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Elucidating the Therapeutic Mechanisms of Agrimonia pilosa Ledeb. Extract for Acute Myocardial Infarction via Network Pharmacology and Experimental Validation

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ABSTRACT

This study aimed to uncover the mechanisms by which Agrimonia pilosa Ledeb. (APL) extract mitigates acute myocardial infarction (AMI) using a combination of network pharmacology and experimental validation. The main bioactive constituents of APL extract were identified through High-Performance Liquid Chromatography (HPLC). Potential targets shared between APL compounds and AMI were predicted via network pharmacology. An AMI model in mice was induced by subcutaneous isoproterenol (Iso) injection, followed by oral administration of APL extract. The study evaluated ECG patterns, cardiac injury markers, oxidative stress indicators, histopathology, PI3K/Akt signaling, and apoptosis-related proteins. Five active compounds were detected in APL extract. Functional enrichment suggested that cardioprotection by APL involves the PI3K/Akt pathway. APL treatment alleviated Iso-induced ST-segment elevation and tachycardia. It also reduced lactate dehydrogenase (LDH), creatine kinase (CK), CK-MB, malondialdehyde (MDA), and reactive oxygen species (ROS), while boosting superoxide dismutase (SOD) activity. Apoptosis of cardiomyocytes was markedly lowered after APL administration. Mechanistically, APL enhanced p-PI3K, p-Akt, and Bcl-2 expression and suppressed Bax, caspase-3, cleaved-caspase-3, and the Bax/Bcl-2 ratio in AMI mice. APL extract confers myocardial protection against Iso-induced AMI, likely through antioxidant and anti-apoptotic mechanisms mediated by the activation of the PI3K/Akt pathway.

Keywords: PI3K/Akt, APL extract, Acute myocardial infarction, Apoptosis, Isoproterenol, Oxidative stress

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Introduction

Acute myocardial infarction (AMI) is characterized by myocardial necrosis resulting from sustained and severe ischemia and hypoxia in the coronary arteries, which may lead to heart failure in critical cases [1]. AMI remains a leading cause of mortality among patients with cardiovascular diseases [2]. Timely reperfusion of occluded coronary arteries is a primary treatment strategy to restore blood flow [3], with therapeutic goals including the rescue of ischemic myocardium, reduction of infarct size, preservation of cardiac function, and rapid management of complications. However, delayed reperfusion may exacerbate myocardial injury despite restoring blood flow [4]. When coronary perfusion fails to meet myocardial demand, a cascade of detrimental changes occurs, including ultrastructural myocardial alterations, metabolic disturbances, oxidative stress, and cardiomyocyte necrosis, with severe cases potentially causing arrhythmia or shock [5].

The isoproterenol (Iso)-induced AMI model is widely used to evaluate cardioprotective interventions [6]. Iso, a β-adrenergic agonist, increases myocardial oxygen consumption by enhancing contraction and heart rate, leading to cardiac overload, impaired microcirculation, and myocardial infarction. Oxidative stress and apoptosis are key mechanisms underlying Iso-induced myocardial injury [7]. Apoptosis is a regulated form of cell death controlled by genetic and environmental factors, playing roles in normal development, cell renewal, and drug-induced cytotoxicity [8, 9]. Dysregulated apoptosis is closely associated with cardiovascular pathologies [10–13].

Oxidative stress can simultaneously trigger cellular damage and apoptosis, where moderate apoptosis can be protective under stress, but excessive oxidative stress can amplify apoptosis and cause pathological injury [14–17]. The phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) signaling pathway is a critical intracellular mechanism that exerts anti-apoptotic effects, partly by regulating apoptosis-related proteins [13, 18].

Agrimonia pilosa Ledeb. (APL) extract, derived from the aerial parts of the plant, contains diverse bioactive compounds with antithrombotic, antioxidant, and anticancer properties, and can scavenge reactive oxygen species and modulate nitric oxide production [19]. Flavonoids from APL exhibit strong free-radical scavenging, DNA protective effects against oxidative damage, antioxidant activity, and aldose-reductase inhibition [20, 21]. Additionally, APL has been reported to improve glucose metabolism and prevent apoptosis in ischemic brain tissue following middle cerebral artery occlusion [22]. However, the cardioprotective effects of APL aqueous extracts in AMI have not yet been studied.

Network pharmacology offers systematic approaches to identify active compounds and targets in traditional Chinese medicine (TCM), enabling comprehensive analysis of multi-component, multi-target, and multi-pathway mechanisms, which can guide modernization research of TCM [23]. APL, as a TCM, contains numerous compounds with potential cardiovascular benefits, yet its specific actions and mechanisms in AMI remain largely unexplored [24].

Building on prior HPLC findings, the present study integrates network pharmacology with experimental validation to investigate the mechanisms of APL extract against AMI. A drug–component–target network was constructed to identify potential targets and pathways, followed by experimental confirmation of its cardioprotective effects in an AMI mouse model.

Materials and Methods

Materials

APL extract (Cat.#: HL-210302) was supplied by Xi'an Huilin Biotechnology Co., Ltd. (Shanxi, China), Iso was obtained from Amylet Scientific Inc. (Michigan, USA), and verapamil (Ver) was purchased from HeFeng Pharmaceutical Co., Ltd. (Shanghai, China). Pentobarbital sodium was acquired from Shanghai Rongbai Biological Technology Co., Ltd. (Shanghai, China). 4% paraformaldehyde solution was purchased from Labgic Technology Co., Ltd. (Beijing, China), and sodium chloride injection was obtained from Shijiazhuang No.4 Pharmaceutical Co., Ltd. (Hebei, China). Shimadzu LC-20AD was used for HPLC analysis (Shimadzu, Tokyo, Japan). Chromatographic-grade acetonitrile, methanol, and formic acid were obtained from Fisher Chemical (Pittsburgh, PA, USA). Standard compounds—ellagic acid (≥98%), (+)-catechin (≥98%), quercetin (≥98%), quercitrin (≥98%), and taxifolin (≥98%)—were obtained from Alfa Biotechnology Co., Ltd. (Chengdu, China).

HPLC detection

For qualitative analysis, APL extract was dissolved in methanol at a concentration of 2 mg/mL. Chromatographic separation was performed on a ZORBAX StableBond C18 column (4.6 mm × 250 mm, 5 μm, Agilent, USA) using 0.2% formic acid aqueous solution as mobile phase A and acetonitrile as mobile phase B, with a flow rate of 1 mL/min and column temperature of 30°C. Detection was carried out with a PDA detector at 254 nm, using an injection volume of 10 μL over 80 minutes. The elution gradient was set as follows: 0–50 min, 5–30% B; 50–70 min, 30–55% B; 70–80 min, 55–95% B.

Target prediction and PPI network construction

Active ingredients identified via HPLC and literature were used to predict potential targets for network pharmacology studies. Targets were retrieved from TCMSP [25], Swiss Target Prediction [26], and DrugBank [27]. AMI-related targets were obtained from GeneCards [28], standardized via UniProt [29], and intersected with component targets. Cytoscape 3.7.2 was used to construct drug–component–target networks. Protein–protein interaction (PPI) networks were generated using STRING [30] with Homo sapiens as the species filter and a high confidence interaction score threshold of 0.700.

Enrichment analysis

The shared targets were analyzed using Metascape (http://metascape.org/) to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment [31]. Core proteins and pathways were

selected based on statistical significance (p-values) and visualized for interpretation. These results, combined with prior literature, guided the selection of experimental indicators for subsequent animal studies.

Experimental animals

Sixty Kunming mice (average weight 25 ± 5 g) were sourced from Hebei Medical University (license number: SCXK (Ji) 2018–004) and maintained under standard laboratory conditions with free access to food and water. Animals were acclimated for one week and fasted overnight before experimentation. All procedures adhered to the ethical guidelines approved by the Ethics Committee of Hebei University of Chinese Medicine (certificate number: DWLL2020073).

Experimental design

Mice were randomly divided into six groups: control (Con), Iso (85 mg/kg), verapamil (Ver, 2 mg/kg), and APL extract low (75 mg/kg), medium (150 mg/kg), and high (300 mg/kg) doses. The medium dose corresponded to the clinical maximum (12 g), adjusted for the 10:1 extraction rate and body surface area. APL extract was administered orally for seven days, followed by two days of subcutaneous Iso injection to induce AMI. The cardioprotective effects of APL extract were then evaluated.

Electrocardiography (ECG) assessment

Twenty-four hours after the last Iso injection, mice were anesthetized with pentobarbital sodium (40 mg/kg) and positioned for ECG measurement. Cardiac electrical activity was recorded using the RM6240BD Biological Signal Collection System (Chengdu Instrument Factory, Chengdu, China).

Measurement of myocardial injury markers

Serum lactate dehydrogenase (LDH, Cat.#: 210101), creatine kinase (CK, Cat.#: 201101), and CK-MB (Cat.#: 201101) levels were quantified as indicators of myocardial injury. Blood samples were collected from the orbital sinus, centrifuged at 3500 rpm for 20 minutes at 4°C, and the supernatant was analyzed using commercial kits per the manufacturer's instructions (Shenzhen Icubio Biomedical Technology Co., Ltd., Shenzhen, China).

Histological evaluation

Following ECG recording, hearts were excised, rinsed in saline, and fixed in 4% paraformaldehyde. Fixed tissues were paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Morphological changes were assessed using light microscopy.

Assessment of oxidative stress

Cryosections of heart tissue (5 μm) were stained with DHE (Cat.#: D7008) and incubated for 30 minutes at 37°C in the dark. After rinsing with PBS, ROS levels were visualized under a fluorescence microscope. Serum malondialdehyde (MDA, Cat.#: 200425) and superoxide dismutase (SOD, Cat.#: 200425) activities were measured using kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) to evaluate oxidative stress.

TUNEL assay for apoptosis

Cardiomyocyte apoptosis was detected using an in situ apoptosis detection kit (Servicebio, Wuhan, China). Tissue sections were rinsed with PBS, fixed in 4% paraformaldehyde, and treated with proteinase K for 10 minutes. Apoptotic nuclei were visualized with green TUNEL staining and imaged at 200× magnification under a fluorescence microscope (Nikon, Japan).

Western blot analysis

Proteins were extracted from heart tissue using RIPA buffer (Servicebio, Wuhan, China) and quantified with an ultra-micro spectrophotometer (METTLER TOLEDO, Shanghai, China). Equal protein amounts (50 μg) were separated by 10% SDS-PAGE and transferred to PVDF membranes. Membranes were blocked in 5% skim milk for 1.5 hours, then incubated overnight at 4°C with primary antibodies targeting GAPDH (Cat.#: AF7021), PI3K (Cat.#: AF6241), p-PI3K (Cat.#: AF3241), Akt (Cat.#: AF6261), p-Akt (Cat.#: AF0016), Bcl-2 (Cat.#: AF6139), Bax (Cat.#: AF0120), caspase-3 (Cat.#: AF6311), and cleaved-caspase-3 (Cat.#: AF7022) (Affinity Biosciences,

Jiangsu, China) at 1:2000. After three washes with TBST, membranes were incubated with secondary antibody (Cat.#: S0001, Affinity Biosciences, Jiangsu, China) for 50 minutes at room temperature. Protein bands were visualized using an Intelligent Image Workstation (6000Plus, Guangzhou Biolight Biotechnology Co., Ltd., Guangzhou, China).

Statistical analysis

All data are expressed as mean ± SEM. Group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. SPSS version 26 was used for analysis, and $p < 0.05$ was considered statistically significant.

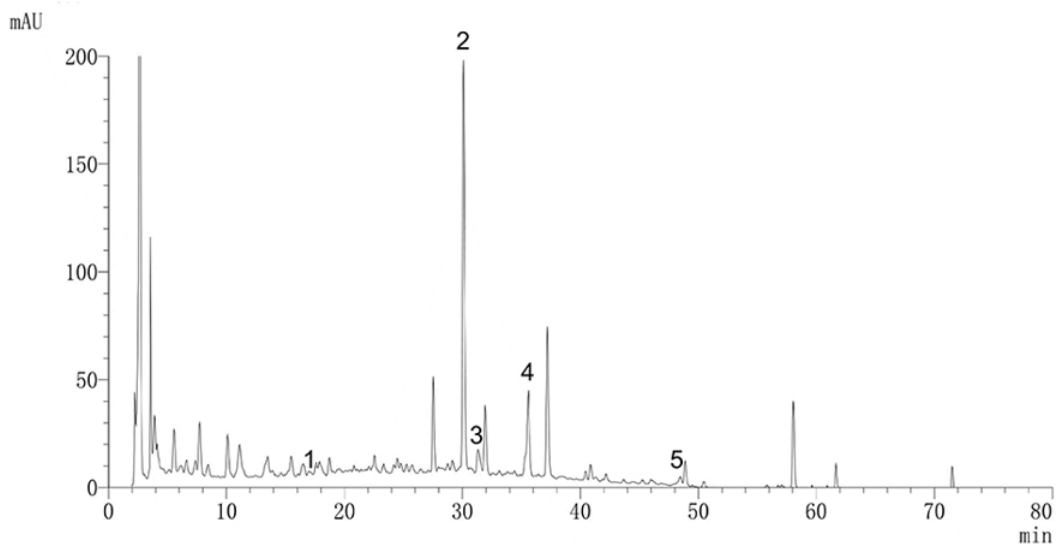
Results and Discussion

Identification of active compounds in APL extract

HPLC analysis **(Figure 1a)** identified five major bioactive constituents in APL extract: (+)-catechin, ellagic acid, taxifolin, quercitrin, and quercetin, with respective concentrations of 24.24 mg/g, 9.76 mg/g, 11.03 mg/g, 9.84 mg/g, and 0.78 mg/g **(Figure 1b)**. Standard curves plotting peak area (Y) versus concentration (X) confirmed a strong linear relationship for all compounds **(Table 1)**.

Table 1. Regression Equation

Name	Regression Equation	r
(+)-catechin	Y = 7E+06X + 842.95	0.999
Ellagic acid	Y = 2E+08X + 11,603	0.999
Taxifolin	Y = 8E+06X - 821.3	0.999
Quercitrin	Y = 5E+07X + 57,491	0.987
Quercetin	Y = 3E+08X + 4191.2	0.999



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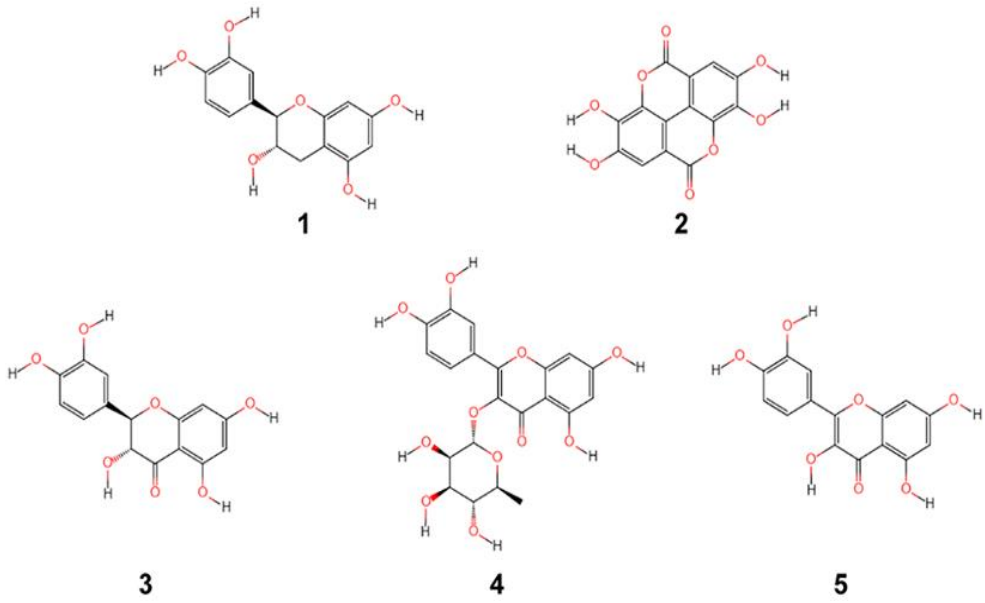
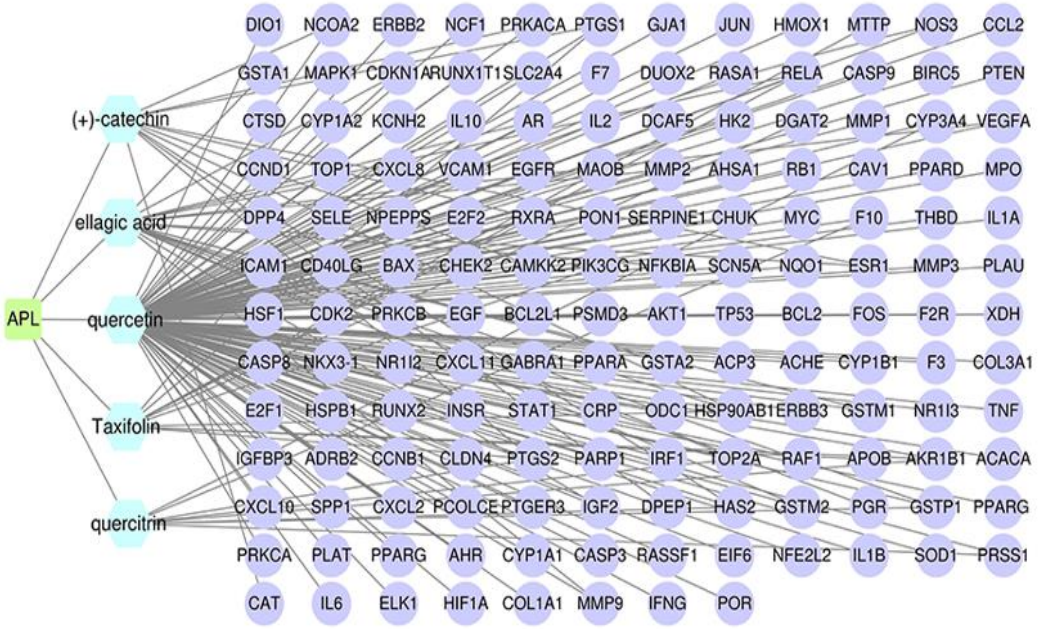


b)

Figure 1. APL Extract. (a) Chromatograms of standard compounds. (b) Identified bioactive constituents: 1. (+)-catechin, 2. ellagic acid, 3. taxifolin, 4. quercitrin, 5. quercetin.

Network analysis

Using data from TCMSP, Swiss Target Prediction, and DrugBank, a total of 152 potential targets were predicted for the five bioactive compounds **(Figure 2a)**. From the GeneCards database, 899 AMI-related targets were retrieved and screened. By overlapping the APL extract targets with AMI-associated targets, 93 common targets were identified **(Figure 2b)**. These intersecting targets were used to construct a protein–protein interaction (PPI) network representing the connections between APL bioactive compounds and AMI **(Figure 2c)**. Further analysis indicated that APL extract exerted its effects primarily through 20 key targets, including AKT1, TNF, and JUN.



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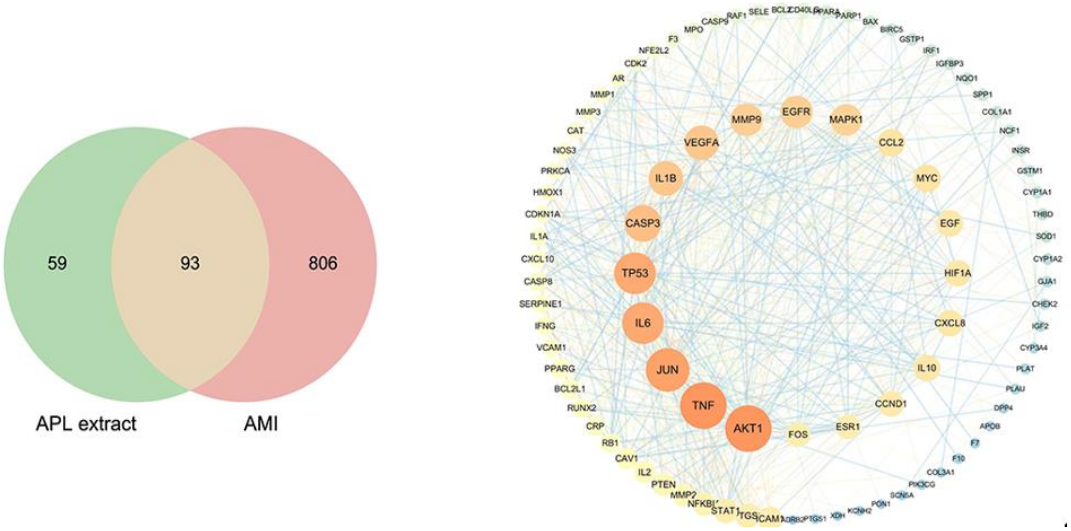
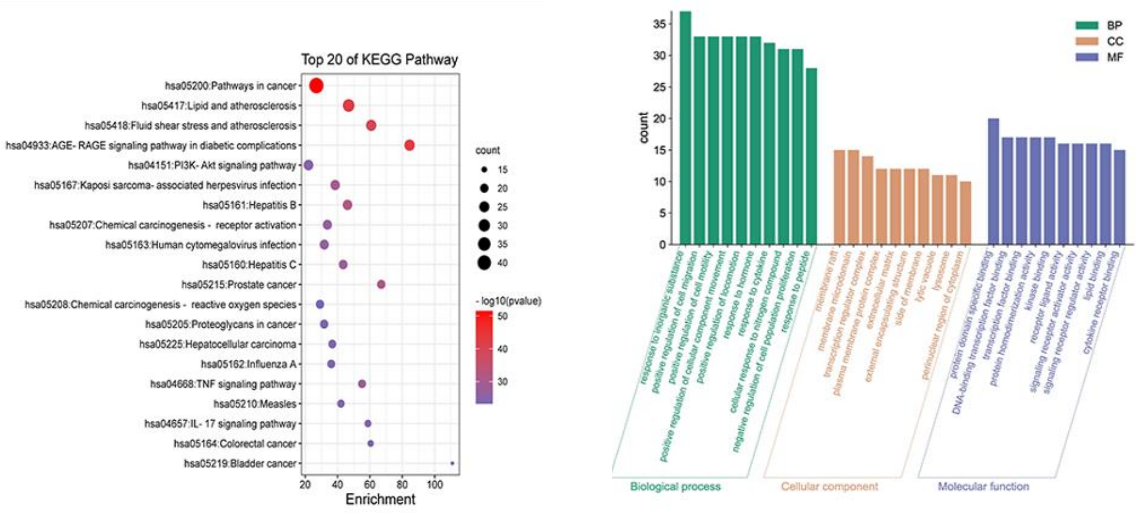


b) c)

Figure 2. Target Identification and Network Mapping. (a) Visualization of the drug–compound–target network. (b) Venn diagram showing the overlap between targets of APL extract compounds and AMI-related proteins. (c) Protein–protein interaction (PPI) network for the shared targets. In the network, the green rectangles represent the drug itself, blue hexagons denote candidate bioactive compounds from APL extract, purple circles indicate the corresponding protein targets, and connecting lines illustrate interactions between these targets.

Enrichment analysis

To explore the underlying mechanisms of APL extract in AMI, GO and KEGG enrichment analyses were conducted. The 93 shared targets between APL bioactive compounds and AMI were associated with 179 KEGG pathways, with the top 20 most significant pathways presented in **Figure 3a**. The analysis suggested that APL extract may exert cardioprotective effects through multiple pathways, including cancer-related pathways, lipid metabolism and atherosclerosis, fluid shear stress responses, AGE-RAGE signaling, and notably the PI3K-Akt pathway.



a) b)

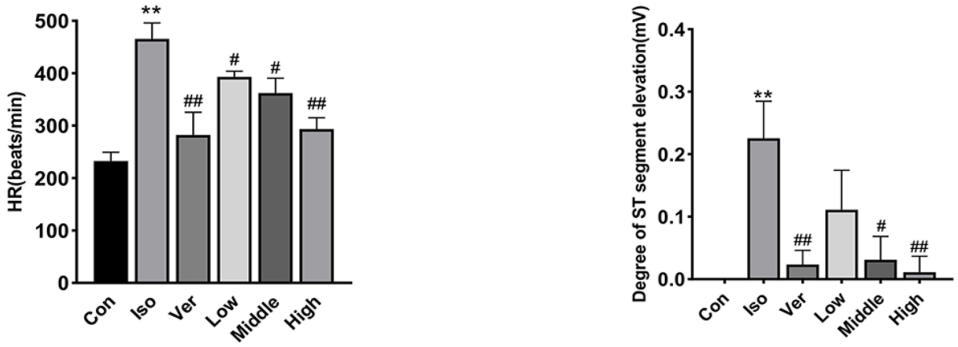
Figure 3. KEGG and GO Enrichment Analyses. (a) KEGG pathway enrichment of candidate targets of APL extract for AMI. (b) GO term enrichment of candidate targets of APL extract for AMI.

GO enrichment revealed that the top 10 terms were significantly associated with biological processes (BP), cellular components (CC), and molecular functions (MF) **(Figure 3b).** The 93 shared targets were mainly involved in responses to inorganic substances, positive regulation of cell migration, and cell motility within biological

processes. At the cellular component level, these targets were primarily located in membrane rafts, membrane microdomains, and caveolae. For molecular functions, the targets were largely associated with cytokine receptor binding, protein domain-specific binding, and DNA-binding transcription factor interactions. Notably, the PI3K/Akt signaling pathway has been identified as a key regulator in AMI pathogenesis, particularly in modulating apoptosis [32]. Based on this, animal experiments were conducted to further explore the role of the PI3K/Akt pathway in AMI.

Impact of APL extract on ECG in AMI mice

Electrocardiography revealed that Iso treatment significantly increased heart rate and induced ST segment elevation compared to the control group ($P < 0.01$). Pre-treatment with APL extract markedly attenuated these changes, reducing both heart rate and ST segment elevation compared to the Iso group ($P < 0.05$ or $P < 0.01$). Furthermore, administration of high-dose APL extract nearly restored heart rate and ST segment to normal levels in Iso-induced AMI mice **(Figure 4)**, indicating that APL extract effectively mitigates ECG alterations associated with myocardial infarction.

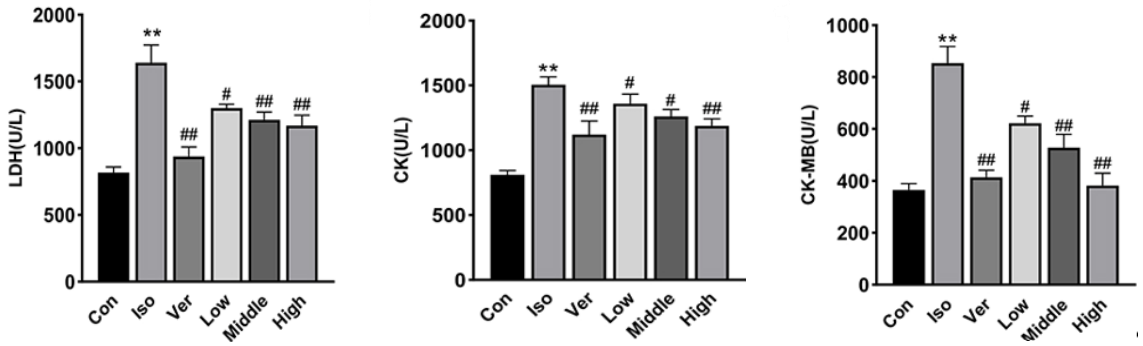


a) b)

Figure 4. Effects of APL Extract on Heart Rate and ST-Segment in Iso-Induced AMI Mice. (a) Heart rate measurements. (b) ST segment elevation. Data are presented as mean ± SEM (n = 10). Statistical differences: **P < 0.01 vs. control group; #P < 0.05, ##P < 0.01 vs. Iso group.

Impact of APL extract on cardiac enzyme levels

Serum analysis revealed that mice receiving Iso exhibited a marked increase in LDH, CK, and CK-MB activities compared with untreated controls ($P < 0.01$). Administration of APL extract prior to Iso exposure led to a pronounced reduction in these cardiac enzyme levels ($P < 0.05$ or $P < 0.01$), indicating that the extract mitigates enzyme leakage caused by myocardial injury **(Figure 5)**. These findings suggest that APL extract can protect the myocardium by limiting the release of key biomarkers associated with cardiac damage.



a) b) c)

Figure 5. APL Extract Attenuated Serum LDH, CK, and CK-MB Levels in Iso-Induced AMI Mice. (a) LDH activity. (b) CK activity. (c) CK-MB activity. Data are shown as mean ± SEM (n = 10). Statistical significance: **P < 0.01 vs. control group; #P < 0.05, ##P < 0.01 vs. Iso group.

Histopathological effects of APL extract in AMI mice

In control mice, myocardial cells displayed normal morphology, tightly organized structures, and uniform density, with no signs of edema, necrosis, or inflammatory infiltration. In contrast, Iso-treated mice showed pronounced morphological changes, including disorganized myocardial fibers, cellular swelling, necrosis, and infiltration by inflammatory cells, confirming the successful establishment of the AMI model.

Treatment with APL extract improved myocardial histology in a dose-dependent manner. Myocardial fibers appeared more regularly arranged, and the extent of necrosis and inflammatory infiltration was markedly reduced. The high-dose group exhibited the most notable improvement, with tissue structure approaching normal morphology **(Figure 6)**. These observations indicate that APL extract can effectively alleviate myocardial pathological damage associated with AMI.

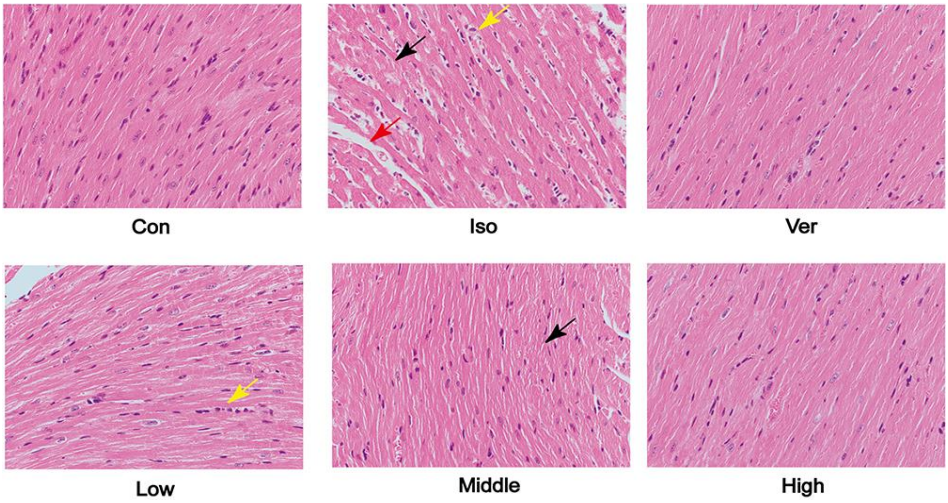
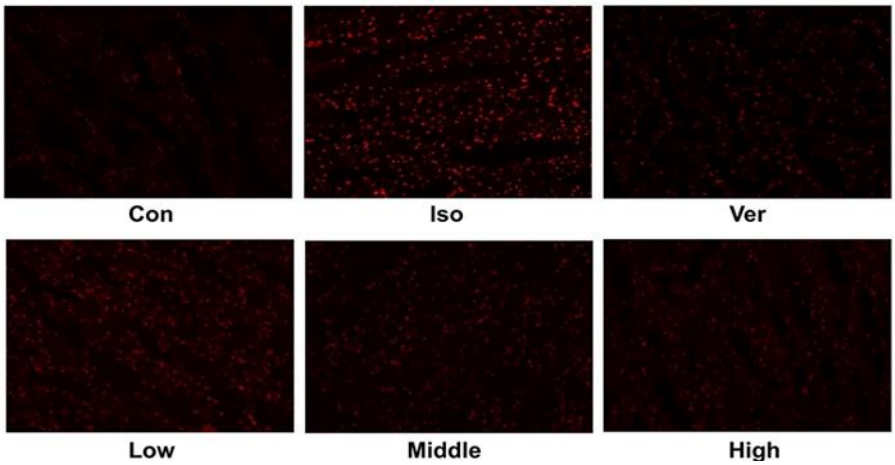


Figure 6. APL Extract Alleviated Iso-Induced Myocardial Injury in Mice. Representative H&E-stained images of cardiac tissue (magnification 400×) illustrate histopathological changes in Iso-treated mice. Red arrows indicate edema, black arrows indicate necrotic cells, and yellow arrows indicate infiltration of inflammatory cells.

Effects of APL extract on oxidative stress markers (ROS, SOD, and MDA)

In the Iso-treated group, mice exhibited significantly reduced SOD activity and markedly elevated ROS and MDA levels compared with the control group ($P < 0.01$), indicating heightened oxidative stress. Administration of APL extract significantly improved antioxidant capacity by increasing SOD activity while lowering ROS production and MDA content ($P < 0.05$ or $P < 0.01$). Notably, the middle- and high-dose treatments demonstrated the most pronounced protective effects against oxidative stress following myocardial infarction **(Figure 7)**.



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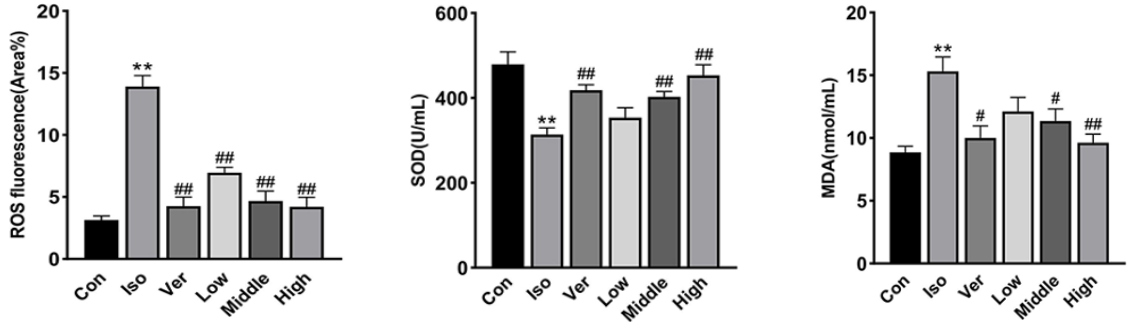

Figure 7. APL Extract Attenuated Iso-Induced Oxidative Stress in Mice. Levels of ROS (a and b, magnification 200×), SOD (c), and MDA (d) were measured in myocardial tissues. Data are expressed as mean ± SEM (n = 10). Statistical significance: **P < 0.01 vs. control group; #P < 0.05, ##P < 0.01 vs. Iso group.

Effect of APL extract on myocardial cell apoptosis

TUNEL staining was performed to evaluate apoptosis in myocardial tissue following AMI and to assess the protective effects of APL extract. Apoptotic cells were absent in the control group. Iso treatment caused a marked increase in apoptosis compared to controls. Pre-treatment with APL extract significantly reduced the number of apoptotic cardiomyocytes, indicating that APL extract effectively inhibits myocardial cell apoptosis in AMI mice **(Figure 8)**.

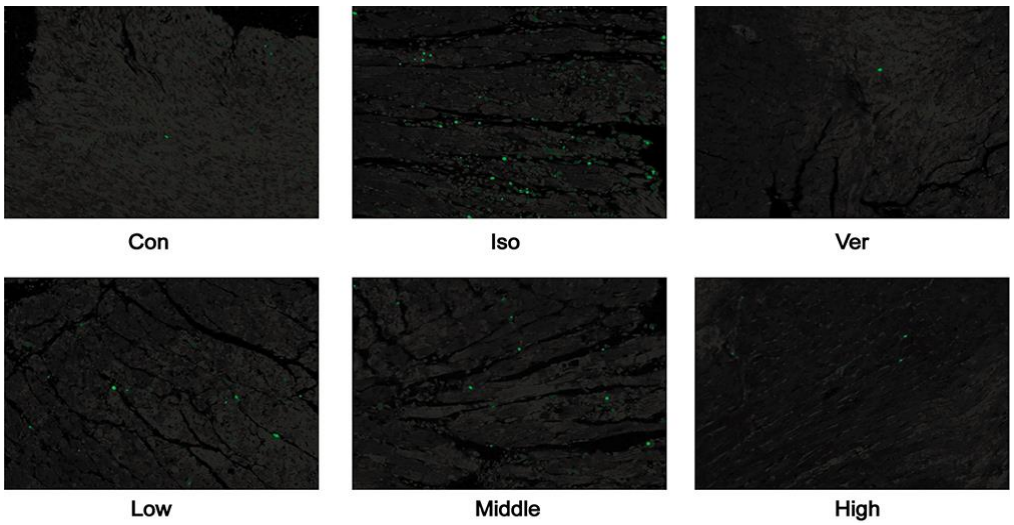

Figure 8. APL extract reduced cardiomyocyte apoptosis in AMI mice, as evidenced by representative immunofluorescent TUNEL staining images (200× magnification).

Effect of APL extract on apoptosis-related proteins in myocardial tissue of AMI Mice

The findings demonstrated that Iso treatment decreased Bcl-2 expression (P < 0.05) and increased Bax, Bax/Bcl-2 ratio, caspase-3, and cleaved-caspase-3 levels (P < 0.05 or P < 0.01). Treatment with APL extract markedly counteracted these effects, reducing Bax, Bax/Bcl-2 ratio, caspase-3, and cleaved-caspase-3 while enhancing Bcl-2 expression (P < 0.05 or P < 0.01). These results suggest that APL extract can suppress apoptosis by promoting anti-apoptotic protein expression and inhibiting pro-apoptotic protein expression **(Figure 9)**, indicating its potential to significantly attenuate Iso-induced myocardial apoptosis in mice.

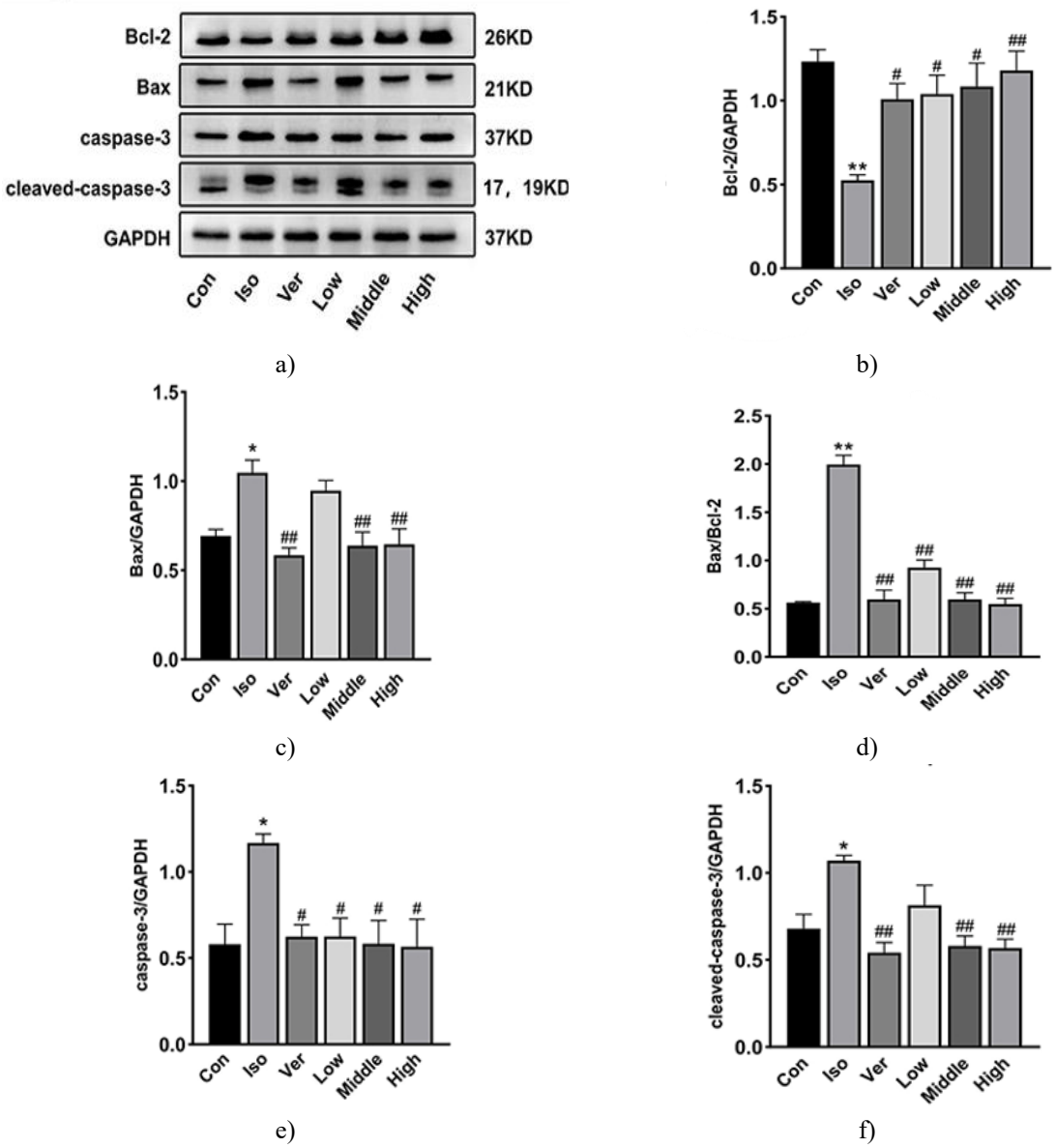

Figure 9. APL extract effectively reduced apoptosis in cardiomyocytes of Iso-induced mice. Western blotting was employed to assess apoptosis-related proteins (a), and quantitative grayscale analysis was performed for Bcl-2 (b), Bax (c), Bax/Bcl-2 ratio (d), caspase-3 (e), and cleaved-caspase-3 (f). Data are expressed as mean ± SEM (n = 3), with statistical significance indicated by *P < 0.05, **P < 0.01 versus the Con group, and #P < 0.05, ##P < 0.01 versus the Iso group.

Impact of APL extract on the PI3K/Akt signaling pathway

To investigate whether APL extract influences the PI3K/Akt pathway, protein levels of PI3K, phosphorylated PI3K (p-PI3K), Akt, and phosphorylated Akt (p-Akt) were analyzed. Total PI3K and Akt levels remained largely unchanged across groups. However, Iso administration markedly reduced p-PI3K and p-Akt levels compared with the Con group (P < 0.01). Treatment with APL extract significantly elevated p-PI3K and p-Akt relative to the Iso group (P < 0.05 or P < 0.01) **(Figure 10)**, indicating that the cardioprotective effect of APL extract may involve activation of the PI3K/Akt signaling pathway.

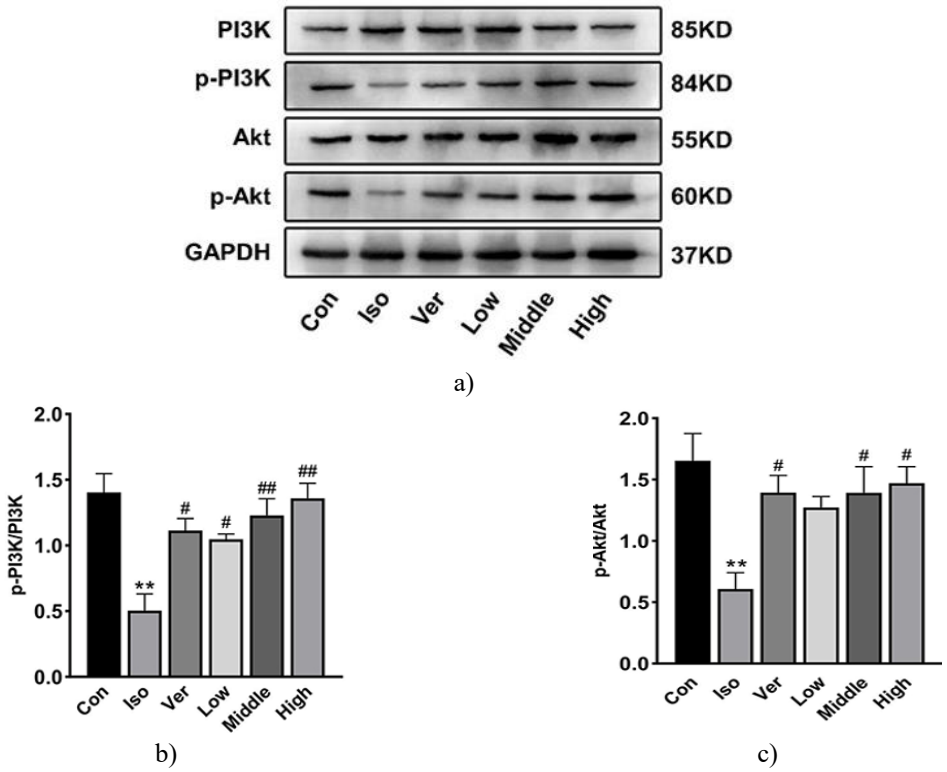

Figure 10. APL extract enhanced PI3K/Akt signaling in Iso-induced mice. Western blotting was employed to assess the protein levels of PI3K, phosphorylated PI3K (p-PI3K), Akt, and phosphorylated Akt (p-Akt) (a), and densitometric analysis was performed for p-PI3K (b) and p-Akt (c). Data are expressed as mean ± SEM (n = 3), with statistical significance indicated as **P < 0.01 versus the Con group and #P < 0.05, ##P < 0.01 versus the Iso group.

Acute myocardial infarction (AMI) frequently leads to irreversible myocardial injury and is associated with high morbidity and mortality rates [33]. While percutaneous coronary intervention and thrombolytic therapy are standard treatments for AMI [34], they may cause adverse effects such as reperfusion injury, emphasizing the need for safer and more effective natural therapeutic agents. Oxidative stress and apoptosis are central contributors to AMI pathogenesis, making compounds with both antioxidant and anti-apoptotic properties particularly valuable for therapy development. Clinical studies have shown that certain natural compounds can confer significant cardioprotection against ischemia/reperfusion injury in AMI patients, likely by reducing oxidative stress and suppressing apoptosis [35, 36]. Previous research suggests that the protective effects of APL extract against cerebral ischemia-reperfusion injury may be linked to its antioxidant capacity [22]. In this study, we first identified the active components of APL extract, then applied network pharmacology to predict its potential targets and pathways in AMI treatment. Experimental validation further confirmed that APL extract provides cardioprotective effects against Iso-induced AMI in mice **(Figure 11)**.

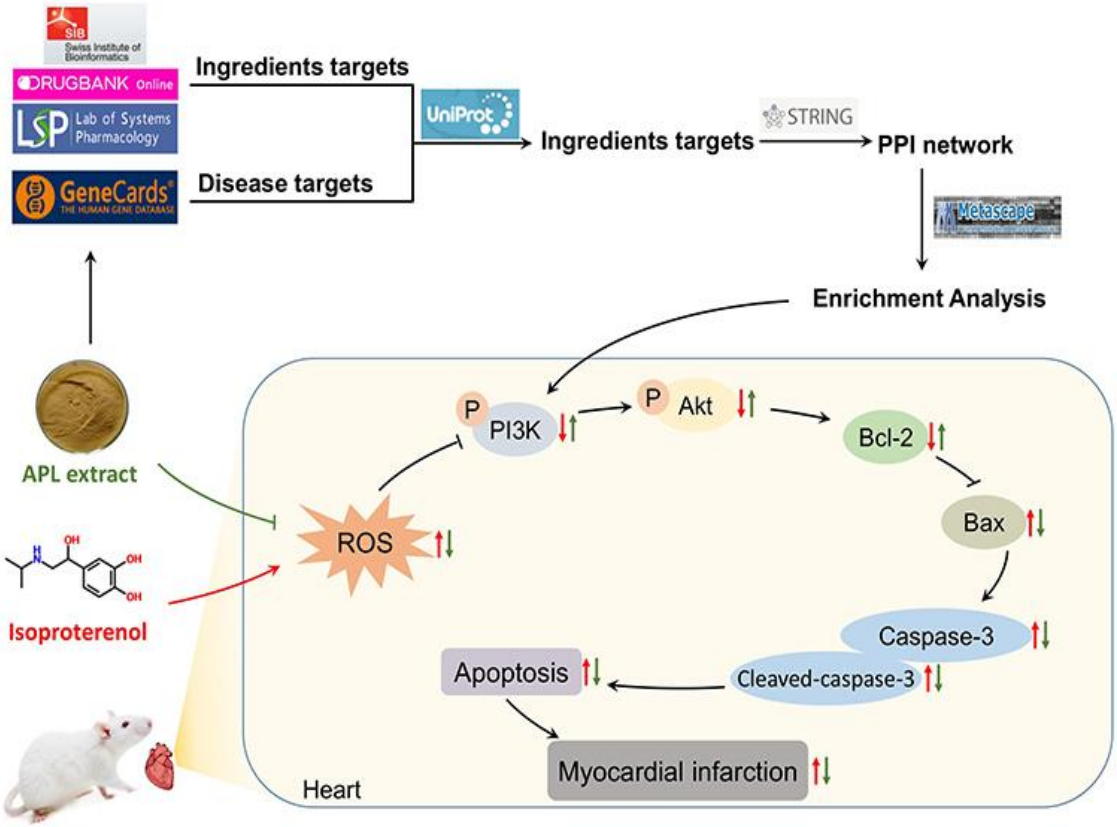

Figure 11. Schematic representation of the potential cardioprotective mechanism of APL extract against Iso-induced AMI.

KEGG and GO enrichment analyses suggested that the cardioprotective effects of APL extract against AMI involve multiple targets and pathways. Based on these findings, the PI3K/Akt signaling pathway was further investigated, as it plays a crucial role in modulating oxidative stress and apoptosis, indicating that APL extract may exert its effects through apoptosis regulation, which was subsequently confirmed by experimental results [37]. Animal studies demonstrated that APL extract significantly protected against Iso-induced AMI.

AMI, a severe outcome of coronary atherosclerotic heart disease, is typically diagnosed using ECG changes, pathological assessments, and biochemical markers. Subcutaneous Iso administration for two consecutive days in Kunming mice caused notable ECG alterations, likely due to persistent loss of cell membrane potential in damaged myocardium [38, 39]. Serum cardiac enzymes, including LDH, CK, and CK-MB, serve as sensitive indicators of myocardial injury [40], as myocardial infarction triggers the release of intracellular enzymes into circulation. Previous studies have shown that Iso markedly elevates LDH, CK, and CK-MB levels, reflecting severe myocardial cell damage [41]. In this study, treatment with APL extract significantly reduced these enzyme levels, confirming its protective effect against post-infarction myocardial injury.

Histological analysis using H&E staining revealed that Iso induced myocardial cell disorganization, morphological alterations, and necrosis. Conversely, administration of APL extract restored cellular architecture and reduced necrosis, suggesting that it may facilitate cardiac remodeling and improve ECG profiles in AMI mice. Iso administration is also known to induce oxidative stress and myocardial apoptosis [42]. Oxidative stress contributes to AMI pathogenesis and is reflected in myocardial markers such as ROS, SOD, and MDA [43]. ROS mediates oxidative damage to biomolecules, while SOD mitigates free radical-induced cellular injury and can inhibit apoptosis. MDA, a lipid peroxidation product, indicates oxidative damage to cardiac membranes [44]. In this study, APL extract increased SOD levels while reducing ROS and MDA, demonstrating its antioxidant effect. Furthermore, TUNEL staining revealed significant myocardial apoptosis in Iso-treated mice, which was markedly reduced by APL extract treatment, confirming its anti-apoptotic activity [45].

The PI3K/Akt pathway regulates critical cellular processes including proliferation, differentiation, and apoptosis [46]. Previous studies have implicated PI3K/Akt signaling in cardiomyocyte apoptosis regulation [47, 48]. PI3K activation triggers Akt phosphorylation, influencing downstream Bcl-2 family gene expression, with Bcl-2 inhibiting Bax activity and preventing caspase-3-mediated apoptosis [49–54]. Consistent with this, APL extract

treatment increased p-PI3K and p-Akt levels, promoting activation of the PI3K/Akt pathway, which in turn enhanced Bcl-2 expression and suppressed Bax, caspase-3, and cleaved-caspase-3 levels, reducing cardiomyocyte apoptosis [55, 56]. Other studies have similarly shown that PI3K/Akt activation alleviates AMI by inhibiting apoptosis [57]. Overall, the cardioprotective effects of APL extract appear to involve both antioxidant and anti-apoptotic mechanisms, likely mediated via PI3K/Akt signaling.

Although this study provides evidence that APL extract protects against Iso-induced myocardial infarction through PI3K/Akt-related pathways, further research using specific inhibitors is required to delineate the precise mechanisms involved.

Conclusion

APL extract exerts cardioprotective effects in AMI mice by attenuating oxidative stress and apoptosis, likely through activation of the PI3K/Akt signaling pathway. These findings highlight the potential of APL extract as a therapeutic candidate for AMI-related cardiovascular diseases.

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