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Platycodin D alleviates myocardial ischemia-reperfusion injury by suppressing aberrant mitophagy

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Abstract: **Background**: Myocardial ischemia-reperfusion (I/R) injury represents a critical complication in cardiovascular diseases, profoundly influencing patient prognosis. **Objectives**: This study endeavors to elucidate the protective mechanisms and effects of Platycodin-D (PD) on myocardial ischemia-reperfusion injury (MIRI). **Methods**: A rat model of myocardial ischemia-reperfusion was employed to assess the impact of PD treatment on cardiac performance, myocardial injury biomarkers (CK-MB, LDH, cTnI), and infarct size. Further mechanistic insights were gained through Western blot and JC-1 staining, which analyzed the modulation of the HIF-1 α /BNIP3 signaling pathway and mitochondrial autophagy by PD. **Results**: PD treatment markedly improved cardiac function, decreased levels of myocardial injury biomarkers (CK-MB, LDH, cTnI), and reduced infarct size. Mechanistically, PD was found to regulate the HIF-1 α /BNIP3 signaling pathway, inhibit mitochondrial autophagy, and enhance mitochondrial function. Western blot and JC-1 staining confirmed that PD increases mitochondrial membrane potential and reduces the number of damaged mitochondria in cardiomyocytes. **Conclusions**: This study underscores the significant protective effects of Platycodin-D against myocardial ischemia-reperfusion injury, presenting a promising therapeutic approach for cardiovascular disease management.

Keywords: Acute myocardial infarction; MIRI; Mitophagy; Myocardial ischemia-reperfusion injury; Platycodin D

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INTRODUCTION

MIRI frequently occurs as a pathological complication in multiple cardiovascular disorders, not only leading to cell death but also causing severe organ damage (Xia, *et al*., 2023). Ischemia occurs when tissues experience insufficient blood supply, leading to a hypoxic state; the subsequent restoration of blood flow can cause further tissue damage, a process known as ischemia-reperfusion injury. MIRI is highly prevalent in ischemic heart disease, mainly driven by atherosclerotic processes (Chen, *et al*., 2024, Wang, *et al*., 2025). The mechanisms underlying MIRI are complex, involving oxidative stress, inflammatory responses and mitochondrial dysfunction. The cessation of blood flow during ischemia leads to myocardial tissue injury, while in the reperfusion phase, blood flow restoration aids myocardial repair but can also trigger further tissue damage. This damage not only exacerbates myocardial cell death but may also lead to long-term adverse cardiac remodeling (Xiang, Yi *et al*., 2024, Zhou, *et al*., 2021). Mounting research implicates mitochondria as key mediators in MIRI pathophysiology (Das, *et al*., 2025, Noguchi, Iwata *et al*., 2025). Mitochondria are not only the cell's powerhouses but also play a key role in regulating apoptosis and autophagy (Prabhu, *et al*., 2023). Mitophagy, a selective form of autophagy, maintains mitochondrial function by clearing damaged mitochondria (Ajoolabady, *et al*., 2022, Bravo-San Pedro, *et al*., 2017). Dysregulation of mitophagy during MIRI can lead to mitochondrial dysfunction, thereby aggravating myocardial injury. Mitophagy activates during MIRI in mice, removing damaged mitochondria, preserving energy and reducing cell death (Yamano, *et al*., 2016). Similarly, in pigs, mitophagy is linked to less apoptosis and better heart function (Roe, *et al*., 2015). It's a key mechanism for mitochondrial quality control, especially in myocardial I/R (Zhu, *et al*., 2020). Acute myocardial infarction (AMI) serves as a primary instigator of ischemia-reperfusion injury in cardiac tissue. The most effective method to limit infarct size and preserve cardiac function and structure post-AMI is to promptly restore blood flow to the occluded coronary artery (Bu, *et al*., 2022). Current treatments for AMI include coronary angioplasty, coronary artery bypass grafting and percutaneous coronary intervention (Yang, *et al*., 2023). Despite these methods' effectiveness in reducing AMI incidence, the morbidity and mortality rates of acute ST-segment elevation myocardial infarction post-reperfusion remain high (Keeley, *et al*., 2003). Studies indicate that the short-term mortality rates for post-percutaneous coronary angioplasty and thrombolytic therapy are 7% and 9%, respectively, with a 1-year post-procedure mortality or heart failure hospitalization rate of 8.6%, largely attributable to ischemia-reperfusion injury. Thus, MIRI is a critical factor affecting patient prognosis, exacerbating infarct size, promoting myocardial cell death and loss and fostering long-term adverse cardiac remodeling (Chen, *et al*., 2022). Hypoxia-inducible factor-1 (HIF-1α) is maintained at low levels under normoxic conditions

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through ubiquitin-proteasome degradation mediated by E3 ubiquitin ligase. In hypoxic environments, HIF-1α levels increase due to inhibition of E3 ubiquitin ligase. HIF-1α is closely related to the extent of myocardial ischemia and adverse prognosis (Glazachev, *et al*., 2017). Studies have found that down regulating HIF-1α expression can reduce hypoxia-induced myocardial cell apoptosis. Additionally, HIF-1α is involved in regulating mitophagy by binding to specific autophagy proteins to remove dysfunctional mitochondria (Jantsch and Schodel, 2015). In recent years, several cardioprotective compounds have been reported to attenuate myocardial ischemia-reperfusion injury by modulating mitophagy. For instance, melatonin activates Apelin/SIRT3 pathway to inhibit mitochondrial autophagy, improving myocardial function (Wang and Lin *et al*., 2024, Wu, *et al*., 2024); Curcumin boosts mitochondrial autophagy via LC3B-mitochondria co-localization (Yang, *et al*., 2013). Resveratrol boosts AMPK/SIRT1/FOXO1, enhances autophagy and mitigates MIRI (Li, *et al*., 2022, Liu, *et al*., 2023). Despite progress, PD's effect on mitophagy in MIRI is unknown.

Platycodin D (PD), a triterpenoid saponin extracted from *Platycodon grandiflorum*, has various pharmacological effects, including anti-inflammatory, hypoglycemic, hypolipidemic, antitumor and immunomodulatory properties (Han, *et al*., 2024, Xie, Zhao *et al*., 2023). The previous experiments have shown that oral administration of PD significantly improves myocardial injury and demonstrates significant attenuation of cardiomyocyte apoptosis in rodent myocardial infarction models (Ma, *et al*., 2024). PD, with antioxidant and anti-inflammatory traits, could be a promising natural agent for mitophagy-driven heart protection (Wang, *et al*., 2024). However, its role in myocardial ischemia-reperfusion injury remains unclear. In summary, MIRI remains a major cause of cardiac dysfunction, with mitochondrial impairment and excessive mitophagy recognized as key pathological events. The hypoxia-inducible factor-1α (HIF-1α)/BNIP3 signaling pathway plays a crucial role in regulating mitochondrial autophagy under ischemic conditions; however, its modulation and therapeutic targeting are not fully understood. PD exhibits antioxidant and anti-apoptotic properties, yet its cardioprotective effects and underlying mechanisms in MIRI have not been clarified. Therefore, this study established a rat model of myocardial ischemia-reperfusion to evaluate the protective potential of PD and to explore whether its effects involve regulation of the HIF-1α/BNIP3 signaling pathway and mitochondrial autophagy. These findings aim to bridge current knowledge gaps and provide insights for developing novel therapies for ischemic heart disease.

MATERIALS AND METHODS

Experimental animals

A cohort of 50 male Sprague-Dawley rats, with body weights ranging from 220 g to 240 g, was procured from Shanghai Keshuo Laboratory Animal Co., Ltd located in Minhang District, Shanghai. The farm holds a Production License (No. SCXK (Hu) 2017-0013). The rats were housed at the Experimental Animal Center of Shanghai Tenth People's Hospital Science and Technology Innovation Park with three rats per cage, controlled environmental settings, with temperature at 22-26°C and humidity at 50-70%. The subjects had constant access to food and water.

Establishment of myocardial ischemia-reperfusion injury model and grouping

The rats underwent anesthesia induction via intraperitoneal administration of 10% sodium pentobarbital (Enxing Biological Technology Co., Ltd, Hubei), with a dosage of 50 mg/kg. The rats were fixed in a supine position and an electrocardiogram was monitored using a multi-channel physiological signal recorder. After shaving the neck and left anterior chest, a longitudinal incision was made in the neck skin and blunt dissection of the soft tissue was performed to expose the trachea, which was connected to a mechanical ventilator for ventilation. Once stable respiration was achieved, a longitudinal incision was made 0.5-1 cm to the left of the sternum, followed by stepwise muscle layer separation and cutting between the 3rd and 4th ribs. After identifying the left anterior descending (LAD) coronary artery and a 4 - 0 suture (Yuyan Scientific Instruments Co., Ltd., Shanghai) was passed through the LAD at 2-3 mm from its origin and around the pulmonary conus. A latex tube (outer diameter ≈ 1.0 mm; length ≈ 3–4 cm) was placed at the ligation site and the suture was tied. The ends of the suture were left outside the chest, the heart was returned to its original position and the chest cavity was closed to prevent pneumothorax. Successful ligation was indicated by ST-segment elevation and anterior left ventricular wall cyanosis. After 30 minutes, the suture was loosened and reperfusion was performed for 1 hour. Successful modeling was defined by the disappearance of cyanosis in the anterior left ventricular ischemic area and a reduction of at least half in ST-segment elevation. In the sham-operated group, the same surgical steps were carried out, but ligation of the LAD was omitted. PD was administered orally (Nanjing Fusu Biological Technology Co., Ltd., Cat No. 58479-68-8). Specifically, PD was administered intraperitoneally at a dose of 25 mg/kg per day for seven consecutive days prior to the induction of myocardial ischemia-reperfusion injury(Chen, *et al*., 2022, Wang and Wang *et al*., 2024). The experimental design comprised three distinct groups: (1) Sham (control group undergoing sham surgery), (2) I/R (positive control group subjected to myocardial ischemia followed by reperfusion) and (3) I/R+PD (treatment group receiving PD administration prior to reperfusion).

Anesthesia and monitoring of respiration

Rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and placed in a supine position on a thermostatic surgical table. Stable respiration was

monitored throughout the operation by observing thoracic movements and using a rodent physiological monitoring system (including respiratory rate, heart rate and body temperature tracking; Panlab OxyletPro, SCIVARO, Shanghai, China). Body temperature was maintained at 37 ± 0.5 °C using a heating pad. Additional doses of anesthetic were administered as needed to maintain a stable anesthetic plane without respiratory suppression (Qiao, *et al*., 2021, Zhong, *et al*., 2024).

Western blot

Western blot analysis was employed to assess the expression profiles of proteins LC3 (ab192890, Abcam), Pink1 (ab186303, Abcam) and Parkin (ab77924, Abcam), with GAPDH (ab8245, Abcam) as the internal control. After quantifying cytoplasmic and mitochondrial proteins from each group, protein samples were combined with buffer, heated at 100°C for 8 minutes and then centrifuged at 4°C for 10 minutes for storage. Samples were loaded onto gels for electrophoresis, transferred to membranes and blocked. Membranes were reacted overnight at 4°C with anti-LC3 (1:1000), anti-Pink1 (1:500) and anti-Parkin (1:500). On the following day, membranes were washed thoroughly. They were then reacted with HRP-conjugated antibodies (ab97057, Abcam, diluted 1:5000) for 1 hour at room temperature. After washing, the membranes were exposed using the ECL detection system (A38554, Thermo Fisher). ImageJ software (v1.54h) was used to analyze the changes in autophagy proteins in myocardial tissue.

JC-1 staining and fluorescence assay for mitochondrial membrane potential

Myocardial tissue samples were harvested and the extraction of cytoplasmic proteins, mitochondrial proteins, as well as high-purity mitochondria was carried out utilizing a mitochondrial isolation kit (HZ3606, Shanghai Huzhen Industrial Co., Ltd.). The mitochondrial membrane potential was assessed through the utilization of the JC-1 fluorescent probe (HZ-9113, Shanghai Huzhen Industrial Co., Ltd.). High membrane potential causes JC-1 to form aggregates in the mitochondrial matrix, producing red fluorescence, while low membrane potential keeps JC-1 in a monomeric form, producing green fluorescence. A 96-well plate was loaded with 180 μL of staining working solution and 20 μL of high-purity mitochondria (total protein usually 10-100 μg). The fluorescence assay determined JC-1 aggregates (excitation: 525 nm; emission: 590 nm) and JC-1 monomers (excitation: 490 nm; emission: 530 nm). Mitochondrial membrane potential was represented by the ratio of JC-1 aggregates to monomers.

Frozen sections

Heart specimens were gathered and sections from the apex and left ventricular wall were placed in embedding medium and promptly frozen by submerging in liquid nitrogen. Subsequently, frozen tissue slices were prepared using a cryostat (CM1860, Leica) at a thickness of 15 μm and stored at -20°C.

Detection of myocardial injury markers

Blood samples (6 ml) were taken from the carotid artery and let stand for 30 minutes and then centrifuged by 3000 rpm for 10 min at 4 °C to separate serum, which was stored at -80°C. Myocardial enzyme levels, including CK-MB (Nanjing Jiancheng Bioengineering Institute, A034), lactate dehydrogenase (LDH) (Shanghai Yubo Biotechnology Co., Ltd., YBSH280) and cardiac troponin I (cTnI) (Wuhan Fine Biotech Co., Ltd., FY-P525364), were determined by automatic biochemical analyzer (Hitachi, Japan, 7150).

Echocardiography

Prior to experimental procedures, food was withheld from the rats for 12 hours while maintaining ad libitum water access. Anesthesia was induced with 1%-3% isoflurane to ensure the rats were pain-free during the experiment. Cardiac function was evaluated utilizing a high-resolution cardiac ultrasound system outfitted with an 18 - 20 MHz high-frequency transducer (Hitachi, Japan). The chest hair of the rats was shaved and conductive gel was applied. The probe was adjusted to obtain optimal cardiac images via the transthoracic approach. Measurements were taken over at least three consecutive cardiac cycles and averaged to ensure accuracy and reliability. Data collection and analysis were performed blindly by trained personnel. Appropriate measures were taken to ensure the rats safely recovered post-experiment.

Data analysis

The experimental data were evaluated using GraphPad Software (version 8) and were represented as mean ± SEM. One-way ANOVA was used for multiple comparisons to analyze protective effects of PD against myocardial injury induced by ischemia-reperfusion (I/R) in rats. $p < 0.05$ was set as statistically significant.

RESULTS

PD reduces myocardial injury following ischemia-reperfusion injury

Fig. 1 demonstrates the cardioprotective potential of PD in a rat model of I/R-induced myocardial injury. To evaluate myocardial damage, we quantified key biochemical indicators including creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and cardiac troponin I (cTnI) in serum samples. As shown in fig. 1, the concentrations of CK-MB, LDH and cTnI were significantly elevated in the positive control group compared to the Sham group, indicating substantial myocardial cell injury due to the ischemia-reperfusion process. In the I/R+PD group, the concentrations of these biomarkers were markedly reduced, with CK-MB and LDH showing particularly significant decreases ($P < 0.05$), demonstrating the notable cardioprotective effect of PD. Specifically, compared to the I/R group, CK-MB levels in the I/R+PD group decreased by approximately 50%, LDH levels by about 40% and cTnI

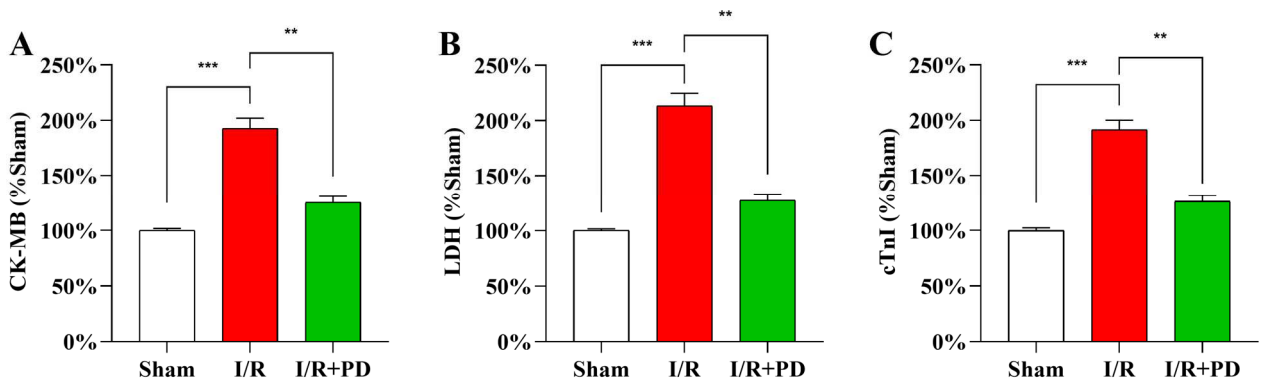

Fig. 1: Protective effects of PD against myocardial injury induced by ischemia-reperfusion (I/R) in rats.
Biochemical markers of myocardial injury, CK-MB (A), LDH (B) and cTnI (C) levels, were measured in Sham, I/R and I/R+PD groups. ($^{*}p < 0.05$ compared to I/R group). n = 8 using one-way ANOVA Kruskal-Wallis test,$^{*}p < 0.05$,$^{**}p < 0.01$,$^{***}p < 0.001$ shown as mean ± SEM.

levels also showed a significant reduction, albeit with a relatively moderate decrease.

PD improves cardiac function in rats

Fig. 2 illustrates the cardioprotective efficacy of PD against I/R-induced myocardial dysfunction through the assessment of cardiac function using echocardiography. The results showed significant protective effects of PD. For the I/R experimental group, echocardiographic results revealed a marked decline in myocardial contractile function, reflected by decreased left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS). Specifically, LVEF and LVFS in the I/R group were significantly lower than those in the Sham group, indicating severe impairment of cardiac function due to ischemia-reperfusion. However, in the case of I/R+PD group, these cardiac function indicators improved significantly. Compared to the I/R group, the I/R+PD group showed substantial increases in LVEF and LVFS, suggesting that PD exerts a reparative effect on damaged myocardium. Additionally, the biochemical marker of myocardial cell injury, cardiac troponin I (cTnI), exhibited a marked increase in the I/R group, while its concentrations significantly decreased after PD treatment. This further corroborates the efficacy of PD in myocardial protection.

PD reduces myocardial infarction area

Fig. 3 provides compelling evidence for the cardioprotective effects of PD against ischemia-reperfusion (I/R) injury. We systematically evaluated the cardiac protective effects of PD through cardiac cross-sectional observation, HE-stained heart sections and infarct size quantification. From the cross-sectional images of the heart in fig. 3A, it is evident that I/R treatment caused significant infarction, characterized by extensive myocardial necrosis and disorganized myocardial cell arrangement. In contrast, the I/R+PD group exhibited a markedly reduced infarct area, with heart morphology more closely resembling normal tissue, indicating significant cardioprotective effects of PD. The reduction in myocardial infarct size suggests that PD effectively mitigates myocardial damage induced by ischemia-reperfusion, demonstrating its notable cardioprotective properties.

At the cellular level, HE-stained heart sections (Fig. 3B) provided additional corroboration of the protective actions exerted by PD. The I/R group showed disorganized myocardial cell arrangement, significant structural damage and extensive inflammatory cell infiltration, indicating severe myocardial injury due to ischemia-reperfusion. In contrast, the I/R+PD group exhibited more orderly myocardial cell arrangement, with significantly reduced cellular structural damage, resembling the Sham group more closely. This indicates that PD significantly reduces myocardial cell damage induced by I/R, preserving the structural integrity of myocardial cells.

In fig. 3C, the quantitative analysis of myocardial infarct size is given. The I/R group had a notably larger area of myocardial infarction than the Sham group, indicating pronounced myocardial damage. In contrast, the I/R+PD group showed a significantly reduced infarct area, with a statistically significant difference compared to the I/R group. This result clearly reflects the protective effects of PD, demonstrating that PD effectively reduces myocardial infarct size, thereby alleviating myocardial damage.

PD reduces myocardial mitochondrial autophagy

Fig. 4 illustrates the impact of PD on mitochondrial autophagy and function in myocardial cells. LC3 is a key protein in the autophagy process, where LC3-I (cytosolic LC3) is lapidated to LC3-II and incorporated into autophagosome membranes during autophagy (Peña-Martinez, *et al*., 2022, Priem, *et al*., 2023). Therefore, LC3 levels are often used as indicators of autophagic activity. In mitochondrial autophagy, LC3 helps identify and encapsulate damaged mitochondria into autophagosomes, which are then transported to lysosomes for degradation (Ren, *et al*., 2024).

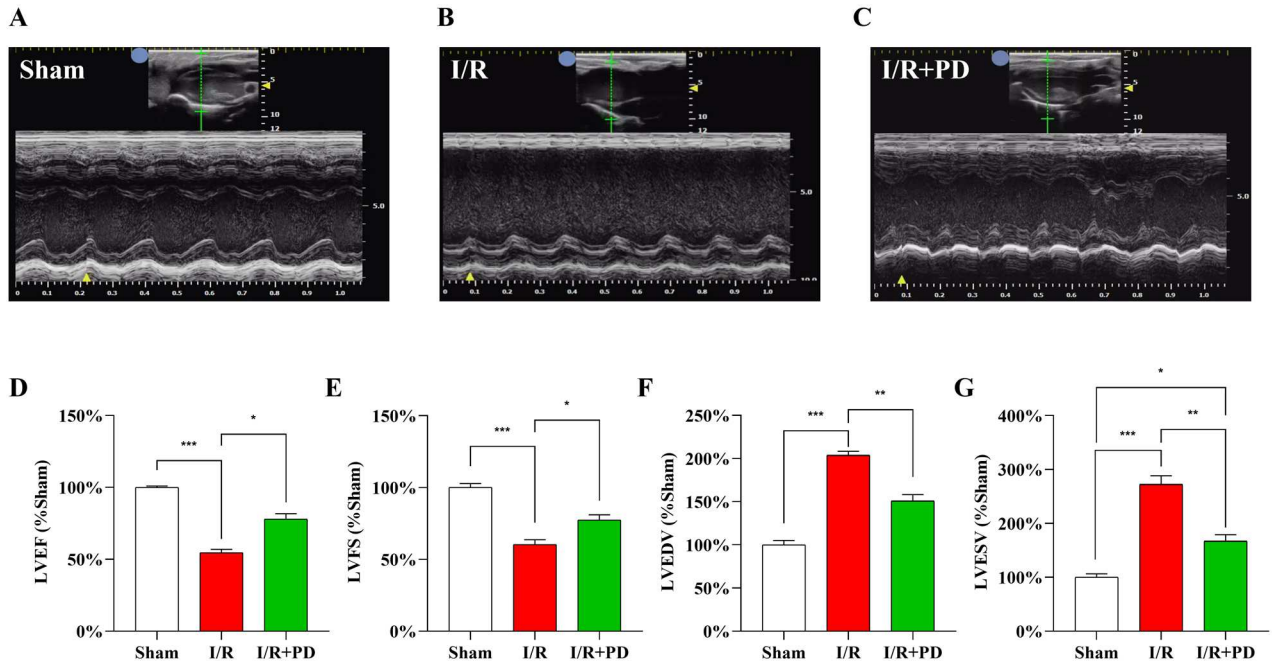

Fig. 2: Protective effects of PD on cardiac function in rats subjected to ischemia-reperfusion (I/R) injury. Echocardiography image of Sham group (A), I/R group (B) and I/R+PD group (C). Echocardiographic assessments showed significant improvements in left ventricular ejection fraction (LVEF) (D) and left ventricular fractional shortening (LVFS) (E) in the I/R+PD group (F) compared to the I/R group (G). Cardiac troponin I (cTnI) levels were significantly lower in the I/R+PD group, indicating reduced myocardial cell injury ($^{*}p < 0.05$ compared to I/R group). $n = 8$ using one-way ANOVA Kruskal-Wallis test,$^{*}p < 0.05$,$^{**}p < 0.01$,$^{***}p < 0.001$ shown as mean ± SEM.

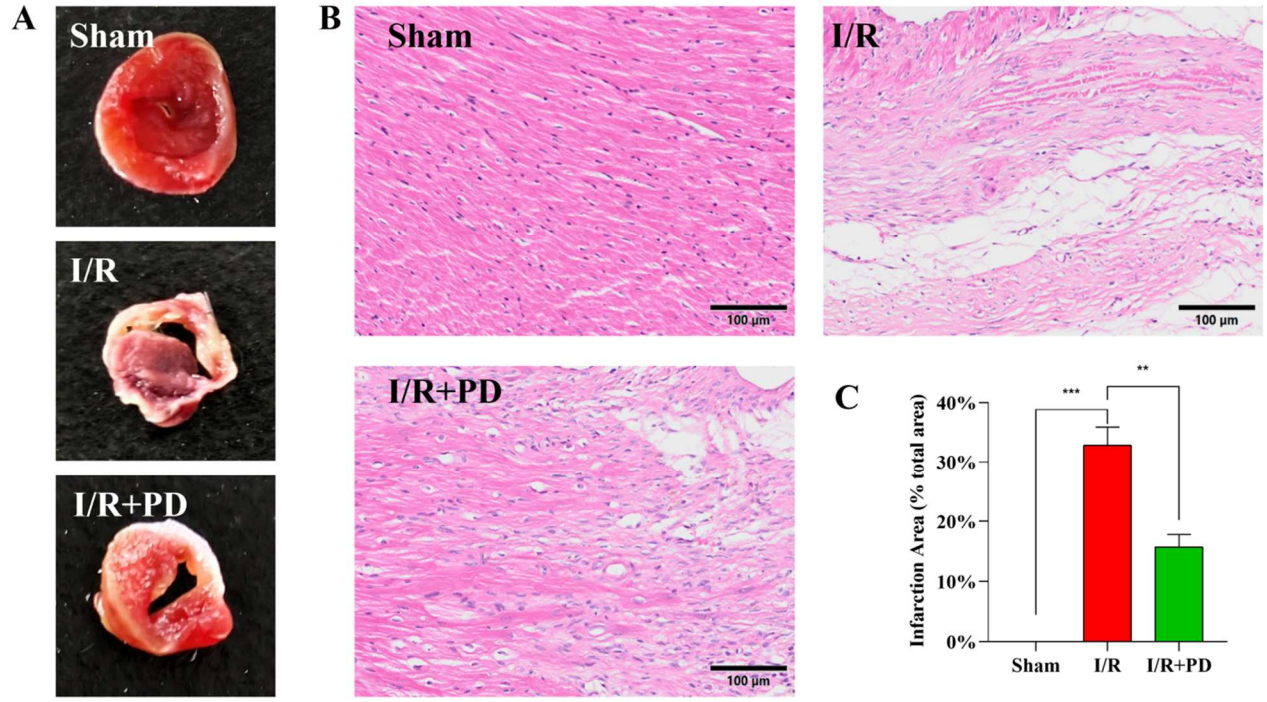

Fig. 3: Evidence of the cardioprotective effects of PD against ischemia-reperfusion (I/R) injury in rats.
(A) Cross-sectional images of the heart showing reduced infarct areas in the I/R+PD group compared to the I/R group. (B) HE-stained sections showing improved myocardial cell arrangement and reduced structural damage in the I/R+PD group. (C) Quantitative analysis of myocardial infarct size showing significant reduction in infarct area in the I/R+PD group compared to

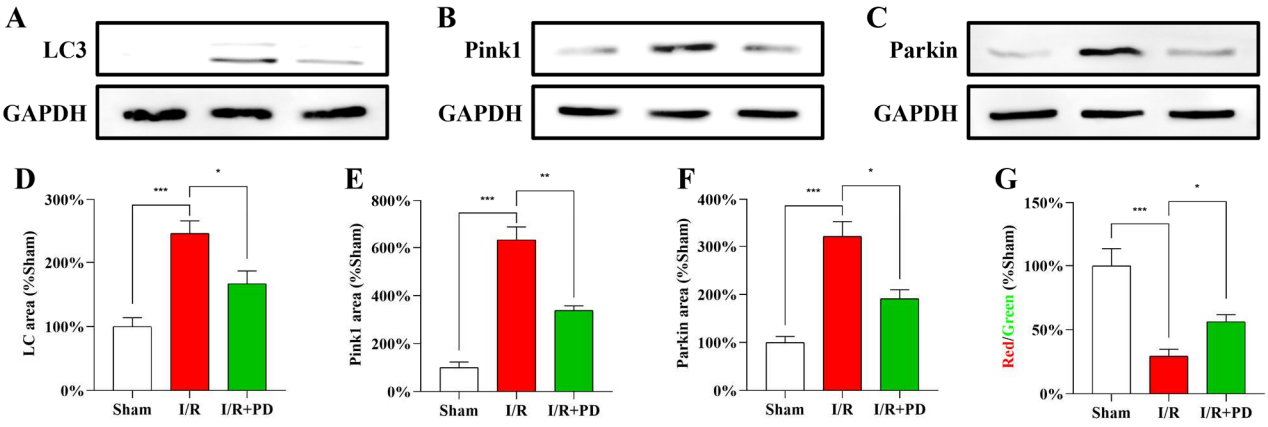

Fig. 4: Effects of PD on mitochondrial autophagy and function in myocardial cells subjected to ischemia-reperfusion (I/R) injury.

(A-C) Western blot analysis showing expression levels of LC3, Pink1, and Parkin in Sham, I/R and I/R+PD groups. (D-F) Quantitative analysis of LC3-II, Pink1 and Parkin expression levels. (G) JC-1 staining showing mitochondrial membrane potential changes in the different groups. ($^{*}p < 0.05$ compared to I/R group). $n = 8$ using one-way ANOVA Kruskal-Wallis test,$^{*}p < 0.05$,$^{**}p < 0.01$,$^{***}p < 0.001$ shown as mean ± SEM.

Western blot analysis results (Fig. 4A-C) and corresponding quantitative results (Fig. 4D-F) reveal the effect of PD on LC3 expression levels. The results show that PD treatment significantly reduces LC3-II expression levels compared to the I/R group, proving that PD may alleviate mitochondrial autophagy by inhibiting autophagic activity.

The Western blot analysis in fig. 4 indicates that Pink1 and Parkin expressions are significantly higher in the I/R group, while it's significantly lower in the I/R + PD group. This suggests that PD mitigates myocardial cell damage by inhibiting mitochondrial autophagy through the down regulation of Pink1 and Parkin expression.

Further evidence of PD's improvement of mitochondrial function is provided by JC-1 staining results (Fig. 4G). JC-1 is a fluorescent probe responsive to membrane potential changes, commonly used to assess mitochondrial membrane potential, an important indicator of mitochondrial function (Jedrzejewski, *et al*., 2025, Shah and Dobrovolskaia, 2024, Zezeski, *et al*., 2025). Given that JC-1 aggregates to produce red fluorescence at high mitochondrial membrane potential and remains as monomers with green fluorescence at low potential, it follows that the red/green fluorescence ratio can be utilized as an indirect approach to evaluate changes in mitochondrial membrane potential.

In the I/R group, JC-1 aggregation is significantly reduced, indicating a decrease in mitochondrial membrane potential, reflecting impaired mitochondrial function. This is characterized by enhanced green fluorescence and reduced red fluorescence, indicating significant mitochondrial depolarization, a hallmark of mitochondrial damage, suggesting severe mitochondrial dysfunction in myocardial cells during ischemia-reperfusion. Conversely, in the I/R+PD group, JC-1 aggregation is significantly increased, indicating a restoration of mitochondrial membrane potential and improved mitochondrial function. This is characterized by enhanced red fluorescence and reduced green fluorescence, indicating a significant recovery of mitochondrial membrane potential. This phenomenon suggests that PD effectively inhibits mitochondrial depolarization, maintaining mitochondrial membrane integrity and function, thereby protecting myocardial cells from ischemia-reperfusion injury.

DISCUSSION

In the present investigation, we systematically explored the cardioprotective properties of PD and its underlying molecular pathways in the context of MIRI. The results indicate that PD ameliorates mitochondrial function by regulating the HIF-1α/BNIP3 signaling pathway, thereby reducing mitochondrial autophagy in myocardial cells. HIF-1α, a key hypoxia response factor, is crucial for the survival of myocardial cells under ischemic conditions. BNIP3, a downstream target of HIF-1α, regulates mitochondrial autophagy activity. Western blot and JC-1 staining results show that PD treatment reduces HIF-1α and BNIP3, decreases the levels of autophagy-related proteins LC3, Pink1 and Parkin and significantly improves mitochondrial membrane potential, indicating effective restoration of mitochondrial function. Furthermore, PD exhibits a notable ability to enhance cardiac function in rats experiencing ischemia-reperfusion. It effectively lowers the concentrations of myocardial injury indicators, including CK-MB, LDH and cTnI and also reduces the size of the myocardial infarct. These results highlight the promising prospects of PD for the treatment of cardiovascular diseases, especially in terms of preventing and alleviating myocardial injury induced by ischemia-reperfusion.

I/R injury manifests as a swift elevation in the levels of cytokines and chemokines, accompanied by a substantial infiltration of leukocytes into the myocardium affected by infarction. These inflammatory responses activate pro-apoptotic signaling pathways, exacerbate myocardial cell apoptosis and disrupt the extracellular matrix integrity of myocardial cells, ultimately determining the infarct size and cardiac function recovery (Kumari, *et al*., 2024). Mitochondrial autophagy is a critical mechanism for maintaining mitochondrial function by selectively clearing damaged mitochondria. The abnormal regulation of mitochondrial autophagy is strongly linked to the death of myocardial cells and impaired cardiac function. Emerging research has indicated that mitochondrial dysfunction significantly contributes to the initiation and advancement of MIRI (Zong, *et al*., 2024). Therefore, strategies to protect mitochondrial structure and function are key to preventing myocardial injury. Mitochondrial autophagy, a selective form of cellular autophagy, is responsible for removing dysfunctional mitochondria. Dysregulated mitochondrial autophagy is a major cause of mitochondrial dysfunction in MIRI (Beg, *et al*., 2024). Insufficient mitochondrial autophagy leads to the accumulation of damaged mitochondria in myocardial cells, causing severe oxidative stress and ultimately leading to apoptosis. Conversely, excessive mitochondrial autophagy can deplete mitochondria in myocardial cells, resulting in insufficient energy supply (Tang, *et al*., 2025, Tang, *et al*., 2024).

PD, a naturally occurring triterpenoid saponin derived from the *Platycodon grandiflorum*, has demonstrated potential therapeutic impacts in MIRI. Previous studies have found that PD significantly improves vascular compliance, reduces cardiac load and enhances myocardial contractile function in ischemia-reperfusion rats (Ho, *et al*., 2024, Peng, *et al*., 2024, Wang and Wang *et al*., 2024). Additionally, it has effects such as promoting lung health, resolving phlegm and benefiting the throat and it has certain antitumor effects (Li, *et al*., 2025). Our study demonstrates that PD-treated rats exhibit significant improvements in cardiac function compared to the ischemia-reperfusion control group, including significant reductions in myocardial injury markers (CK-MB, LDH, cTnI) and marked improvements in echocardiographic cardiac function indicators (such as LVEF and LVFS). These results indicate that PD has significant protective effects against MIRI-induced myocardial injury. Further molecular mechanism studies reveal that PD regulates the HIF-1α/BNIP3 signaling pathway, reducing mitochondrial autophagy in myocardial cells.

PD plays a positive role in improving cardiac function following I/R. The significant increases in LVEF and LVFS suggest that PD can effectively restore the contractile function of damaged myocardium and reduce the extent of myocardial cell injury. Furthermore, the reduction in cTnI levels further supports the cardioprotective mechanism of PD. As a specific marker of myocardial cell injury, the decrease in cTnI levels indicates the stabilizing effect of PD on myocardial cell membranes. This result is consistent with the changes in CK-MB and LDH, suggesting that PD exerts a significant protective effect by mitigating both myocardial necrosis and apoptotic cell death. These findings collectively demonstrate that PD has a significant effect in alleviating I/R-induced myocardial injury, providing strong support for its potential clinical application in the treatment of cardiovascular diseases. PD, as a potential therapeutic agent, shows promise in improving cardiac function by reducing myocardial cell injury.

These multidimensional pieces of evidence collectively support the treatment prospects of PD in ischemic heart diseases. PD, as a potential cardioprotective agent, not only reduces myocardial infarct size but also improves myocardial cell structure and preserves the integrity of myocardial cells, providing a solid foundation for its clinical application. Furthermore, the dose-dependent cardioprotective effects of PD provide important dosage-effect information for future clinical trial designs. These results indicated that PD has significant effects in mitigating myocardial injury induced by ischemia-reperfusion. PD significantly reduces myocardial infarct size and improves myocardial cell structure, showcasing its great potential as a cardioprotective agent.

Upstream regulatory mechanisms of autophagy show that PD decreases the expression of Pink1 and Parkin proteins, which are critical factors for identifying and tagging damaged mitochondria for degradation. Pink1 is a mitochondrial kinase associated with familial Parkinson's disease (Narendra and Youle, 2024). Under normal conditions, Pink1 expression on the mitochondrial inner membrane is low due to rapid degradation. However, when mitochondria are damaged, Pink1 stabilizes and accumulates on the outer membrane, recruiting and activating downstream Parkin protein, triggering the selective autophagy of mitochondria (Jin and Li, 2025). Parkin is an E3 ubiquitin ligase also associated with certain genetic forms of Parkinson's disease (Basilone, *et al*., 2025). Parkin activation relies on Pink1 activation and its movement to the outer membrane of damaged mitochondria (Pereira, *et al*., 2023). Activated Parkin adds ubiquitin tags to specific proteins on damaged mitochondria, marking them for degradation. Through this mechanism, Parkin promotes the selective degradation of damaged mitochondria (Coukos and Krainc, 2024, Nguyen, Padman *et al*., 2016). PD's regulation of this pathway is crucial for maintaining mitochondrial quality and function in myocardial cells, especially under stress conditions like ischemia-reperfusion.

Restoration of mitochondrial membrane potential also implies improved energy metabolism functions and the regulation of intracellular calcium homeostasis and

apoptosis signaling. By improving mitochondrial membrane potential, PD may reduce apoptosis due to mitochondrial dysfunction, further protecting myocardial cells. JC-1 staining results clearly indicate that PD effectively restores mitochondrial function impaired by ischemia-reperfusion, showcasing its significant role in protecting mitochondrial function, maintaining myocardial cell energy metabolism and ensuring cell survival. This provides strong experimental support for PD as a potential cardioprotective agent and theoretical basis for its application in cardiovascular disease treatment.

PD protects mitochondria in myocardial cells, enhances mitochondrial tolerance and reduces the number of damaged mitochondria, thereby maintaining energy metabolism and cell survival. JC-1 staining and Western blot results show that PD improves mitochondrial function, reduces the number of damaged mitochondria in myocardial cells, which is crucial for maintaining energy metabolism and cell survival. Further mechanistic studies suggest that PD may alleviate myocardial damage and protect myocardial cells by regulating the HIF-1α/BNIP3 signaling pathway, reducing mitochondrial autophagy activity and improving mitochondrial function. Overall, this study demonstrates that PD significantly alleviates myocardial injury induced by ischemia-reperfusion by regulating autophagy-related signaling pathways, inhibiting mitochondrial autophagy and improving mitochondrial function. The study provides new molecular evidence of PD's cardioprotective mechanism and a theoretical ground for its potential application in ischemic heart disease treatment.

This study shows PD's cardioprotective role against MIRI but has limitations. Experiments were only in vivo, lacking in vitro mechanistic studies on mitochondrial autophagy for direct evidence. It mainly focused on one pathway, while others in myocardial injury should be explored. Also, translational studies in large animals or clinical settings are needed to assess PD's use in humans, which will aid its development as a therapy. Further research is needed for clinical translation, including evaluating its pharmacokinetic and safety profile and conducting trials. Exploring its potential in other cardiovascular conditions and its molecular targets is also worthwhile.

CONCLUSION

In conclusion, this study reveals a new mechanism by which PD exerts protective effects in a MIRI model by reducing mitochondrial autophagy by the regulation of the HIF-1α/BNIP3 signaling pathway. These findings not only provide new strategies for treating cardiovascular diseases but also open new avenues for the application of traditional Chinese medicine in modern medicine. We look forward to future research that will further elucidate the specific mechanisms of PD's cardioprotective effects, offering novel theoretical insights and practical methodologies for the management of cardiovascular conditions.

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Author's contribution
J. L. conceived the idea and conducted the experiment. C. X. contributed to the writing and revisions. All authors reviewed the manuscript.

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Data availability statement
All data generated or analysed during this study are included in this published article.

Ethical approval
All animal experimental protocols were approved by the Ethics Committee of Experimental Animals at Shanghai Tenth People's Hospital (Ethics No. SHDSYY-2024-187237).

Conflict of interest
All authors have given approval of this manuscript. The authors declared that they have no financial or other contractual agreements that might cause conflicts of interest.

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