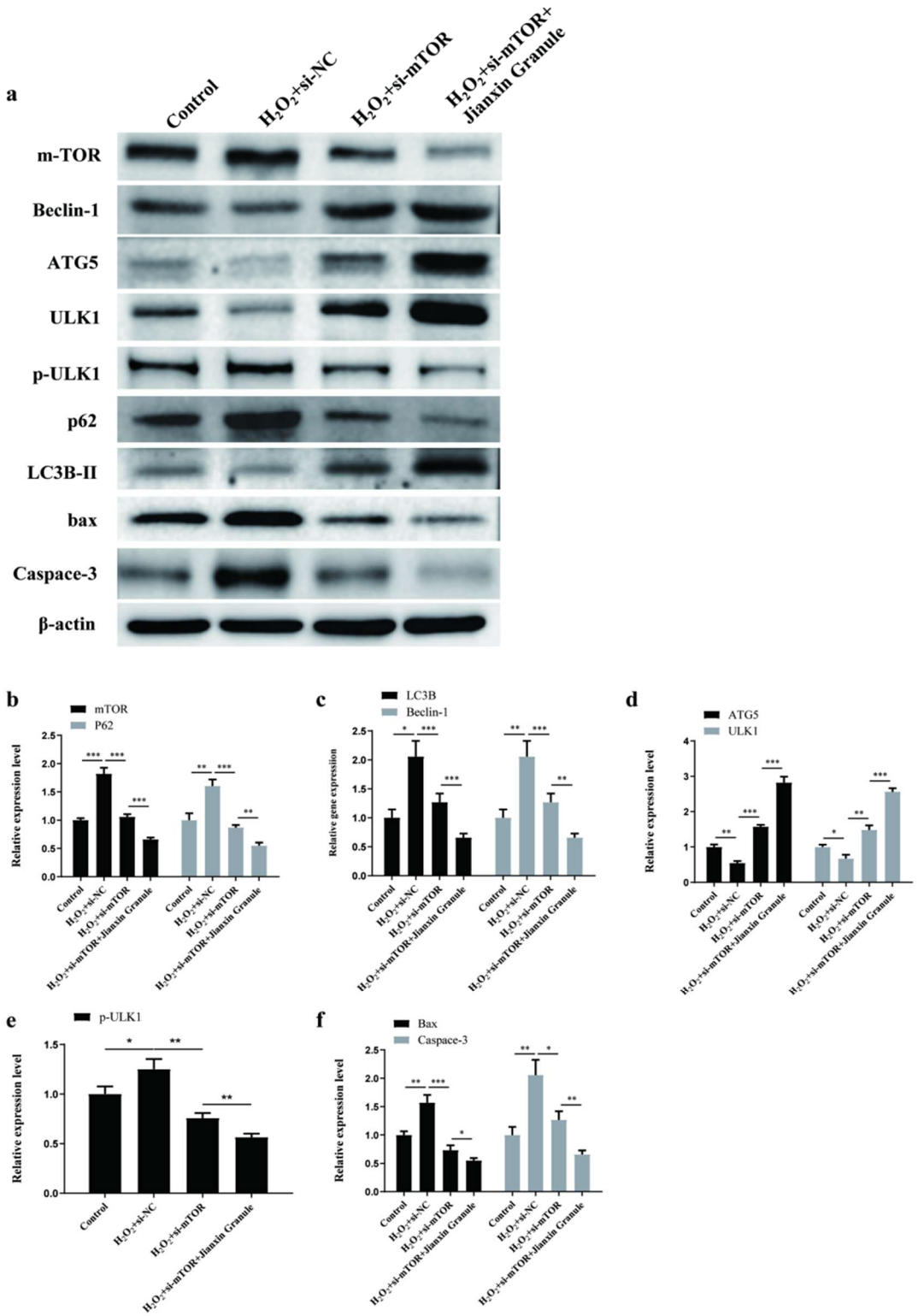

Fig 8: Western blot and quantitative analysis of autophagy and apoptosis markers under oxidative stress and therapeutic interventions. (a) Western blot images showing the expression levels of autophagy-related proteins (m-TOR, Beclin-1, ATG5, ULK1, p-ULK1, p62, LC3B-II) and apoptosis-related markers (bax, Caspase-3) across experimental groups under oxidative stress (H_2O_2) with or without therapeutic interventions; (b) Quantitative statistical analysis of mTOR and P62; (c) LC3B and Beclin-1; (d) ATG5 and ULK1; (e) p-ULK1; (f) Bax and Caspase-3 expression levels normalized to β-actin. Significance markers: *p < 0.05, **p < 0.01, ***p < 0.001.

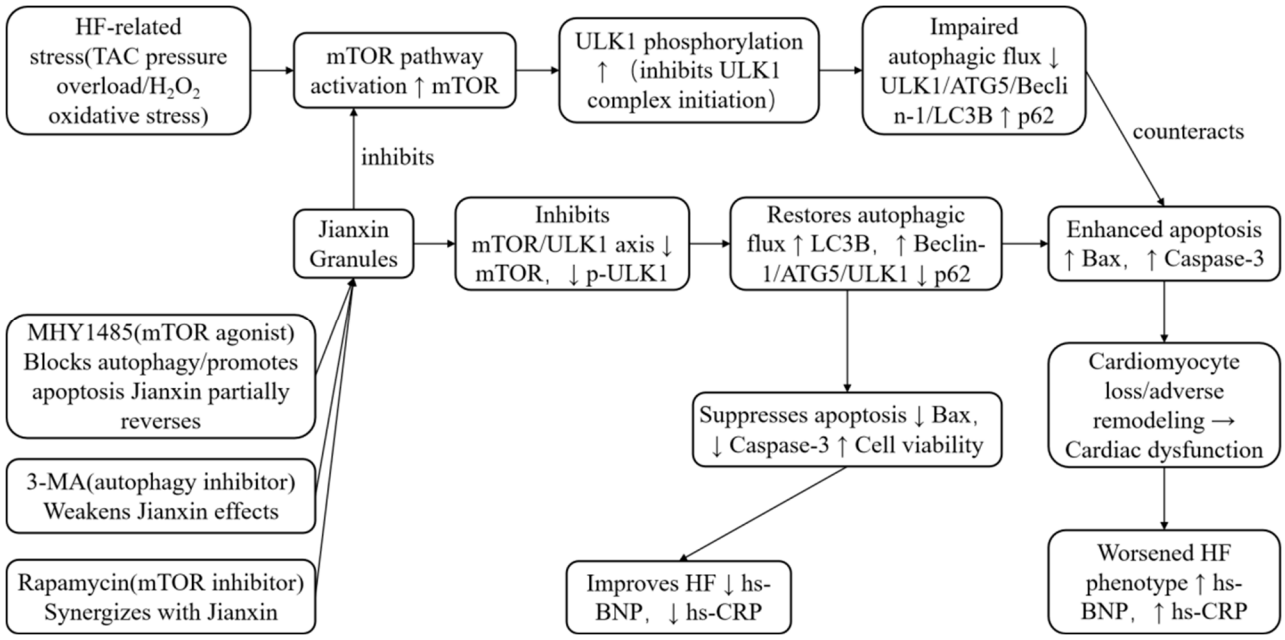

Fig. 9: The mechanism of action of Jianxin Granules in heart failure

This observation supports previous studies, which demonstrated that TCM-induced autophagy activation can attenuate apoptosis, especially under stress conditions (Yang *et al*., 2020). Jianxin Granules, as a multi-component TCM formulation, exemplifies the characteristic complexity of TCM therapies, in which multiple bioactive constituents may simultaneously target different aspects of disease progression. This multi-target approach is considered advantageous for managing complex conditions like HF, as it can modulate various molecular pathways, including reducing oxidative stress, exerting anti-inflammatory effects, and improving mitochondrial function. However, this complexity also presents challenges in understanding the precise mechanisms of action. The study partially addresses this by demonstrating the role of Jianxin Granules in regulating autophagy via mTOR and related signaling cascades (e.g., the ULK complex and the Beclin-1 complex). Still, a more detailed exploration using advanced molecular techniques such as omics-based analysis or network pharmacology could provide a deeper understanding of the synergistic effects of these components.

The study's animal and cell models provide robust evidence for the therapeutic effects of Jianxin Granules, including enhanced autophagic flux and reduced apoptosis. These results are corroborated by previous studies, such as Lv *et al* demonstrated that multi-target TCM interventions can effectively improve myocardial autophagy and ameliorate HF symptoms in rat models (Lv *et al*., 2020). However, while preclinical evidence is promising, translating these findings to clinical applications requires careful consideration of dosing, safety and efficacy in human trials.

One notable aspect of the study is the use of an autophagy inhibitor (3-MA) to clarify further the role of autophagy in Jianxin Granules' cardioprotective effects. The partial reversal of benefits in the Jianxin Granules + 3-MA group suggests that autophagy plays a significant, though not exclusive, role in the observed effects. This finding underscores the importance of autophagy in TCM-induced cardioprotection and aligns with the broader scientific consensus that optimal autophagy levels are protective, whereas excessive autophagy can be detrimental.

The present study provides a strong foundation for further investigation into the therapeutic potential of Jianxin Granules in HF management. Based on the findings, several directions for future research and clinical applications can be proposed: Given the encouraging results in preclinical models, the next logical step is to conduct well-designed clinical trials to evaluate the safety and efficacy of Jianxin Granules in HF patients. The trials should be randomized, double-blinded and include long-term follow-up to assess outcomes such as survival rates, hospitalization rates and quality of life. Advanced techniques, such as proteomics, genomics and metabolomics, can help identify specific molecular targets of Jianxin Granules' components and their interactions with autophagy-related pathways. This would provide a more comprehensive understanding of how each element contributes to the overall therapeutic effect. One of the challenges with TCM formulations is the variability in composition and dose. Standardizing the active components of Jianxin Granules and optimizing their doses for maximum efficacy and safety will be crucial for successful clinical translation. Given the multi-target and potentially synergistic effects of Jianxin Granules,

integrating them with conventional HF treatments (e.g., beta-blockers, ACE inhibitors) could enhance overall therapeutic outcomes. Combination therapy studies could investigate potential synergistic effects and help refine treatment regimens for better patient outcomes.

While the study offers substantial insights, it is essential to acknowledge certain limitations. The use of animal models, while informative, does not fully capture the complexity of human HF. Additionally, while Jianxin Granules showed significant benefits in autophagic flux and reduced apoptosis, their effects on other HF-related pathways (e.g., inflammation, fibrosis) remain unexplored. Further studies should incorporate broader biomarker analyses to provide a more holistic view of the effects of Jianxin Granules on HF progression.

The mechanism described in the article is shown in Fig. 9.

CONCLUSION

The study provides valuable evidence supporting the potential of Jianxin Granules as a novel therapeutic approach for HF, emphasizing their regulatory role in autophagy via the mTOR pathway. The findings align with the existing literature, reinforcing the notion that TCM formulations, with their multi-component and multi-target characteristics, hold promise for managing complex diseases such as HF. Continued research efforts, both preclinical and clinical, will be critical in translating these findings into effective, safe and standardized treatments for HF patients.

Acknowledgments
None.

Authors' contributions
Jun Chen and Jinjian Guo: Conceptualization, study design, supervision, project administration, writing—review and editing and funding acquisition/resources. Yong Wang and Feixiang Huang: Investigation (*in vivo* and *in vitro* experiments), data acquisition, data curation; Yirong Chen: Formal analysis, statistics, visualization, writing—review and editing. Jun Chen and Yong Wang: Writing—original draft. All authors: Data verification and interpretation, final approval of the manuscript and accountability for the work.

Funding
This study was supported by the Fujian Province Health and Wellness Program for Training Young and Middle-Aged Key Personnel (Grant No. 2020GGA068), Fujian Provincial Natural Science Foundation (Grant No. 2022J01835) and National Natural Science Foundation of China (Grant No. 82574851).

Data availability statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval
All procedures were performed in compliance with institutional animal care standards and were approved by the Fujian University of Traditional Chinese Medicine Ethics Committee (No. SYXK2020-0002, dated: 2020.06.29). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The authors declared no conflicts of interest.

Supplementary data
https://www.pjps.pk/uploads/2026/06/SUP1781344457.pdf

REFERENCES

Ajoolabady A, Chiong M, Lavandero S, Klionsky DJ and Ren J (2022). Mitophagy in cardiovascular diseases: Molecular mechanisms, pathogenesis and treatment. *Trends Mol Med,* **28**(10): 836-849.

Bellagambi FG, Petersen C, Salvo P, Ghimenti S, Franzini M, Biagini D and Lomonaco T (2021). Determination and stability of N-terminal pro-brain natriuretic peptide in saliva samples for monitoring heart failure. *Sci Rep,* **11**(1): 13088.

Chen M, Zhong G, Liu M, He H, Zhou J, Chen J and Zhou H (2023). Integrating network analysis and experimental validation to reveal the mitophagy-associated mechanism of Yiqi Huoxue (YQHX) prescription in the treatment of myocardial ischemia/reperfusion injury. *Pharmacol Res,* **189**: 106682.

Dai Y, Chen Y, Wei G, Zha L and Li X (2021). Ivabradine protects rats against myocardial infarction through reinforcing autophagy via inhibiting PI3K/AKT/mTOR/p70S6K pathway. *Bioengineered,* **12**(1): 1826-1837.

Dong Y, Chen H, Gao J, Liu Y, Li J and Wang J (2019). Molecular machinery and interplay of apoptosis and autophagy in coronary heart disease. *J Mol Cell Cardiol,* **136**: 27-41.

Farag A, Mandour AS, Hendawy H, Elhaieg A, Elfadadny A and Tanaka R (2023). A review on experimental surgical models and anesthetic protocols of heart failure in rats. *Front Vet Sci,* **10**: 1103229.

Gao G, Chen W, Yan M, Liu J, Luo H, Wang C and Yang P (2020). Rapamycin regulates the balance between cardiomyocyte apoptosis and autophagy in chronic heart failure by inhibiting mTOR signaling. *Int J Mol Med,* **45**(1): 195-209.

Jin L, Zhou Y, Han L and Piao J (2020). MicroRNA302-367-PI3K-PTEN-AKT-mTORC1 pathway promotes the development of cardiac hypertrophy through controlling autophagy. *In Vitro Cell Dev Biol Anim,* **56**(2): 112-119.

Khan M S, Shahid I, Bennis A, Rakisheva A, Metra M and Butler J (2024). Global epidemiology of heart failure. *Nat Rev Cardiol,* **21**(10): 717-734.

Liao M, Xie Q, Zhao Y, Yang C, Lin C, Wang G and Zhu L (2022). Main active components of Si-Miao-Yong-An decoction (SMYAD) attenuate autophagy and apoptosis via the PDE5A-AKT and TLR4-NOX4 pathways in isoproterenol (ISO)-induced heart failure models. *Pharmacol Res,* **176**: 106077.

Lv S, Yuan P, Dong J, Lu C, Li M, Qu F and Zhang J (2020). QiShenYiQi pill improves the reparative myocardial fibrosis by regulating autophagy. *J Cell Mol Med,* **24**(19): 11283-11293.

Miyamoto S (2019). Autophagy and cardiac aging. *Cell Death Differ,* **26**(4): 653-664.

Nakai A, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M and Otsu K (2007). The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. *Nat Med,* **13**(5): 619-624.

Ouyang Q, Zhang L, Chen Y, Liu L, Chen M, Yan J and Guo J (2025). Role of jianxin granules in heart failure: Evidence from network pharmacology, molecular docking and experimental verification. *Minerva Cardiol Angiol,* **73**(3): 285-295.

Savarese G, Becher PM, Lund LH, Seferovic P, Rosano GMC and Coats AJS (2023). Global burden of heart failure: A comprehensive and updated review of epidemiology. *Cardiovasc Res,* **118**(17): 3272-3287.

Sciarretta S, Maejima Y, Zablocki D and Sadoshima J. (2018). The role of autophagy in the heart. *Annu Rev Physiol,* **80**: 1-26.

Tham YK, Bernardo BC, Ooi JY, Weeks KL and McMullen JR (2015). Pathophysiology of cardiac hypertrophy and heart failure: Signaling pathways and novel therapeutic targets. *Arch Toxicol,* **89**(9): 1401-1438.

Wang T, Hou B, Qin H, Liang J, Shi M, Song Y and Jia Z. (2023). Qili Qiangxin (QLQX) capsule as a multi-functional traditional Chinese medicine in treating chronic heart failure (CHF): A review of ingredients, molecular, cellular and pharmacological mechanisms. *Heliyon,* **9**(11): e21950.

Wang Y and Zhang H (2019). Regulation of autophagy by mTOR signaling pathway. *Adv Exp Med Biol,* **1206**: 67-83.

Xia W, Huang ZJ, Shi N, Feng YW and Tang AZ (2023). Dosage selection and effect evaluation of sodium pentobarbital in tree shrew anesthesia. *Lab Anim,* **57**(3): 283-292.

Yang JJ, Zhang XH, Ma XH, Duan WJ, Xu NG, Chen YJ and Liang L (2020). Astragaloside IV enhances GATA-4 mediated myocardial protection effect in hypoxia/reoxygenation injured H9c2 cells. *Nutr Metab Cardiovasc Dis,* **30**(5): 829-842.

Yongzhong C, Hui C, Luting Z, Wei G, Yiqing H, Yiru G and Qiufang O (2024). Mechanism of jianxin granules in the treatment of heart failure based on proteomics and metabolomics. *Chin Med,* **19**(1): 165.

Zhao J, Shi W, Zheng Y, Wang J, Yuan M, Anwar Y and Wu J (2024). Mechanism of mTOR/RILP-regulated autophagic flux in increased susceptibility to myocardial ischemia-reperfusion in diabetic mice. *Front Pharmacol,* **15**: 1506401.