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Liqi Huoxue Dripping Pill protects ischemia and reperfusion-induced myocardial injury through mechanism involving inhibition of necroptosis in rat

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Abstract: Liqi Huoxue Dripping Pill (LHDP), a traditional Chinese herbal formulation, has been shown in recent studies to possess cardioprotective effects. This study aimed to investigate the effects of LHDP on myocardial ischemia-reperfusion injury (MIRI) in rats and their underlying mechanisms. MIRI model rats were administered varying doses of LHDP or Necrostin-1 (Nec-1s, an inhibitor of necroptosis) for 7 days. Echocardiography was performed to observe ventricular remodeling and systolic function. The infarct and fibrosis sizes were evaluated by pathological staining. The serum inflammatory factor levels were evaluated by enzyme-linked immunosorbent assay (ELISA). To investigate the effects of LHDP on necroptosis in I/R-treated hearts, we conducted analysis with quantitative real-time polymerase chain reaction (q-PCR) and Western blotting (WB). LHDP not only alleviated adverse ventricular remodeling and cardiac dysfunction induced by I/R, but also reduced inflammatory factor levels the serum levels of TNF-α, IL-6 and IL-1β. Moreover, LHDP suppressed the expression of necroptosis-related molecules and these functions like Nec-1s. The study demonstrates that treatment with LHDP was conducive to reducing the inflammatory response and improving myocardial function following MIRI. The myocardial protective function of LHDP may be associated with the inhibition of necroptosis.

Keywords: Chinese herbal drugs, Liqi Huoxue Dripping, ischemia and reperfusion-induced myocardial injury, necroptosis.

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INTRODUCTION

An extremely dangerous disorder that might pose a threat to human health and lead to mortality is myocardial infarction (MI). It is primarily caused by blood clots obstructing coronary blood flow (Vaduganathan *et al*., 2022). In the development of MI, ischemia and hypoxia lead to myocardial cells damage/necrosis and the concomitant accumulation of inflammatory cells and secretion of inflammatory factors further aggravate the development of cardiomyopathy hypertrophy and myocardial fibrosis, which may lead to heart failure (Dauerman and Ibanez, 2021). Clinical approaches such as percutaneous coronary intervention (PCI) are effective in regaining myocardial blood flow. These interventions can alleviate the sustained injury caused by hypoxia and ischemia (Sabatine and Braunwald, 2021). Unfortunately, myocardial ischemia-reperfusion (I/R) can lead to additional cardiac tissue damage, with up to approximately half of the final myocardial injury area being affected, which greatly reduces the therapeutic effect of blood flow reconstruction (Davidson *et al*., 2019, Algoet *et al*., 2023). At present, mechanical physiotherapy (hypothermia, ischemia postconditioning, etc.) and drug therapy (atrial natriuretic peptide, cyclosporine A) can only provide limited relief for myocardial I/R injury (MIRI). The original intention and primary objective of this study is to explore more practical therapeutic strategies to inhibit myocardial damage caused by I/R.

MIRI involves a series of complex biological dysfunctions, including increased production of inflammatory response, calcium overload and reactive oxygen species. These factors promote the death of myocardial cells further causes an expanding myocardial infarction and micro vascular disruption, which ultimately results in the impairment of the cardiac pumping function (Del Re *et al*., 2019). Numerous studies have focused on apoptosis and necrosis of cardiomyocytes. However, the essential role of necroptosis as a regulatory mechanism that initiates I/R injury has been widely confirmed (Maslov *et al*., 2022). Necroptosis is induced by activated death receptors, including DNA damage, tumour necrosis factor receptor (TNFR) and multiple types of cellular stress. In the tissue microenvironment of MIRI, various stimulators interact with TNFR to initiate necroptosis vis the receptor interacting protein kinase1 (RIPK1), RIPK3, and mixed lineage kinase domain-like (MLKL) which has been recognized to be tightly implicated in the inflammatory process. Meanwhile, many pro-inflammatory cytokines can trigger necroptosis in return (Yuan and Ofengeim, 2024). Animal and pre-clinical studies have demonstrated that RIPK1 inhibitors such as Nec-1s can suppress necroptosis and effectively alleviate I/R injury (Cao and Mu, 2021, Weisel *et al*., 2020).

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Many natural compounds have been shown to play a role in the treatment of cardiovascular diseases (Munir *et al*., 2021, Yang and Qian, 2021). Liqi Huoxue Dripping Pill (LHDP) is a modern pharmaceutical compound that is manufactured based on the therapeutic principle of eradicating blood stasis and promoting blood circulation. It had received approval from China Food and Drug Administration (No. Z20120037) for the treatment of cardiovascular conditions in 2012 (Gao *et al.*, 2018). This formulation is the composed of the Litsea lancilimba Merr. (Da guo mu jiang zi) essential oil, Ligusticum (Chuan xiong), Allium macrostemon bunge (Xie bai) and Blumea balsami era (L.) DC. (Ai pian). Clinically, LHDP has been effectively utilized to treat angina due to coronary heart disease (Wang *et al.*, 2019). A multi-center study evaluated the safety and efficacy of LHDP in 2433 elderly patients with chronic stable angina pectoris. After 4 weeks of treatment, the study reported a 71.5% symptom relief rate, a 84.4% reduction in nitroglycerin use, and a 57.08% effective rate on electrocardiogram findings, and 45 adverse events (3.95%) were recorded. Furthermore, patients' quality of life showed significant improvement (Han *et al*., 2021). A network pharmacology analysis suggested that modulation of inflammation could play a role in the drug's efficacy in treating MIRI, with a key pathway identified as the TNF signaling pathway (Zou *et al*., 2022). A recent report showed that LHDP decreased serum levels of interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in rats with chronic heart failure (CHF) induced by isoproterenol (Chen *et al*., 2018). Therefore, it is reasonable to assume that LHDP offers a defensive benefit to against cardiac damage caused by I/R. To clarify this hypothesis and investigate the specific mechanism of LHDP in cardiovascular disease treatment, we established a rat MIRI model. In addition, we analyzed whether LHDP alleviated I/R-induced cardiomyocyte necroptosis through the RIPK1/RIPK3/ MLKL signaling axis. This study provides a theoretical and experimental foundation for the clinical utilization of LHDP. Moreover, it offers new therapeutic methods for mitigating I/R-induced necroptosis.

MATERIALS AND METHODS

Animals

We purchased Male Sprague-Dawley (SD) rats (180-200 g, SPF grade, certificate SCXK 2019-0008) from Hua Fu Biotechnology Co. Ltd. (Beijing, China). The rats were kept in Central Laboratory of Gui Zhou Provincial People's Hospital under standard conditions with free water intake and a standard diet. All rats were administered under the regulations of the National Institutes of Health.

Protocol and group

Via an intraperitoneal injection of 0.3% pentobarbital at a dosage of 50 mg per kilogram, all rats were anesthetized. Continuous BL-420 electrocardiographic monitoring was performed on the rats. 60 SD rats were randomly divided into 6 groups, with 10 rats per group. A thoracotomy was performed at the left fourth intercostal space to expose the heart after the pericardium was opened. The descending branch of the left anterior descending (LAD) coronary artery was ligated with a silk suture. Reperfusion was initiated 30 minutes later by loosening the ligature. In the Sham group, the LAD coronary artery was threaded but not tied. Based on prior research findings and body surface area conversions from clinical doses documented in relevant literature *(*Qiao *et al., 2022,* Mao *et al*., 2024), the Nec-1s group received 1.65 mg/kg/d of Nec-1s via intraperitoneal injection, serving as the positive control. The LHDP groups received intragastric doses of 43.75 mg/kg/d, 87.5 mg/kg/d, or 175 mg/kg/d of LHDP. The Sham and I/R groups were administered intragastric 2 ml/kg/d of normal saline. All groups underwent continuous administration for 7 days following model establishment.

We then monitored the rats using continuous BL-420 electrocardiography. Thoracotomy was carried out within the left fourth intercostal space, to expose the heart following pericardial opening. The descending branch of the left anterior coronary artery was ligated using a silk suture. After 30 minutes, reperfusion was achieved by releasing the knot. Rats were intragastrically injected with or without LHDP at doses of 43.75 mg/kg/d, 87.5 mg/kg/d, or 175 mg/kg/d per day for 7 days. Another group of rats was injected intraperitoneally with 1.65 mg/kg of Nec-1s per day for 7 days, with Nec-1s serving as a positive control group. Rats that underwent surgery without ischemic insult were defined as the Sham group. All rats were euthanized by cervical dislocation following anesthesia

Echocardiography

Echocardiography was performed before sacrifice using an EPIQ 7C echocardiography machine (PHILIPS Medical America) equipped with an S8-3 (10-12 MHz) transducer. Both the parasternal long-axis and short-axis views provided M-mode pictures.

Pathological staining

The rat cardiac tissues were immediately frozen after sacrifice, then sliced into 6 pieces and incubated in a 1% TTC solution. Subsequently fixed in a 10% formalin solution for 24 h. Using Image J (NIH, USA), the whole area of the myocardium and the infarct region were measured. Other hearts were preserved in 4% in 4% paraformaldehyde. Treated hearts were incubated with HE or Masson. Nikon microscope was utilized to take photographs.

Western blotting analysis

Cardiac tissue proteins were isolated in RIPA buffer with 0.1% PMSF. After determining protein content in tissue lysates using a BCA protein assay kit, 25 µg of protein

was separated on 10% SDS-PAGE gels. The proteins were then transferred to PVDF membranes, blocked for an hour with blocking buffer, and incubated with anti-GAPDH, anti-RIPK1, anti-RIPK3, anti-MLKL, anti-Phospho-RIPK1 (Tyr384), anti-Phospho-RIPK3 (Ser316), and anti-Phospho-MLKL (Ser358) for 18 h, followed by incubation with IgG-HRP for 2 h. The UVP BioSpectrum Imaging System detected the signals using enhanced ECL reagents. ImageJ software was utilized to calculate the intensity of all signals.

Quantitative real-time PCR

Cardiac tissues RNA was extracted using RNAiso Plus following the protocol. Complementary DNA was generated using a cDNA synthesis kit adhering to the guidelines. Then, the RT-PCR was amplified using SYBR Green PCR Master Mix. Melting curve analysis was used to monitor the PCR product purity, with each sample tested in triplicate. The primer sequences used are listed in table 1.

Enzyme-linked immunosorbent assay (ELISA) analysis

After MIRI, rats were treated with or without LHDP (43.75 mg/kg/d, 87.5 mg/kg/d or 175 mg/kg/d) or Nec-1s for 7 days, blood collected from the abdominal aorta of the rats was centrifuged at 4 °C, 2500 r/min for 15 minutes. The supernatants were stored at -80°C. The concentrations of serum TNF-α, IL-6, and IL-1β were determined using ELISA kits. The processes were implemented following the manufacturer's directions.

Ethical approval

The trials were approved by the Animal Experiment Ethics Committee of Guizhou Provincial People's Hospital (Examination and approval No.2021[366]).

STATISTICS ANALYSIS

Data from three separate experiments (mean ± SEM) were analyzed with GraphPad Prism 9. To compare groups, a one-way ANOVA was used, followed by Tukey's post hoc test. A *P*-value of <0.05 was judged statistically significant.

RESULTS

LHDP improved cardiac morphology and left ventricular contraction function in the post-MIRI hearts

Echocardiographic assessments were performed to evaluate cardiac function and left ventricular remodeling, aiming to explore the potential therapeutic effects of LHDP on MIRI. As shown in fig. 1, treatment with LHDP (87.5 mg/kg/d or 175 mg/kg/d) significantly alleviated I/R induced ventricular remodeling and cardiac dysfunction, which was confirmed by improved LVEDV, LVESV, LVmass, LVmassI, EF%, and FS %. We also found that 43.75 mg/kg/d of LHDP was insufficient to improve cardiac dysfunction induced by I/R. Given the key role of necroptosis in MIRI, we further explored whether Nec-1s could reduce this injury. As expected, Nec-1s administration significantly improved myocardial function after I/R. High-dose LHDP had a similar therapeutic effect to necroptosis inhibitors (Nec-1s) and is superior than low-dose.

LHDP reduced infarct size, fibrosis and myocardial damage in post-MIRI hearts

It is widely accepted that the severity of MIRI can be determined by measuring the myocardial infarct size and fibrosis area (fig. 2 A, B) (Dauerman and Ibanez, 2021, Heusch, 2020). Through TTC and Masson staining, we found less myocardial infarction size and fibrosis area in Higher doses LHDP or Nec-1s-treated MIRI rats than in low doses and untreated rats. Moreover, H&E staining showed that treatment with LHDP or Nec-1s resulted in weakened myocardial cell damage and relatively less inflammatory cell infiltration after MIRI (fig. 2C). In addition, high doses of LHDP were more effective than low doses in alleviating myocardial infarct size, fibrosis area, and inflammatory pathology. These results suggest that LHDP could shield the myocardium from I/R.

LHDP reduces inflammatory cytokines in serum of MIRI

We explored the possibility that LHDP's protective effect against I/R-induced myocardial damage is linked to reduced inflammatory cytokines. As shown in fig. 3, LHDP treatment significantly lowered the serum levels of TNF-α, IL-6, and IL-1β, suggesting that LHDP may prevent the production of inflammatory cytokines, thereby protecting the heart from I/R injury. Higher doses of LHDP have a stronger therapeutic impact than lower doses and are comparable to Nec-1s.

LHDP inhibits proteins associated with necroptosis in rat exposed to I/R treatment

To further explore whether LHDP can inhibit necroptosis, WB was used to identify related protein expressions. In fig. 4, phosphorylation levels of RIPK1, RIPK3 and MLKL in LHDP-treated groups were lower than MIRI group. Real-time PCR results showed that LHDP or Nec-1s treatment significantly inhibited the mRNA levels of RIPK1, RIPK3 and MLKL in MIRI rats (fig. 4), suggesting that the palliative effect of LHDP on MIRI is dependent or at least partially dependent on the inhibition of necroptosis. The impact of high-dose LHDP is comparable to Nec-1s and superior to low-dose.

TNF-α leads to the initiation and phosphorylation of RIPK1/RIPK3. They form the RIPK1-RIPK3 necrosome that can phosphorylate MLKL. P-MLKL transfers from the cytoplasm to the membrane to elicit inflammatory response via recruiting a large number of leukocytes and damage associated molecular pattern (DAMP). Cardiomyocytes swell and plasma membrane rupture ultimately.

Table 1: Sequences of Primers for RT-PCR

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
RIPK1	CCACTTTGGAACAACGGAGTAT	GAGTCATCTGGTGGTGCCAAG
RIPK3	AAGTTATGGCTCAATGGTGCG	AAGATTCACCATAGCCTTCACCT
MLKL	TCCCACAAGATTTCCAAGTCAA	GCCTCACTATTCCAACACTTTCG
GAPDH	CTGGAGAAACCTGCCAAGTATG	GGTGGAAGAATGGGAGTTGCT

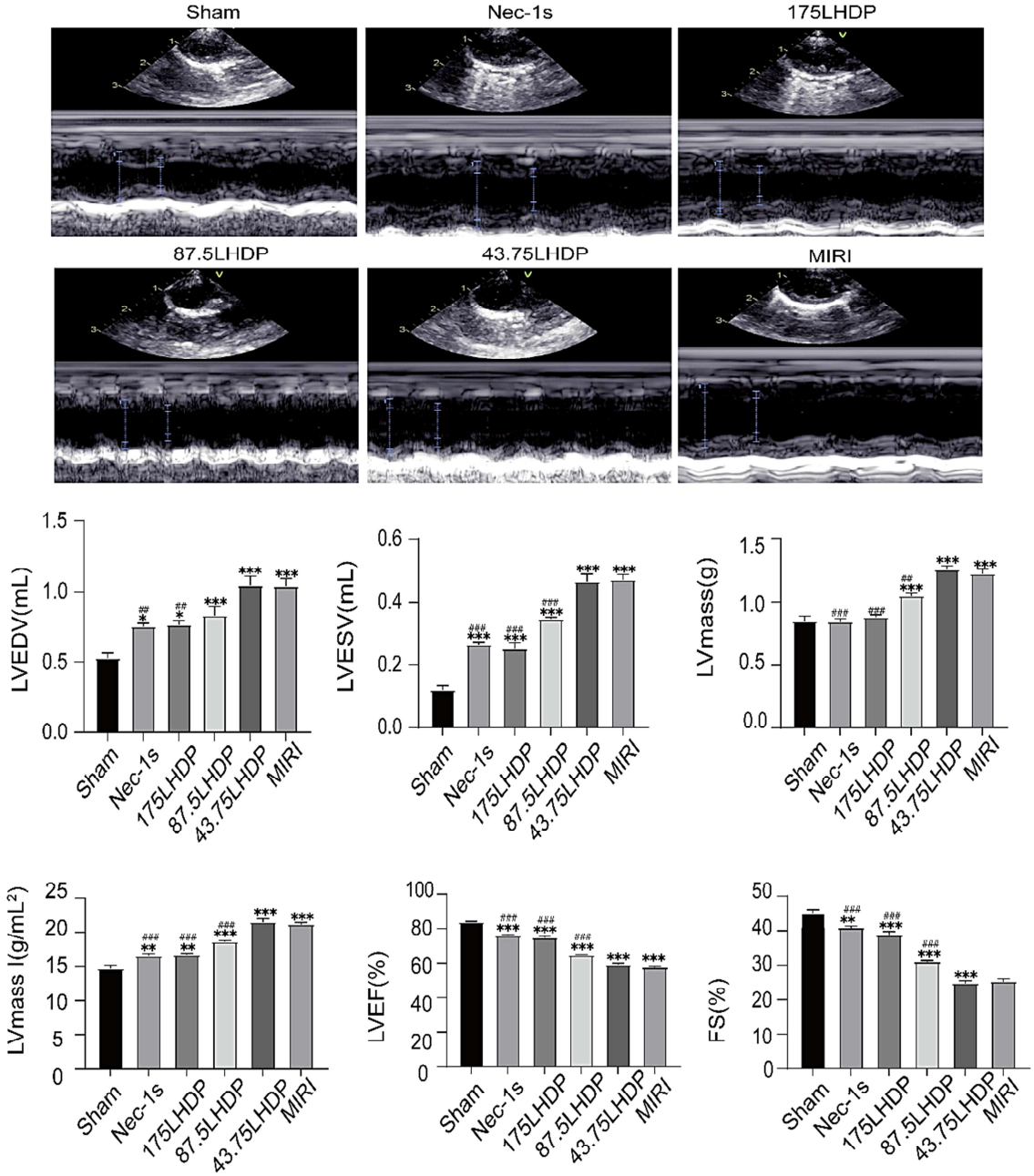

Fig. 1: Effect of LHDP on cardiac morphology and left ventricular contraction function. MIRI rats were intragastrically injected with or without LHDP (43.75mg/kg/d, 87.5, or 175mg/kg/d) for 7 days and other MIRI rats were injected intraperitoneally with 1.65 mg/kg/d of Nec-1s per day for 7 days. Rats that underwent surgery without ischemic insult were defined as Sham. Echocardiography was performed to evaluate: (A) LVEDV, (B) LVESV, (C) LVmass, (D) LVmassI, (E) EF% and (F) FS % in the hearts of these rats. Data are presented as the mean ± SEM (n=10). * P<0.05, ** P<0.01, *** P<0.001 vs. Sham group; # P<0.05; ## P<0.01; ### P<0.001 vs. I/R group.

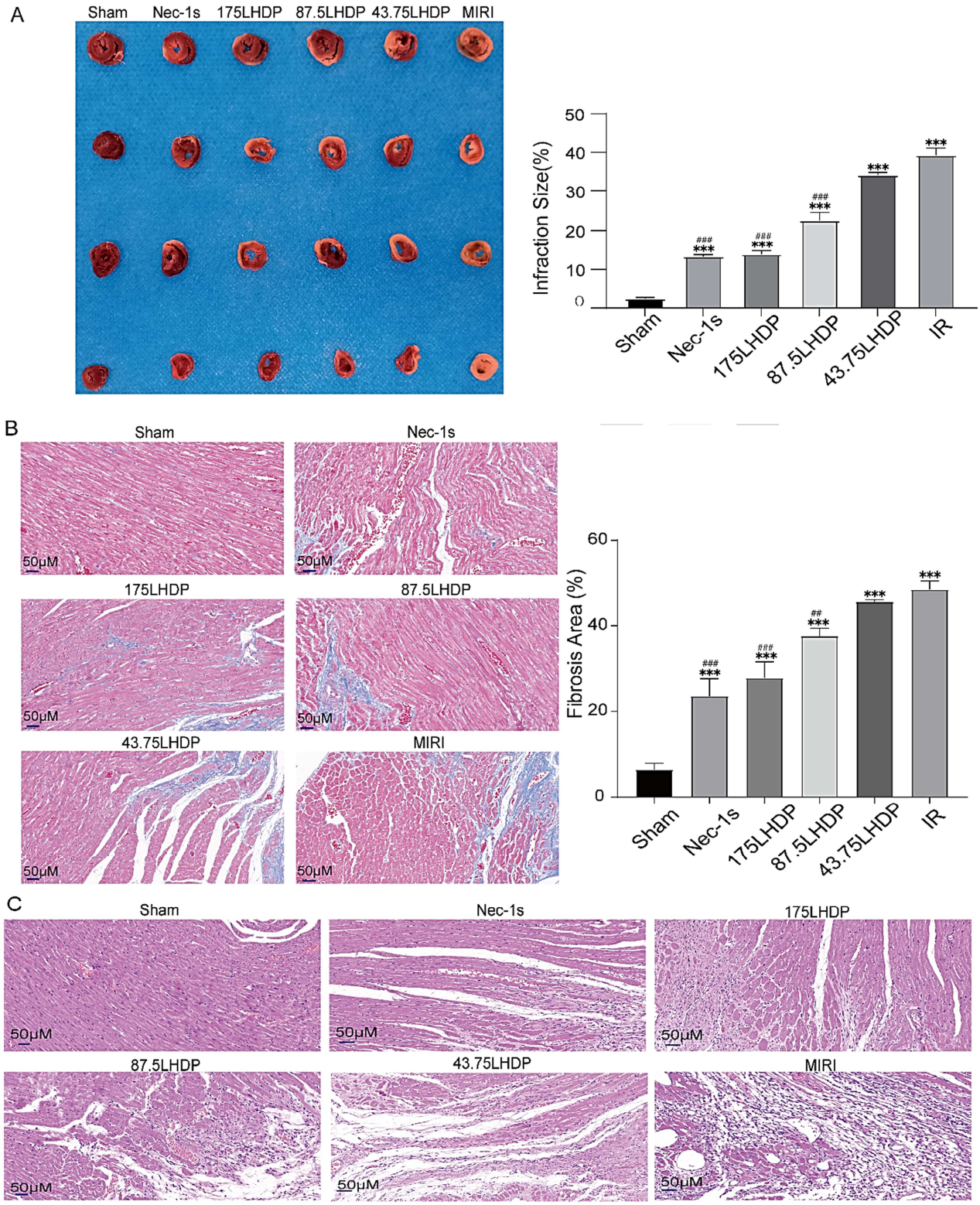

Fig. 2: LHDP reduces myocardial infarction size. After MIRI rats were treated with or without LHDP (43.75 mg/kg/d, 87.5 mg/kg/d or 175 mg/kg/d) or Nec-1s for 7 days, hearts were collected to measure myocardial infarct size and pathological change through TTC (A)/Masson (B)/H&E staining (C). Scale bar = 50 μm. Data are presented as the mean ± SEM (n=3). * P<0.05, ** P<0.01, *** P<0.001 vs. Sham group; # P<0.05; ## P<0.01; ### P<0.001 vs. I/R group.

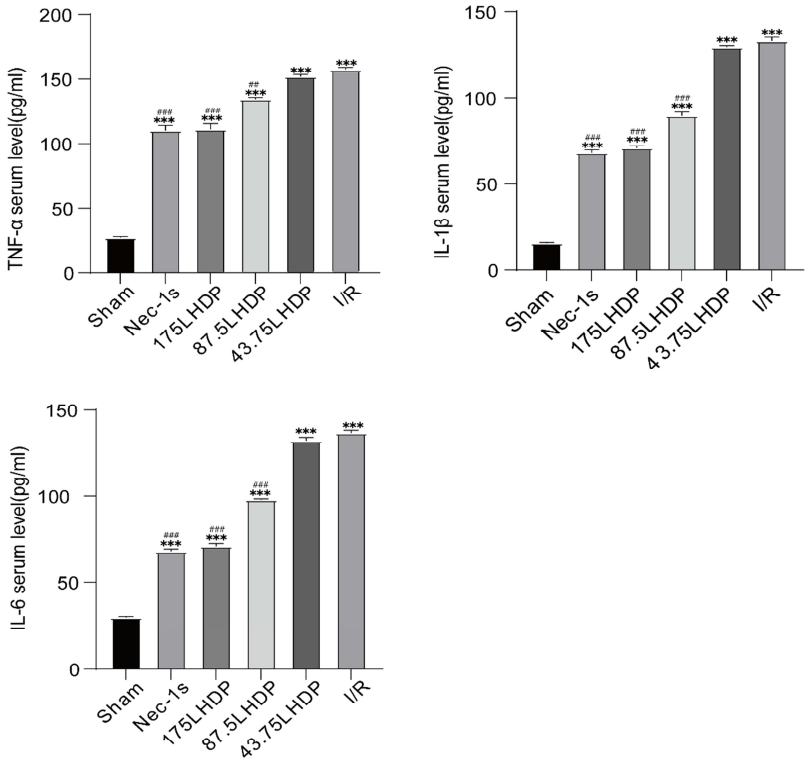

Fig. 3: LHDP reduces inflammatory cytokines in serum of MIRI rats. MIRI rats were given LHDP (43.75mg/kg/d, 87.5 mg/kg/d, or 175 mg/kg/d) or Nec-1s for 7 days. Serums were drawn in order to measure the amounts of (A) TNF-α, (B) IL-6 and (C) IL-1β by ELIAS. The data (n=5-8) are shown as the mean ± SEM. **P*<0.05, ** *P*<0.01, *** *P* <0.001 vs. Sham group; # *P*<0.05; ## *P*<0.01; ### *P*<0.001 vs. I/R group.

DISCUSSION

In recent years, the incidence of myocardial stunning and arrhythmias after revascularization has not been markedly reduced. This status is relevant to the lack of standard therapies for MIRI (Hadanny *et al*., 2021). Numerous studies have highlighted that mitigating inflammation and necroptosis via the RIPK1/RIPK3/MLKL signaling pathway can effectively reduce I/R-induced cardiomyocyte death (Maslov *et al*., 2022, Cao and Mu, 2021). Previous investigations have demonstrated the cardioprotective effects of LHDP in heart disease; however, its specific role in MIRI remains unclear. Our findings in this work suggest that I/R damage can lead to impaired left ventricular geometry and function, companied by elevated levels of inflammation, and proteins relevant to necroptosis. These effects were mitigated by treatment with LHDP or Nec-1s. Our research offers proof for the first occurrence that LHDP can safeguard the rat heart against MIRI and this effect may be associate with the inhibition of necroptosis.

Litsea lancilimba Merr, a member of the Lauraceae family and genus Litsea, is the main component of LHDP. This plant is mainly distributed in Guizhou and Yunnan in China, as well as in the tropical regions of the Americas. *Litsea* has diverse pharmacological functions, such as anti-inflammatory, anti-arrhythmic and analgesic owing to the abundant resources of *alkaloids, flavonoids* and others (Muhammad *et al*., 2022). Because of its multifaceted biological activity, LHDP has the advantage of potently releasing angina caused by coronary heart disease like other Chinese material medicine, such as Compound Danshen Dripping Pill (Liao *et al.*, 2019), which has been utilized in the treatment of heart disease globally. In 2020, Gao analyzed some indices of curative effect on angina pectoris in patients who had taken LHDP and Compound Danshen Dripping Pill (Gao *et al., 2020)*. The results showed that LHDP can effectively relieve angina. Unexpectedly, in the terms of reducing angina pectoris symptoms, LHDP was superior to Compound Danshen Dripping Pill. Numerous pre-clinical and clinical studies demonstrated that LHDP can exert cardioprotective effects by modulating inflammation levels (Lin *et al.*, 2019). A clinical study by Jiang (Jiang *et al*., 2023) demonstrated that compared with Atorvastatin monotherapy, the combination of LHDP and Atorvastatin significantly improved clinical symptoms, electrocardiographic efficacy, endothelial function, oxidative stress markers and inflammatory factors in patients with unstable angina pectoris. These findings highlight the potential synergistic effect of LHDP in managing unstable angina pectoris. Our study discovered that LHDP reduced the levels of inflammatory cytokines, which is consistent with these investigations.

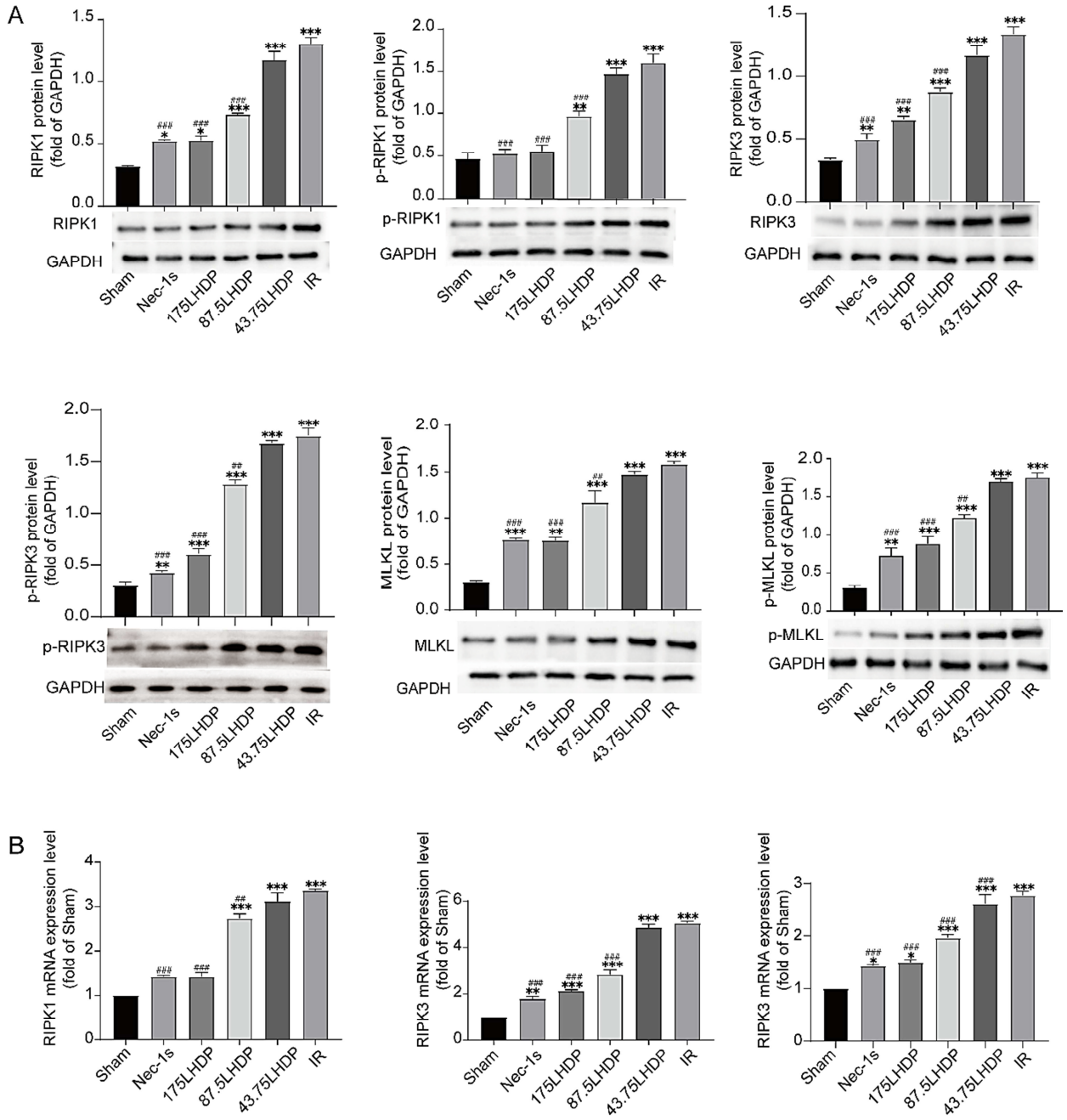

Fig. 4: LHDP inhibits necroptosis in MIRI. After MIRI rats were treated with or without LHDP (43.75 mg/kg/d, 87.5 mg/kg/d or 175 mg/kg/d) or Nec-1s for 7 days, hearts were collected to detect the expression of (A) RIPK1, (B) p-RIPK1, (C) RIPK3, (D) p-RIPK3, (E) MLKL and (F) p-MLKL by WB. The expression of (G) RIPK1, (H) RIPK3 and (I) MLKL by Real time-PCR. The mean ± SEM (n = 3) is used to show the data. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. Sham group; #*P*<0.05; ##*P*<0.01; ###*P*<0.001 vs. I/R group.

It is well known that inflammation is a significant promoter to the pathophysiology of MIRI. Acute and chronic inflammation can damage the cardiac configuration and systolic function (Yao *et al*., 2022). In the damaged MIRI, the hyperactive inflammatory response stimulates a combination of adhesion molecules and chemokines. IL-1β and IL-6 recruit inflammatory leukocytes and regulate remedial reaction signaling, such as myofibroblast activation (Frangogiannis, 2020). Various studies have demonstrated that the inhibition of inflammation can provide defense against I/R injury, including succeeding negative ventricular remodeling and extended fibrotic transformation exceeding the original extent of infarction (Meyer *et al*., 2022). Consistent with these studies, our research found that the application of LHDP in MIRI model rats successfully reduced the range

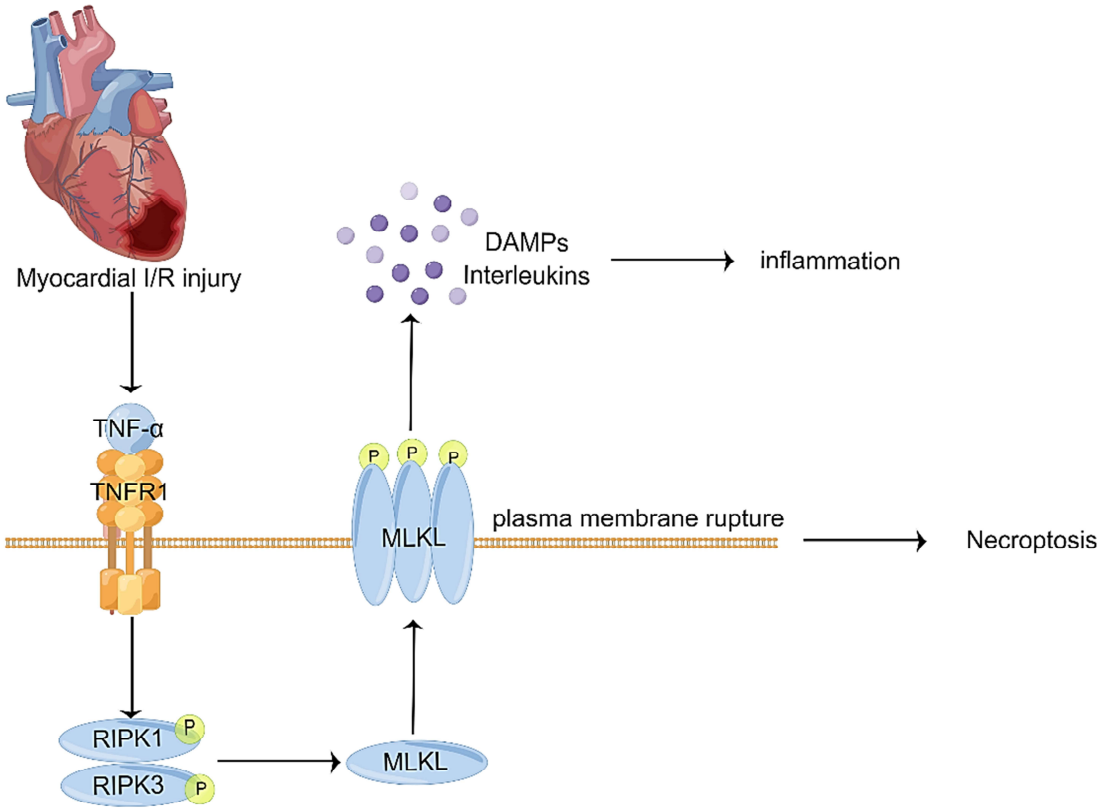

Fig. 5: (Source: By Figdraw) Necroptosis caused by I/R of heart

of damaged cardiomyocytes and restored ejection fraction (fig. 2 and 1). Along with decreased levels of inflammatory cytokines in the serum (fig. 3). These findings indicate that the anti-inflammatory properties of LHDP play a role in its cardioprotective effects. Moreover, we observed a remarkable decline in the expression of necroptosis-related molecules with treatment of LHDP.

There are multiple mechanisms of cardiomyocyte injury involved in I/R, which mainly include necrosis, apoptosis, and necroptosis. Necrosis initiates cell death by inducing autolysis in impaired tissues. Although the mitochondria are damaged in apoptosis, the sarcolemma remains intact. Therefore, this type of cell death does not induce inflammation (Heusch, 2020; Xu *et al*., 2024) conducted in vivo and in vitro experiments to investigate the effects of LHDP on apoptosis in human umbilical vein endothelial cells (HUVECs) induced by low shear stress. The results demonstrated that LHDP reduced the apoptosis rate of HUVECs and modulated the expression of YAP, TAZ, JNK1/2 and Caspase-3, while upregulating Bcl-2 expression. These findings suggest that LHDP can inhibit low shear stress-induced apoptosis in HUVECs. Necroptosis triggers cell death in a specific intracellular programmer with morphological characteristics including fracture of the plasma membrane, cell swelling and organelle dysfunction. Many studies have provided powerful evidence that suppressing necroptosis and inflammation introduced by necroptosis can prevent adverse remodeling and decrease the incidence of heart failure (Newton *et al*., 2021). Consequently, prevention of necroptosis could be an innovative approach for the management of ischemic-reperfusion damage.

Activated RIPK1 executes necroptosis by stimulating and phosphorylating RIPK3. They form a supramolecular complex (the RIPK1-RIPK3 necrosome) that can phosphorylate MLKL (Ma *et al*., 2021). P-MLKL transfers from the cytoplasm to the membrane to increase sodium influx and elicit inflammatory response via recruiting a large number of leukocytes and damage associated molecular pattern (DAMP). Subsequently, the intracellular osmotic pressure increases with continuously increasing sodium ion concentrations, leading to sustained water infiltration. Cardiomyocytes swell and plasma membrane rupture ultimately (fig. 5). Myocardial enzymes are released into the serum as a result of cell membrane disruption. Numerous studies have indicated that Nec-1s a familiar inhibitor of RIPK1 can inhibit I/R injury by decreasing the expression of RIPK1/RIPK3/MLKL (Cao and Mu, 2021). However, its clinical value remains unclear. Therefore, the search for new therapies, particularly from Chinese material medicine, to protect the heart from I/R injury via the reduction of necroptosis is a novel endeavor. Recently, several research efforts have revealed that a number of

plant extracts can suppress I/R - or H/R-induced injury, which are related to the regulation of the RIP1K/RIP3K/MLKL signaling pathway (Chen *et al.*, 2020, Huang *et al.*, 2019). Consistent with these reports, in this study, we observed that the expression of necroptosis-related mRNA and proteins was significantly decreased following treatment with LHDP and Nec-1s.

We observed that the levels of inflammatory cytokines had not decreased to the normal post-MIRI. That may have relationship with the complex process of recovery after IR. This process can persist to 28 days in rodents that includes three overlapping stages (inflammation, proliferation, and maturation). To observe the advantageous effects of LHDP in acute and chronic injury induced by I/R, the time point of the study should set less than 48 hours and beyond 7 days. This study is the first to show that LHDP exerts myocardial protective effects by reducing inflammation, and this function may be related to the inhibition of necroptosis by RIPK1/RIPK3/MLKL. However, our study did not reveal whether LHDP suppressed necroptosis was the direct effect or the result of reducing proinflammatory cytokines. Additionally, LHDP is manufactured using multiple plants. It is activated through multiple mechanisms. In the next step of our research, the possibility that other forms of MIRI-induced cell death are connected to the protective mechanism of LHDP needs to be further explored. Furthermore, to comprehensively assess LHDP's efficacy, our future investigations will include direct comparisons with established pharmacological treatments for MIRI. Additionally, long-term studies in larger animal models will be conducted to provide critical evidence before considering LHDP for clinical application.

CONCLUSION

LHDP reduces the number of damaged cardiomyocytes and reinstates the ejection fraction of the left ventricle, which is induced by MIRI. These defense mechanisms are connected to LHDP's anti-inflammatory properties. Moreover, suppressing the RIPK1-RIPK3-MLKL signaling pathway in necroptosis may be involved in this protective mechanism.

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