Myocardial Lschemia Reperfusion Injury is Alleviated by Curcumin-peptide Hydrogel via Upregulating Autophagy and Protecting Mitochondrial Function

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Research

Keywords: ischemia-reperfusion injury, autophagy, apoptosis, curcumin, hydrogel

Posted Date: August 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-51338/v1

Version of Record: A version of this preprint was published on January 28th, 2021. See the published version at https://doi.org/10.1186/s13287-020-02101-y.

Abstract

Background: Ischemia-reperfusion injury (IRI) is an important factor limiting the success of cardiac reperfusion therapy. Curcumin has a significant cardioprotective effect against IRI, can inhibit ventricular remodeling induced by pressure load or MI, and improve cardiac function.

Patient: However, the poor water solubility and low bioavailability of curcumin restrict its clinical application. In this study, we prepared and evaluated a curcumin-hydrogel (cur-hydrogel) to reduce cardiomyocyte apoptosis and reactive oxygen species formation induced by hypoxia-reoxygenation injury, promote autophagy, and reduce mitochondrial damage by maintaining the phosphorylation of Cx43.

Results: Meanwhile, cur-hydrogel can restore cardiac function, inhibit myocardial collagen deposition and apoptosis, and activate JAK2/STAT3 pathway to alleviate myocardial ischemia-reperfusion injury in rats.

Conclusion: The purpose of this study is to elucidate the protective effects of cur-hydrogel on myocardial ischemia-reperfusion injury by regulating apoptosis, autophagy and mitochondrial injury in *vitro* and in *vivo*, which lays a new theoretical and experimental foundation for the prevention and reduction of IRI.

Introduction

The reperfusion therapy of acute myocardial infarction can lead to more serious dysfunction after myocardial ischemia, resulting in the decrease of myocardial diastolic and systolic function, the decrease of ventricular threshold and the shortening of refractory period, which can be manifested as malignant arrhythmia, cardiac insufficiency and even sudden death(B et al., 2015; B et al., 2017). The mechanism of ischemia-reperfusion injury is not completely clear. At present, the mechanisms with more evidence include oxidative stress, intracellular calcium overload, vascular endothelial injury, endoplasmic reticulum stress, inflammatory response(Y et al., 2020a; Y et al., 2020b). To develop effective drugs or treatments to reduce myocardial ischemia-reperfusion injury has become a key problem to be solved urgently.

Curcumin is a polyphenolic compound extracted from the rhizome of curcuma. As an effective ingredient of traditional Chinese medicine turmeric, curcumin has a variety of pharmacological effects, such as anti-inflammation, anti-oxidation, anti-apoptosis, anti-fibrosis, anti-tumor, cardiovascular protection and so on. Studies have shown that curcumin has a protective effect on myocardial ischemia-reperfusion injury and can reduce oxidative stress injury and cardiomyocyte apoptosis(W et al., 2012a). Curcumin can also inhibit angiotensin II-mediated myocardial fibrosis(XF et al., 2015a) and cardiac non-benign ventricular remodeling caused by left ventricular pressure overload(QH et al., 2004) by regulating the expression of angiotensin receptor. It can also reduce myocardial hypertrophy caused by diabetic cardiomyopathy and improve cardiac function(W et al., 2012b). Curcumin can also improve the cardiac function of rats after myocardial infarction in a dose-dependent manner(Y et al., 2014), and can prevent the progression of heart failure after myocardial infarction(A et al., 2018b). In the treatment of heart failure, curcumin can inhibit myocardial fibrosis and inhibit the activation of myocardial fibroblasts by regulating TGF-

β/Smads signaling pathway to reduce collagen synthesis and reduce ventricular remodeling after heart failure(AM et al., 2019). However, curcumin has poor water solubility, easy oxidation in vitro and low bioavailability. For example, in the human pharmacokinetic test, volunteers take a large dose of curcumin 12 grams per day, but the blood concentration is too low to be detected, and its metabolic degradation is very fast. These shortcomings seriously restrict the wide clinical application of curcumin(B and G, 1978; JR and ZY, 2014).

In order to increase the water solubility and bioavailability of curcumin, various dosage forms of curcumin carriers have been developed, such as polymer nanoparticles, polymer micelles, microemulsions, microspheres, liposomes and polymer hydrogels(H et al., 2008). These carriers greatly improve the water solubility and bioavailability of curcumin by encapsulation, chemical bond linking or physical adsorption. The degradation products of small molecular hydrogels of polypeptides are amino acids, which have the advantages of easy degradation, high biocompatibility, non-toxicity and safety, and have been widely used in biological fields such as three-dimensional cell culture, drug controlled release, biosensor and so on. At present, cur-hydrogels are mainly made of high molecular hydrogels (such as galactose, chitosan, gelatin, chitin and polyacrylamide gelatin, etc.) or covalently linked to cur-hydrogels(Z and S, 2012). It has been studied that polypeptide Nap-GFFYG-RGD and curcumin monomer were assembled into small molecular polypeptide hydrogel, which significantly improved the anti-tumor effect(J et al., 2014a).

In this study, we prepared a kind of hydrogel as curcumin carrier in order to improve the bioavailability of curcumin in the treatment of cardiac ischemia-reperfusion injury.

Methods And Materials

2.1 Animals

Healthy male SD rats were selected and fasted 12 hours before the animal experiment. The rats were anesthetized by pentobarbital sodium according to their body weight. The ECG were monitored and recorded by electrocardiograph.

Cut the skin of the rat neck with aseptic scissors, fully expose the trachea, intubate the trachea with an indwelling needle, connect the small animal ventilator, give artificial ventilation (pressure 3kpa, frequency 70 times / min) to assist breathing, open the chest in the left fifth auxiliary room beside the sternum, separate the pericardium, fully expose the heart, the left coronary artery is located between the left atrial appendage and the pulmonary artery, and use a small curved needle at 1-2 mm under the left atrial appendage. The left anterior descending branch of the coronary artery was punctured at the upper 1/3 of the left anterior descending branch (note that the needle depth was not more than 1 mm, and the width was not more than 3 mm). The color of the anterior wall of the distal end of the coronary artery was observed to become purplish red. ECG monitoring showed that the ST segment in lead I, II, avL of ECG elevated 0.2mV as a sign of successful operation. 40uM curcumin or cur-hydrogel was injected to

ventricular wall at the bilateral 1mm of the ligation site with an insulin injection needle (30 G), and then the chest was closed and sutured.

2.2 Cardiac hemodynamic measurement

A small latex balloon filled with water was inserted into the left atrium by polyethylene intubation and entered into the left ventricle. The pressure transducer was connected to measure the left ventricular diastolic pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), and the maximum rate of rise and fall of left ventricular pressure. The data were input into the computer to analyze and record them.

2.3 Cardiac ultrasound examination of cardiac function.

Five mice in each group were randomly selected for cardiac ultrasound 24 hours after reperfusion. After intraperitoneal injection of pentobarbital sodium, the mice were fixed on a constant temperature plate at 37 ℃. The chest was evenly smeared with ultrasonic coupling agent and detected by Mindray DP-50 ultrasound system. Each mouse was measured for 10 consecutive cardiac cycles and the left ventricular ejection fraction (LVEF) and left ventricular short axis shortening rate (LVFS), LVEF=[(LVEDV-LVESV)/LVEDV]×100%,LVFS=[(LVEDD-LVESD)/LVEDD]×100% .

2.4 Evans blue staining.

The coronary artery was ligated in situ, and retrograde perfusion was performed through the aorta with 2-3 ml Evans Blue (1%) under a certain pressure (80mmHg). Under the action of staining, the non-ischemic heart showed blue, which supported the display of ischemic AAR (risk zone) myocardium.

2.5 HE/MASSON staining

The experimental animals were euthanized by injecting excessive pentobarbital sodium. The heart was removed immediately, and part of the myocardial tissue was fixed in paraformaldehyde, and ethanol was used for gradient dehydration, followed by transparent treatment and paraffin embedding. After the embedded myocardial tissue was cooled and solidified, it was cut into thin slices with a thickness of 5 μ m. The myocardial tissue of rats was stained with HE (hematoxylin-eosin staining) and collagen was stained with Masson.

2.6 Determination of biochemical Indexes of Myocardial tissue.

The myocardial tissue was homogenized at 60 Hz for 60 s for 2 times in an automatic sample grinder, and the supernatant was centrifuged at 3500 r / min for 10 min at 4 ℃. The samples were separated and stored in the refrigerator at-80 ℃ for the determination of content and the activities of SOD, CAT and GPS,GR, respectively. After strictly following the instructions of the kit, the OD values were determined by enzyme labeling instrument.

2.7 Enzyme colorimetric method.

The activities of Ca^{2+}-ATPase and Na^{+}-K^{+} -ATPase in aorta were determined by enzyme colorimetry (Nanjing Jiancheng Institute of Biological Engineering, China). Strictly according to the instructions of ultra-trace Ca^{2+}-ATPase and Na^{+}-K^{+} -ATPase detection kit, the activities of enzyme in myocardial tissue homogenate were determined.

2.8 Preparation of cur-hydrogel

The peptide RADA16-I (Ac-RADARADARADARADA-CONH2) stored at 4°C and was synthesized by Shanghai Bootech BioScience & Technology (Shanghai, China).

The method of configuration of curcumin-RADA16-I solution was as follows: appropriate curcumin and RADA16-I were placed in 10 ml vials, and water was added tovials, obtained curcumin solution with concentration of 5.0×10^{3} M and RADA16-I solution with concentration of 5.8×10^{5} M (0.1 mg/ml). The solution is left in a mixing pan for about 5 days.

2.9 Dynamic Light Scattering (DLS)

The particle size distribution of the RAD16-I-PY suspension was measured by dynamic light scattering particle size analyzer (Nano-ZS90, Malvern, UK). The suspension is shaken vigorously before measurement.

2.10 Drug release kinetics in vitro.

Under the simulated environment of physiological temperature in *vitro*, the release time and rate of curcumin from co-assembled hydrogel were observed. It was detected by high performance liquid chromatography-mass spectrometry (LC-MS).

2.11 Hypoxia-reoxygenation model of cardiomyocytes

H9C2 cardiomyocytes were cultured in DMEM medium containing 10% serum and 1% penicillin-streptomycin at 37 ℃ and 5% CO_2 incubator. According to the experimental group, the original complete culture medium was replaced by serum-free low-sugar DMEM medium, and H9c2 cardiomyocytes were placed in an anoxic incubator with 37 ℃, 5%CO_2 and 2% O_2 for 12 hours. After hypoxia, the culture medium was replaced by complete culture medium and reoxygenated in an incubator of 37 ℃ and 5% CO_2 for 12 hours to establish a cell HR model.

2.12 MTT assay

H9C2 cells were plated in 96-well plates and we used MTT assay to detect the cell viability. MTT (0.5 mg/mL; Beyotime Biotechnology, China) was added after curcumin treatment and incubated for 3 h at 37°C. And 150 μL DMSO was added and incubated for 15 min. We measured the absorbance at 490 nm.

2.13 Flow cytometry.

The Annexin V-FITC/PI apoptosis detection kit was purchased from Solebao Company(Beijing, China), and an appropriate amount of logarithmic growth phase cells were washed twice with pre-cooled PBS. The cells were suspended with 500 ul of bound buffer, mixed with 5 ul of annexin V-FITC and PI, respectively, and placed at room temperature for 15 min. The apoptosis rate was determined by flow cytometry.

2.14 Single fluorescent GFP-LC3 plasmid transfection.

After transient transfection of GFP-LC3 plasmid into H9C2 cells, the changes of green bright spots of GFP-LC3 in each group were observed under double fluorescence microscope, and 5 visual fields were randomly selected in each experimental group to take pictures.

2.15 TUNEL staining.

According to the instructions, the cells or tissues were successively added with biotin labeling solution and 3,3-diaminobenzidine carbon tetrachloride (DAB) chromogenic solution and used PBS. Finally, the 3DHISTECH Pannoramic SCAN system was used to scan 5 non-overlapping visual fields in each group. The apoptotic nuclei and the total number of apoptotic nuclei in the visual field were calculated by Image J software. The apoptosis rate of cardiomyocytes = the number of apoptotic nuclei / the total number of nuclei × 100%.

2.16 DCFH-DA probe staining.

DCFH-DA probe (Sigma, USA) is used to detect the formation of reactive oxygen species. The cardiomyocyte culture medium was removed, diluted DCFH-DA (10uM) probe 1ml was added and incubated at 37 ℃ in 5%CO_2 incubator for 20 min. Rinse with PBS for 3 times × 1 min to fully remove the DCFH-DA probe that did not enter the cell. Then, the fluorescence intensity was detected by laser confocal microscope.

2.17 Determination of mitochondrial membrane potential.

JC-1 reagent (T3168) was purchased from Semel Fisher Technology Co., Ltd. (China). Strictly follow the instructions. 1 μg/mL JC-1 working solution was prepared and incubated at 37 ℃ for 20 min,PBS. Five visual fields were selected for each group and photographed under confocal microscope.

2.18 Western blot.

Take appropriate amount of cells in logarithmic growth phase, after RIPA cleavage, extract total protein, BCA method. After quantitative denaturation, protein electrophoresis-membrane transfer-closure-□ anti-incubation-□ anti-incubation-development exposure were carried out according to the operation steps. The expression of the target protein was expressed by the ratio of the gray value.

2.19 Statistical analysis

All data is presented as a mean ± S.E.M. Statistical analysis was performed using a one-way ANOVA. P-value < 0.01was considered as statistically significant

Results

3.1 Construction and characterization of cur-hydrogel

Cur-hydrogel is a yellow translucent jelly-like hydrogel, which exists stably with time. Cur-hydrogel can form nanofibers with different thickness, the diameter is about 1000nm(Figure 1A), and interweave and winding each other to form a three-dimensional network structure. The cur-hydrogel has good mechanical properties and stability and can slowly release curcumin monomer in vitro(Figure 1B).□Figure1□

3.2 The protective effect of cur-hydrogel on cardiomyocyte injury induced by HR was better than curcumin.

We treated the cells with different concentrations of curcumin or curcumin-loaded hydrogels (20, 40, 60 μM) for 24 hours, followed by hypoxia-reoxygenation injury. MTT results showed that both curcumin

group and curcumin-loaded hydrogel group had inhibitory effect on cell death, and the effect of cur-hydrogel group was better at the same concentration(Figure 2A).

We chose 40 uM curcumin group or curcumin-loaded hydrogel group to inhibit cell death. The results of flow cytometry (Figure 2B) and TUNEL staining (Figure 2C) showed that cur-hydrogel could reduce apoptosis better at the same concentration. The results of Western blotting showed that curcumin or curcumin-loaded hydrogel decreased the protein levels of Bid, Bax, caspase-3 and caspase-9 induced by hypoxia-reoxygenation, and the effect of cur-hydrogel was better than that of curcumin group (Figure 2D). Figure2

As shown in figure 3A, both curcumin and cur-hydrogels can inhibit cell ROS production, and the effect of cur-hydrogel is better at the same concentration. The changes of p38 MAPK/NF-kB in ROS-related pathway were determined by Western blotting. Hypoxia-reoxygenation injury increased the expression of pamp T-p38 MAPK and NF-kB, while cur-hydrogel significantly decreased the expression of these proteins (Figure 3B). Figure3

Curcumin has been reported to affect the level of autophagy. In this study, mRFP-GFP-LC3 adeno-associated virus was used to transfect H9C2 cardiomyocytes labeled LC3. As shown in figure 4A, the expression of autophagosomes decreased due to hypoxia-reoxygenation injury, and cur-hydrogel could promote autophagy more than curcumin. In figure 4B, the transformation of autophagy marker protein LC3-I to LC3-II was further detected by western blot, and the level of p62 protein was measured. Compared with curcumin, cur-hydrogel could promote the transformation from LC3-I to LC3-II and inhibit the expression of p62. Figure4

We used JC-1 probe to detect the degree of mitochondrial depolarization after cur-hydrogel treatment. In HR group, the mitochondrial membrane potential decreased (red fluorescence decreased). After curcumin treatment, the decrease of mitochondrial membrane potential was alleviated, and after Cur-Hydrogel treatment, the decrease of mitochondrial membrane potential was further alleviated. Hydrogel had no effect on mitochondrial membrane potential as in the control group (Figure 5A). Western blotting showed that p-Cx43/Cx43 ratio was decreased by HR treatment, p-Cx43/Cx43 ratio was improved by curcumin treatment, and p-Cx43/Cx43 ratio was further improved by cur-hydrogel group (Figure 5B). Figure5

3.3 Cur-hydrogel was superior to curcumin in relieving myocardial ischemia-reperfusion injury in rats.

We established a rat model of myocardial ischemia-reperfusion and treated with curcumin or hydrogel curcumin. First of all, we measured the left ventricular diastolic blood pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), and the maximum rate of rise and fall of left ventricular pressure (dp/dt max). The results showed that cur-hydrogel could significantly restore the cardiac function of model mice, and its effect was better than that of curcumin (Table 1). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) coordinate to prevent excessive level

of reactive oxygen species in cells, which is collectively referred to as protective enzyme system. cur-hydrogel can increase the activity of these enzymes more than curcumin and play a protective role in cardiomyocytes (Table 2). The activities of Ca^{2+}-ATPase and Na^{+}-K^{+} -ATPase in myocardial sarcoplasmic reticulum were determined. cur-hydrogel could significantly restore the activity of Ca^{2+}-ATPase and Na^{+}-K^{+} -ATPase in model mice (Table 3).Table1-3

Left ventricular ejection fraction (EF) and left ventricular shortening fraction (FS) were detected by echocardiography. The results showed that cur-hydrogel could restore the cardiac function of model rats (Figure 6A, B).The results of Evans blue staining showed that cur-hydrogel reduced the area of myocardial infarction (Figure 6C).

The myocardial tissue of rats was stained with HE and collagen was stained with masson. The results showed that cur-hydrogel could restore the orderly arrangement of cardiomyocytes and inhibit the excessive deposition of collagen (Figure 6D).

Tissue TUNEL staining showed that, consistent with the results in vitro, heart injury led to the increase of apoptosis, curcumin could reduce the occurrence of apoptosis to some extent, but cur-hydrogel had better effect (Figure 6E).

JAK2/STAT3 is an important intracellular signal transduction pathway, which involves a variety of pathological and physiological processes such as apoptosis, cell proliferation and differentiation. Western blot detection of myocardial homogenate showed that cur-hydrogel activated JAK2/STAT3 signal pathway in rat cardiomyocytes and played a role in myocardial protection (Figure 6F). The above results showed that the effect of cur-hydrogel was better than that of curcumin.Figure6

Discussion

Cardiac rational ventricular remodeling caused by myocardial infarction is one of the most common clinical causes of heart failure, which accelerates researchers' efforts to develop new treatments and techniques to inhibit ventricular remodeling after myocardial infarction(G et al., 2007). Among all kinds of biomaterials, injectable hydrogel has the advantages of good biocompatibility, degradability, high water solubility and injectability, so it has a good development prospect and research value in the repair and treatment of myocardial infarction. Simple injection of hydrogel can provide mechanical support and structural filling for the damaged cardiac structure, increase the thickness of the ventricular wall in the infarcted area to prevent the ventricular wall from gradually thinning, dilating or even rupturing, and improve cardiac function(J et al., 2014b). It can also be used as a carrier for carrying cytokines such as TIMP-3, VEGF or FGF-2 and drug delivery(A et al., 2017; M et al., 2016).

Curcumin is an effective ingredient of turmeric, a traditional Chinese medicine, and has been used in China for thousands of years. Current studies have shown that it has a good protective effect on cardiovascular diseases, including diabetic cardiomyopathy(K et al., 2019), hypertension(E et al., 2019), cardiac hypertrophy(R et al., 2018), myocardial ischemia/reperfusion(A et al., 2018a) and heart failure(K

et al., 2020). In addition, curcumin inhibits the progression of cardiomyocyte hypertrophy, apoptosis and fibrosis by reducing inflammation and oxidative damage in cardiomyocytes and general heart tissue(C et al., 2015). Curcumin is a known P300 inhibitor, in the rat model of diabetic cardiomyopathy, curcumin can improve cardiac contractile function by inhibiting cardiomyocyte hypertrophy and cardiac fibrosis, and reduce the expression of TGF-β1(A et al., 2014). Oral curcumin can reduce the progression of myocardial fibrosis induced by angiotensin II in rats, and this protective effect is related to the decrease of TGF-β1 expression and smad2/3 phosphorylation level in rat heart(XF et al., 2015b). This study further confirmed that curcumin released from hydrogel can reduce the formation of reactive oxygen species, restore mitochondrial function, improve cardiac function, inhibit myocardial apoptosis and activate JAK2/STAT3 pathway.

Previous studies have shown that curcumin is linked to the peptide to form compound I, which can greatly improve its water solubility, and the solubility in PBS can reach 10mg/ml, showing a more effective anti-tumor effect(C et al., 2014). In this study, we successfully prepared curcumin small molecular peptide hydrogel. Hydrogel can improve the water solubility and bioavailability of curcumin. small peptide hydrogel can be degraded naturally in vivo, and the degradation product is small molecular amino acid, which is needed by the body and has high histocompatibility and safety. It can be used as a good extracellular matrix repair material to provide mechanical and functional support for left ventricular wall and improve cardiac structure and function.

JAK2/STAT3 is an important intracellular signal transduction pathway, which plays an important role in cellular stress, regulation of immunity, proliferation, apoptosis, inflammation and tumor(AV et al., 2017). In myocardial tissue, JAK2/STAT3 signal pathway is closely related to the protective effect of myocardium. Studies have shown that JAK2/STAT3 activation inhibited cardiomyocyte apoptosis(Y et al., 2005).

In general, our study showed that cur-hydrogel could reduce cardiomyocyte apoptosis, ROS formation and mitochondrial damage in *vitro*, and the effect was better than that of curcumin. Compared with curcumin, cur-hydrogel can effectively improve cardiac function (FS,EF), inhibit left ventricular dilatation, inhibit ventricular remodeling and collagen synthesis, and activate JAK2/STAT3 pathway to protect injured myocardium in rat ischemia-reperfusion model, which may become a feasible scheme for clinical treatment of myocardial infarction.

Conclusion

In general, our study showed that cur-hydrogel could reduce cardiomyocyte apoptosis, ROS formation and mitochondrial damage in *vitro*, and the effect was better than that of curcumin. Compared with curcumin, cur-hydrogel can effectively improve cardiac function (FS,EF), inhibit left ventricular dilatation, inhibit ventricular remodeling and collagen synthesis, and activate JAK2/STAT3 pathway to protect injured myocardium in rat ischemia-reperfusion model, which may become a feasible scheme for clinical treatment of myocardial infarction.

Abbreviations

Ischemia-reperfusion injury (IRI)

curcumin-hydrogel (cur-hydrogel)

liquid chromatography-mass spectrometry (LC-MS)

Superoxide dismutase (SOD)

catalase (CAT)

glutathione peroxidase (GPX)

glutathione reductase (GR)

Declarations

- **Ethics approval and consent to participate**

This study has been approved by the animal experimental ethics committee of Affiliated Hospital of Guilin Medical University

- **Consent for publication**

Not applicable

- **Availability of data and materials**

Not applicable

- **Competing interests**

The authors declare that they have no competing interests

- **Funding**

The authors received no funding for this work.

- **Authors' contributions**

Chilin Liao performed the majority of experiments and analyzed the data; Yang Liu performed the molecular investigations; Mengzhao Huang designed and coordinated the research; Huayong Liu wrote the paper.

- **Acknowledgements**

The authors received no funding for this work

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Tables

Table 1. Changes of cardiac hemodynamics

Group	LVDP(mmHg)	LVEDP(mmHg)	+dp/dtmax(mmHg/s)	+dp/dtmin(mmHg/s)
Sham	98.26±8.59	1725±15.36	1802.45±157.24	1326.57±100.78
IR	61.52±25.92 *	1043.26±98.25 *	1236.26±118.46 *	862.34±56.08 *
IR+40μM Cur	74.25±16.94 #	1291.05±113.05 #	1369.34±105.34 #	969.78±87.49 #
IR+40μMCur Hydrogel	81.93± 8.33 #	1481.23±122.45 #	1527.57 ± 144.28 #	1149.78±94.26 #

Table 2. Detection of biochemical indexes in myocardial tissues

Group	SOD	CAT	GPX	GR
Sham	153.86± 13.67	47.45±3.79	67.39±45.55	51.42±4.93
IR	48.38±25.49 *	13.45±1.72 *	23.29±42.43 *	15.83±1.33 *
IR+40μM Cur	94.25±8.88#	22.15±2.62#	44.37±3.86#	39.58±13.55#
IR+40μMCur Hydrogel	135.43±11.35#	38.93±3.06#	52.98±4.93#	44.78±24.25#

Table 3. The activity of $Ca2^{+}$ ATPase and Na^{+}-k^{+}-ATPase

Group	$Ca2^{+}$ ATPase ($umol\cdot mg^{-1}\cdot h^{-1}$)	Na^{+}-k^{+}-ATPase ($umol\cdot mg^{-1}\cdot h\cdot^{1}$)
Sham	1.65±0.14	3.28±0.25
IR	0.594±0.05 *	1.274±0.15 *
IR+40μM Cur	0.874±0.07 #	2.064±0.19 #
IR+40μMCur Hydrogel	1.214±0.11 #	2. 664±0.25 #

Figures

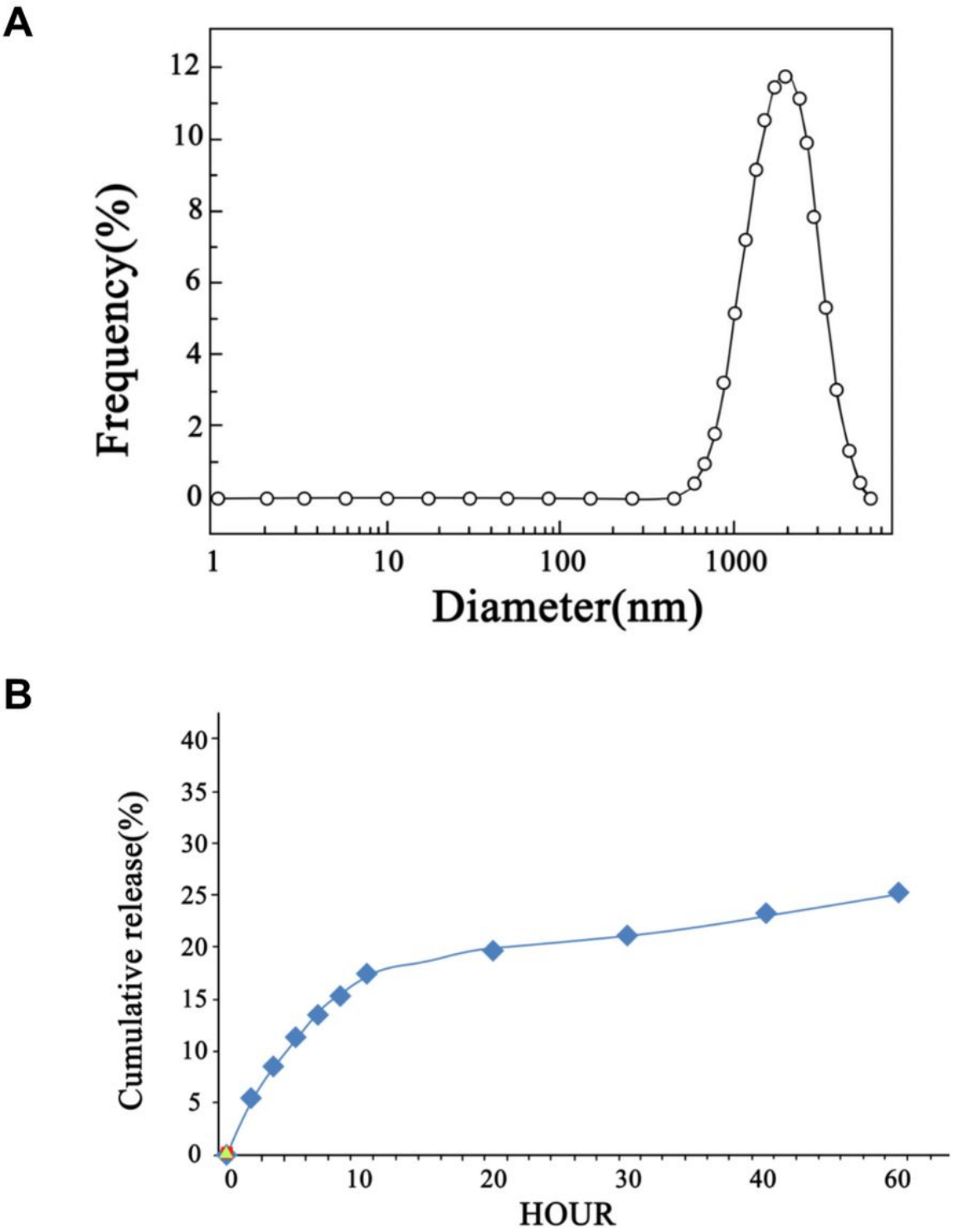

Figure 1

Characteristics of cur-hydrogel. (A) DLS was used to detect the particle size of curcumin hydrosol. (B)The cumulative release amount of curcumin from hydrogel to PBS solution (37 °C pH 7.4).

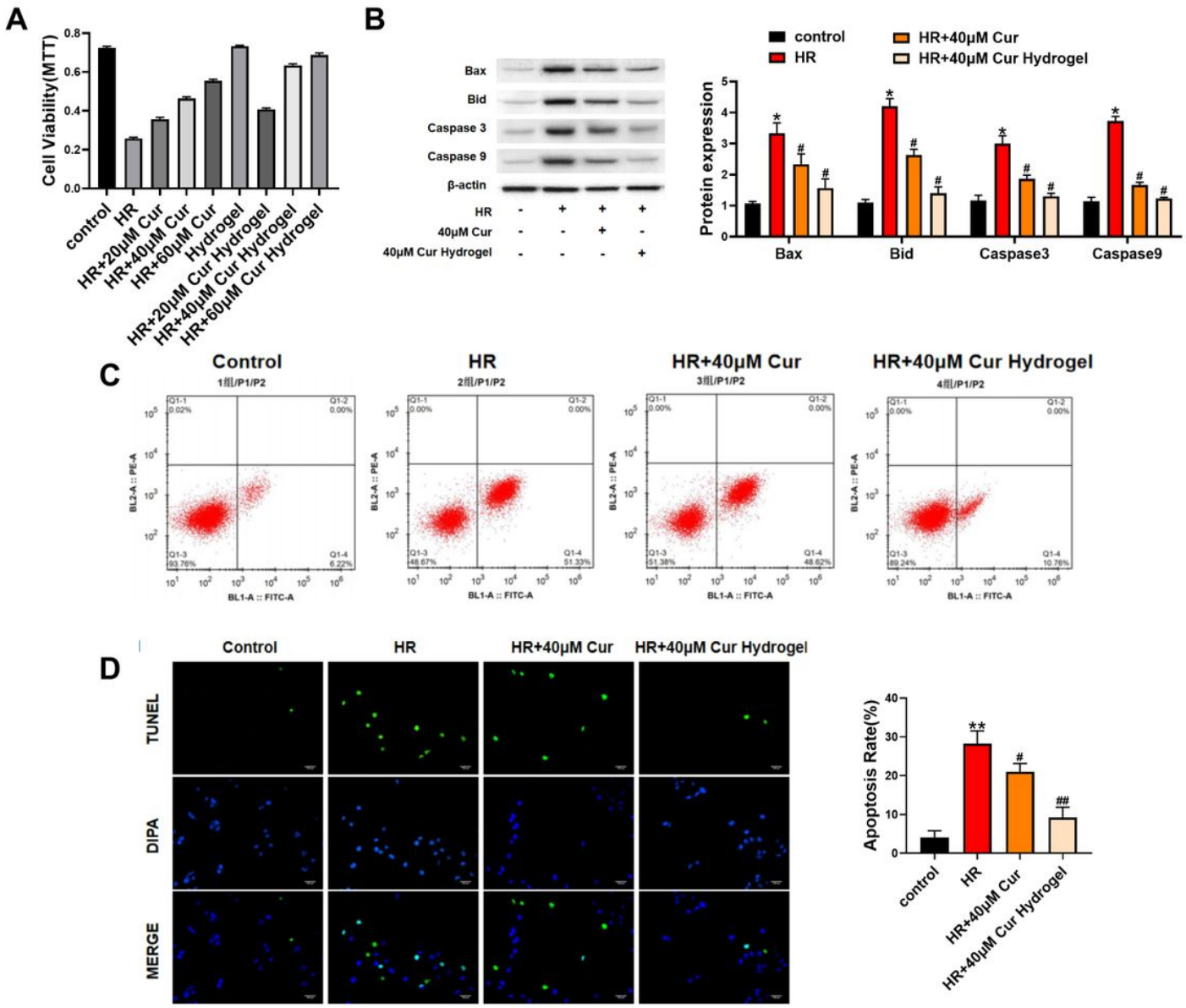

Figure 2

Cur-hydrogel attenuated cardiomyocyte apoptosis induced by HR. (A) The effect of curcumin and cur-hydrogel on cell survival rate was evaluated by MTT. n=5,*p<0.05, **p<0.01 vs control. (B) Apoptosis-related proteins were detected by western blot. n=3, *p<0.05 vs control; #p<0.05 vs HR. (C) Annexin V-FITC/PI apoptosis kit was used to detect cell apoptosis. (D) TUNEL staining and statistical chart. (Bar=150 μm) n=5, **p<0.01 vs control; #p<0.05, ##p<0.01 vs HR.

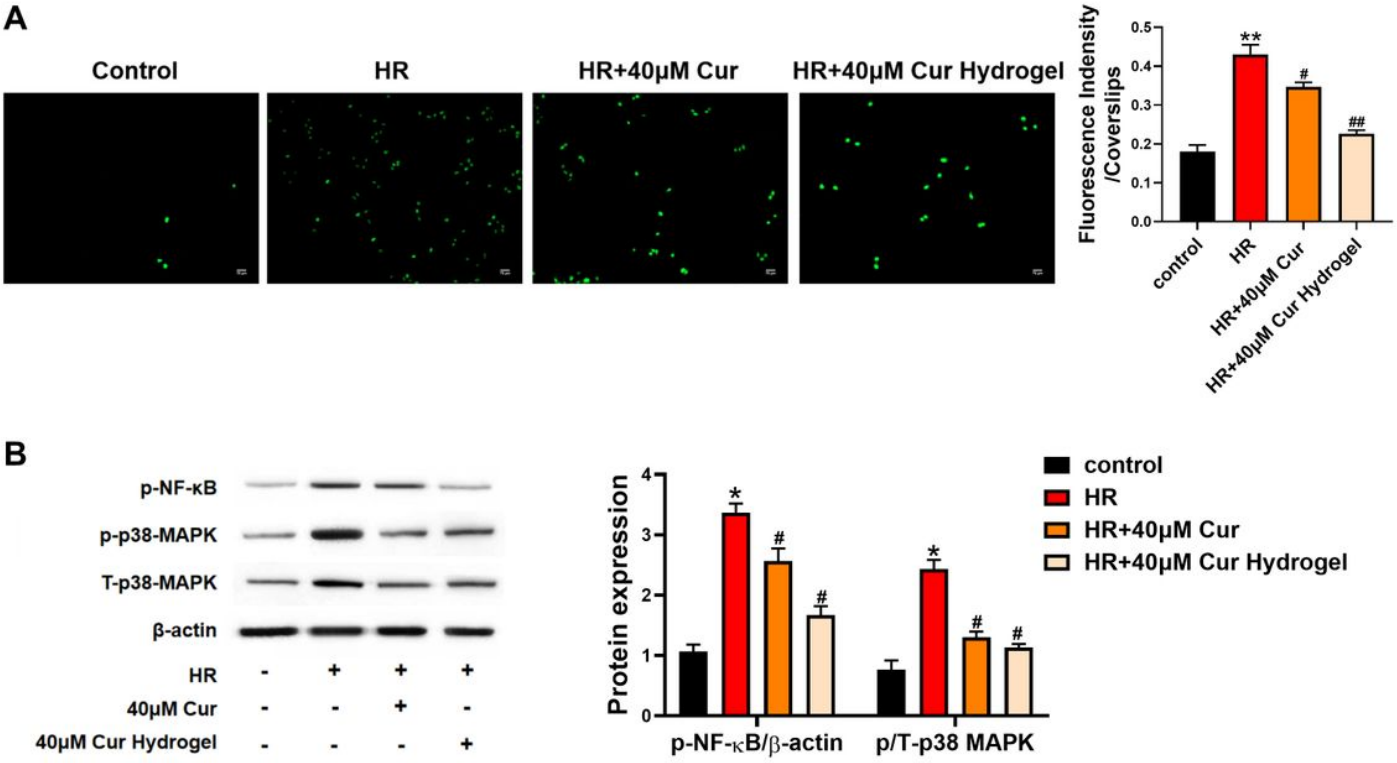

Figure 3

Cur-hydrogel inhibited the formation of reactive oxygen species induced by HR. (A) Detection of reactive oxygen species formation by DCFH-DA staining. (Bar=75μm) n=3, **p<0.01 vs control; #p<0.05, ##p<0.01 vs HR. (B)p-p38 MAPK and p-NF-kB protein levels were evaluated by western blot. n=3, *p<0.05 vs control; #p<0.05 vs HR.

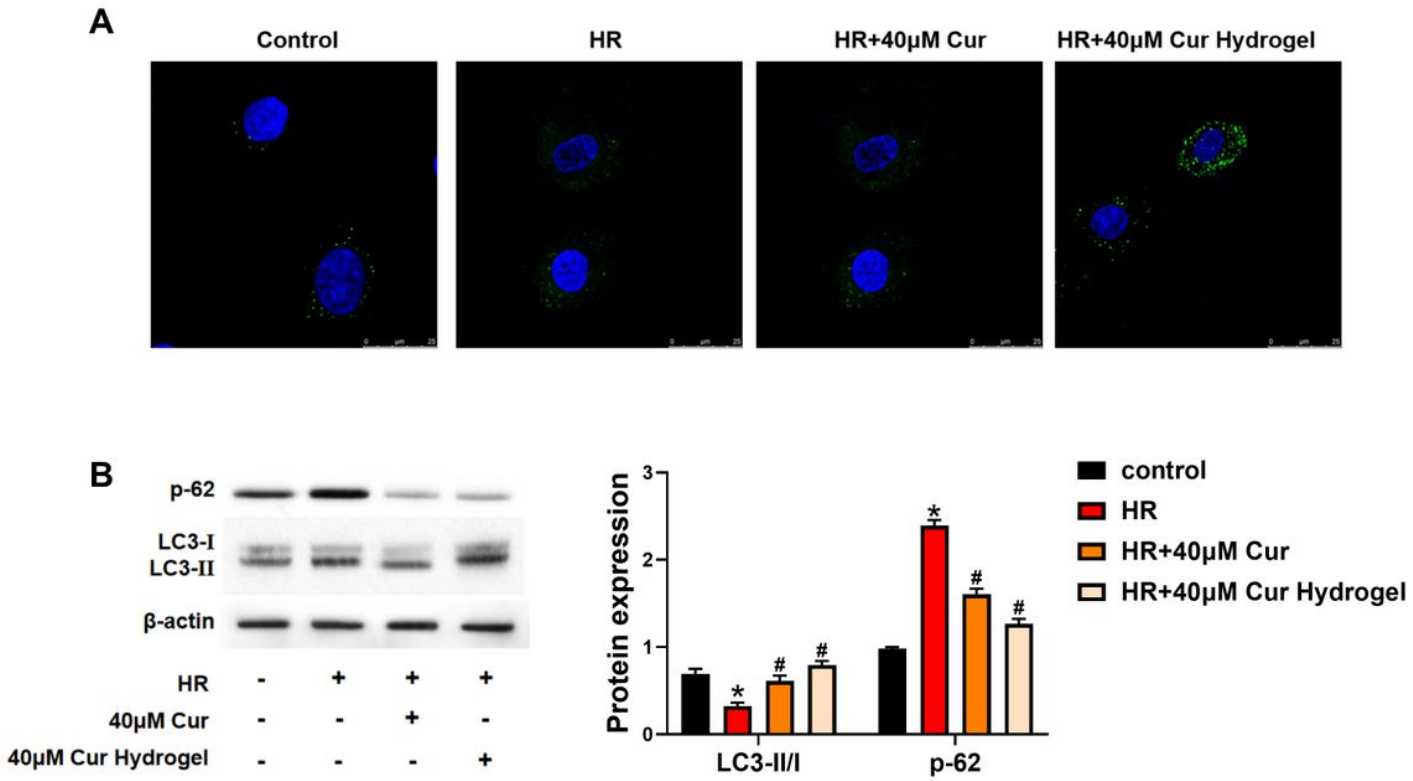

Figure 4

Cur-hydrogel promoted autophagy of injured cardiomyocytes. (A) GFP-LC3 staining was used to assess autophagy (Bar=25μm). (B) p62 and LC3 protein levels were analysed by western blot. n=3, *p<0.05 vs control; #p<0.05 vs HR.

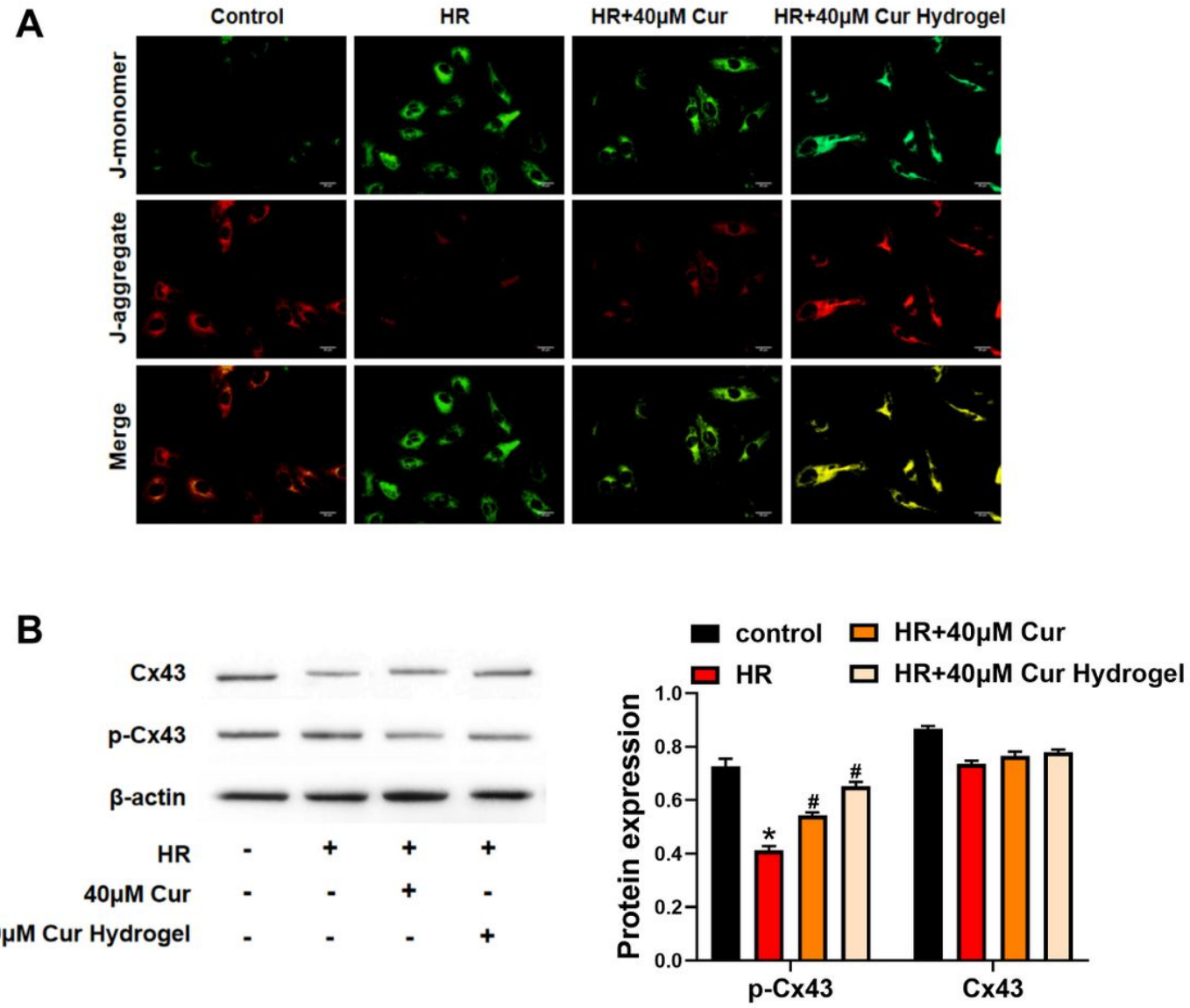

Figure 5

Cur-hydrogel restored mitochondrial function. (A) JC-1 staining was used to analysis mitochondrial membrane (Bar=20μm). (B) Protein level of Cx43 and phosphorylated Cx43 were assessd by western blotting. n=3, *p<0.05 vs control; #p<0.05 vs HR.

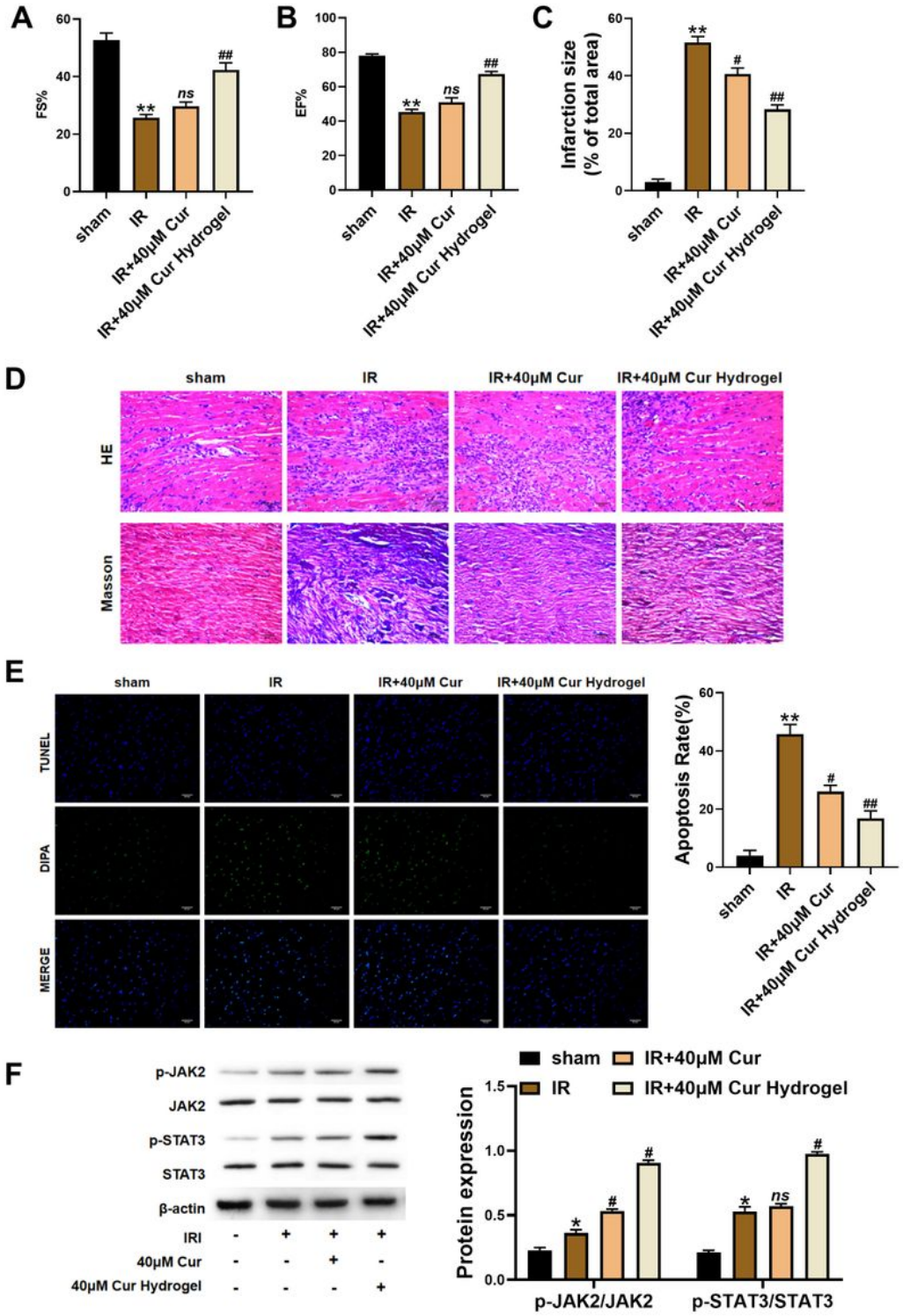

Figure 6

Cur-hydrogel was better than curcumin in improving myocardial ischemia-reperfusion injury in rats. (A-B)Ejection fraction (EF%) and(B)fractional shortening (FS%) examined by echocardiography. n=5, **p<0.01 vs sham; ##p<0.01 vs IR; ns=nonsignificant. (C) Statistical chart of myocardial infarction area.n=5, *p<0.05 vs sham; #p<0.05, ##p<0.01 vs IR. (D) HE and Masson staining of rat myocardial

tissue (Bar=40μm). (E)TUNEL staining and statistical chart. (Bar=40 μm) n=5, *p<0.05 vs sham; #p<0.05, ##p<0.01 vs IR. (F)Protein level of JAK2/STAT3 by western blotting. n=3, *p<0.05 vs sham; #p<0.05 vs IR.