

Please see Corrigendum

Liquiritin ameliorates acute myocardial infarction via the COX-2/NLRP3 signaling pathway: network pharmacology and experimental validation

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Abstract

Liquiritin is beneficial to cardiovascular diseases, including myocardial hypoxia/reoxygenation (H/R) injury, myocardial hypertrophy, and acute myocardial infarction (AMI). However, the mechanism of liquiritin on AMI is unknown. This study is of great significance for elucidating the mechanism of liquiritin in preventing and treating AMI and developing cardiovascular protective drugs. Network pharmacology and molecular docking were used to screen the targets of liquiritin. AMI rats were built by coronary left anterior descending ligation. Echocardiography was used to monitor cardiac function; HE staining was used to detect pathological changes, and cardiac enzymes were detected. The expressions of COX-2, NLRP3, Caspase-1, ASC, and GSDMD were detected by RT-PCR and Western Blot. ELISA was used for the detection of IL-1 β and IL-18 expression. COX-2 was detected as the most considerable protein in the PPI network. Molecular docking predicted that liquiritin has a high binding affinity with COX-2. AMI model rats showed significantly lower EF and FS ($P < 0.01$) and increased LVIDd and LVIDs ($P < 0.01$), which were improved by liquiritin. Liquiritin significantly reduced cardiac pathological changes and decreased LDH, CK, cTn-I, and BNP levels. Liquiritin reduced the mRNA and protein expressions of COX-2, NLRP3, Caspase-1, ASC, and GSDMD. Liquiritin inhibited IL-1 β and IL-18 overexpression. Liquiritin has a better effect against AMI, and its mechanism is related to inhibiting the COX-2/NLRP3 signaling pathway.

Keywords: acute myocardial infarction, COX-2/NLRP3 signaling pathway, liquiritin, molecular docking, network pharmacology

Introduction

Acute myocardial infarction (AMI) can cause irreversible damage to functional myocardial cells, negative

remodeling of the myocardium, and progressive deterioration of cardiac function (Zhou *et al.*, 2023). Despite the progress made in the treatment of myocardial infarction, many patients with AMI still die prematurely, and

those who survive are still at risk of developing heart failure, suggesting that current AMI therapy still lacks key pathophysiologic mechanisms and effective drugs (Gong *et al.*, 2021). Ventricular remodeling (VR) after AMI plays a very important role in the development of chronic heart failure (CHF) (Frantz *et al.*, 2022). In particular, VR after AMI can further lead to the onset and progression of CHF. Therefore, the curtailment of VR after AMI can significantly reduce the incidence of CHF.

Network pharmacology is a research field based on the disciplines of systems biology, genomics, and proteomics, which combines computational analysis with *in vivo* and *in vitro* experiments and integrates a large amount of information to discover new drug targets and molecular mechanisms, which is particularly applicable to multi-component and multi-targeted traditional Chinese medicine (TCM) combinations for the treatment of chronic diseases (Yang *et al.*, 2022; Zhao *et al.*, 2023). Network pharmacology can be used to predict the mechanism of natural products for treating diseases, which can help to quickly find the target of natural products (Li *et al.*, 2017).

During the last two decades, natural products have been recognized as ideal candidates for preventing and treating diseases due to their lack of apparent toxicity and versatile biological properties (Rao *et al.*, 2019). The efficacy and mechanism of natural products in treating AMI have major advantages. Licorice is an important traditional Chinese herb derived from the dried roots and rhizomes of the *Glycyrrhiza* plants, which is used worldwide in food and medicine (Wahab *et al.*, 2021; Yang *et al.*, 2017). Because of its sweet taste, licorice is an essential ingredient in foods such as candy, chewing gum, and beverages (Liu *et al.*, 2022). Liquiritin is a flavonoid derived from licorice, a widely used traditional Chinese medicine with the effects of anti-oxidative stress, anti-inflammation, and anti-apoptosis (Qin *et al.*, 2022). Liquiritin has good effects against septic cardiomyopathy, myocardial hypoxia/reoxygenation (H/R) injury, myocardial hypertrophy, diabetic cardiomyopathy, and AMI, and therefore, it is more often used in CVDs (Zhang *et al.*, 2016; Mou *et al.*, 2021; Thu *et al.*, 2021; Aiyasiding *et al.*, 2022). Previous studies have found that the effect of liquiritin against AMI was associated with its inhibition of the TLR4/MyD88/NF- κ B signaling pathway (Zhou *et al.*, 2022). However, the mechanism of liquiritin in the preventing and treating AMI is not clear.

This study utilized network pharmacology and molecular docking to predict the most likely targets of liquiritin on AMI. Finally, the predicted targets of liquiritin against AMI were experimentally validated using *in vivo* experiments to illustrate the mechanism of liquiritin fully.

Methods

Network pharmacology

Acquisition of liquiritin and targets

Liquiritin was collected in the PubChem database and saved the structure in the SDF format. Swiss Target Prediction database was used for the target prediction of liquiritin (the screening condition: Probability > 0) (Gfeller *et al.*, 2014).

Acquisition of disease target

The “Acute myocardial infarction (AMI)” keyword is used to obtain human genes from the GeneCards, NCBI, and DisGeNET databases. The targets obtained from the GeneCards database were screened by median values according to scores to obtain more relevant targets (Gfeller *et al.*, 2014).

Construction of “ingredient-disease-target” network

Common targets for liquiritin and AMI were collected by Venny (version 2.1). Cytoscape software (version 3.7.2) was used to construct and visualize the “component-disease-target” network.

Protein-protein interaction (PPI) network construction

The potential targets of liquiritin in treating AMI were imported into the STRING database (<https://string-db.org/cgi/input.pl>). The protein type was set to “Homo sapiens” and PPI network data was obtained. The results were visualized using Cytoscape software, the topological properties were analyzed by a network analyzer, and the core targets of liquiritin in treating AMI were screened.

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) pathway analysis

GO enrichment analysis focuses on three areas, including biological processes (BP), cellular components (CC), and molecular functions (MF), with corrected $P < 0.05$ were selected as screening indexes. KEGG pathway enrichment analysis was performed on the common targets of drugs and diseases, and the corrected $P < 0.05$ was screened using the String database. R software (version R 4.0.3), clusterProfiler, enrichplot, and ggplot2 were used to draw the histogram and bubble diagram.

Molecular docking

CB-dock website was used to predict the binding affinity of liquiritin and COX-2. (1) Cyclooxygenase-2 (COX-2) (PDB ID: 5KIR) was downloaded from RCSB (Orlando & Malkowski, 2016). We optimized the COX-2 with a focus on water molecules and hetatoms. One hundred fifty-four water molecules and 454 other hetatoms were deleted from the uploaded COX-2. (2) The structure of liquiritin was downloaded from Pubchem and then optimized for the lowest energy. (3) COX-2 and liquiritin

were input to CB-Dock for molecular docking (Liu *et al.*, 2020). NS-398, a specific inhibitor of COX-2, was used as a control. The VINA score represents the binding affinity between liquiritin and COX-2, with higher scores indicating better binding affinity.

In vivo experimental validation

Drugs and reagents

Liquiritin (551-15-5) was purchased from Nanjing Spring & Autumn Biological Engineering Co.Ltd. Trizol (G3013) and SYBR (G3326-15) were purchased from Servicebio Biotechnology Co.Ltd. (Wuhan, China). Interleukin (IL)-1 β (MM-0047R1), IL-18 (MM-0194R1), and brain natriuretic peptides (BNP, MM-0067R1) were bought from Meimian (Jiangsu, China). Lactate dehydrogenase (LDH, A020-2-2) and creatine kinase (CK, A032-1-1) were purchased from Nanjing Jiancheng Bioengineering Institute (China). Rat cardiac troponin I (cTn-I, Cat.# RX302234R) was bought from Quanzhou Ruixin Biotechnology Co., Ltd (China). Anti-Cleaved-caspase-1 (AF4005), anti-ASC (DF6304), anti-tubulin(AF7011), and anti-COX-2 (AF7003) were purchased from Affinity Biosciences. Anti-NLRP3 AB263899) and anti-GSDMD (ab219800) were purchased from Abcam. Anti-GAPDH (380626) was purchased from ZEN-BIO Science (Chengdu, China).

Animal modeling

As previously described, AMI model rats were constructed by ligating the left anterior descending (LAD) coronary artery. Briefly, Sprague-Dawley rats (male, 180 \pm 20g, provided by Liaoning Changsheng Biotechnology Co., Ltd, with manufacturing license SCXK (Liao) 2020-0001 were anesthetized with isoflurane (Pilz *et al.*, 2020). The left thoracic cavity of the rats was opened and ligated with 6-0 silk thread 2 mm below the left atrial appendage. The rats in the sham group only underwent thoracotomy with perforation and no ligation. This study was approved by the Animal Ethics Review Committee of Anhui University of Chinese Medicine (No. AHUCM-2023012).

Drug treatment

Two weeks later, rats with AMI were randomly assigned to three groups: model (i.g. normal saline), liquiritin (i.g. 20 mg/kg) (Han *et al.*, 2022), captopril (i.g. 4.375 mg/kg) groups. All interventions were conducted for four weeks.

Echocardiography for cardiac function

All rats were anesthetized with 1% sodium pentobarbital by intraperitoneal injection. Two-dimensional M-mode echocardiography was applied to examine cardiac function in all groups of rats, and after locating the ventricles, the LV motion curves were recorded. The parameters

of ejection fraction (EF), fractional shortening (FS), left ventricular internal diameter diastolic (LVIDd), left ventricular internal diameter systolic (LVIDs), left ventricular end-diastolic volume (LVEDV), and left ventricular end-systolic volume (LVESV) were measured.

Hematoxylin-eosin (HE) staining for pathological changes

HE staining was used to observe the pathological changes.

Enzyme-linked immunosorbent assays (ELISA) for IL-1 β , IL-18, BNP and cTnI

After blood collection, the rats were left for 2 h, centrifuged for 10 min (3000 rpm/min), and the supernatant was collected and frozen at -20 °C. The contents of IL-1 β , IL-18, BNP, and cTnI in the serum of rats were detected according to the kit instructions.

Colorimetric assay for LDH and CK

Rat serum LDH and CK activities were assayed according to the kit instructions. Fluorescence was measured on a SpectraMax i3x microplate reader.

RT-PCR for mRNA expression

Rat myocardial tissue was taken, total RNA was extracted by Trizol reagent, then Eppendorf BioPhotometer Plus quantified RNA, and RNA purity was determined by absorbance at 260 and 280 nm. RNA was reverse transcribed into cDNA using a Mastercycler[®] nexus gradient (Germany). The cDNA was then subjected to real-time fluorescence quantification using a LightCycler[®] 96 PCR instrument (Roche, Switzerland). The amplification procedure consisted of pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 15 s, annealing at 60 °C for 60 s, and PCR at 40 cycles. The melting curve is from 60 °C to 95 °C. The mRNA expression levels of the genes were analyzed by the 2^{- $\Delta\Delta$ C_q} method using β -actin as a control. The primer sequence for COX-2 (Forward: 5'-CACATTTGATTGACAGCCCACCAAC-3', Reverse: 5'-AGTCATCAGCCACAGGAGGAAGG-3'), NLRP3 (Forward: 5'-GAGCTGGACCTCAGTGACAA TGC-3', Reverse: 5'-AGAACCAATGCGAGATCCTGAC AAC-3'), ASC (Forward: 5'-ATGGTTTTGCTGGATGC TCTGTAT-3', Reverse: 5'-CAGCAACATCCTGGCAG TCATCC-3'), Caspase-1 (Forward: 5'-GCACAAGAC TTCTGACAGTACCTTCC-3', Reverse: 5'-GCT TGGGCACTTCAATGTGTTTCATC-3'), GSDMD (Forward: 5'-CAGCAGGCAGCATCCTTGAGTG-3', Reverse: 5'-CCTCCAGAGCCTTAGTAGCCAGTAG-3'), and β -actin (Forward: 5'-CCCATCTATGAGGG TTACGC-3', Reverse: 5'-TTTAATGTACGCACGAT TTC-3') were used in the study.

Western blot for protein expression

Total protein was extracted after the cardiac tissue had been dissolved in lysate. Lysates were separated on 10%

to 15% polyacrylamide gels and then transferred to PVDF membranes. The PVDF membrane was blocked with 5% skim milk and then incubated with COX-2 (1:1000), NLRP3 (1:1000), ASC (1:1000), Cleaved-Caspase-1 (1:500), GSDMD (1:1000), Tubulin (1:5000) and GAPDH(1:5000) at 4 °C overnight. The secondary antibody was incubated overnight at 4 °C and then treated with the primary antibody for 2 h at room temperature. The ECL chemical substrate luminescence kit was used to determine the protein band density, and the Tanon5200 imaging system (Tanon, China) was used to photograph the protein bands.

Statistical analysis

The data were presented as mean±standard deviation (SD), and SPSS 26.0 was used for statistical analysis. One-way ANOVA was used to determine the difference. $P<0.05$ was considered statistically significant.

Results

Network pharmacology results

Integration of liquiritin and AMI targets

Seventy-four potential targets of liquiritin were obtained. Disease targets were then searched in the GeneCards, NCBI, and DisGeNET databases, and a total of 2200 genes associated with AMI were obtained. Finally, the common targets of liquiritin and AMI were obtained and plotted by Venn diagrams, that is, 40 targets were the potential targets of liquiritin for the treatment of AMI (Figure 1).

PPI network and core target analysis

Protein interaction information obtained from the String database was put into Cytoscape to generate a PPI network (Figure 2). The network has 40 nodes and 94 edges with an average degree of 4.7. PTGS2 (COX-2), ESR1, and PPARG were the top 3 targets. COX-2 was considered the most important target in the PPI network for relevance to AMI.

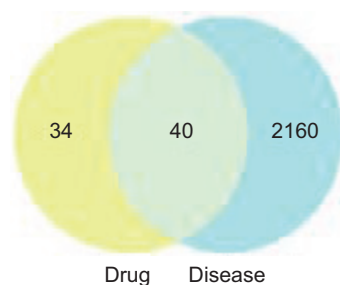


Figure 1. The Venn diagram of the common targets of liquiritin and AMI. The 40 common targets for liquiritin in the treatment of AMI.

GO enrichment analysis

GO enrichment analysis revealed 473 BP terms, 22 CC terms, and 39 MF terms. The top 10 entries in each section were shown according to the number of enriched genes (Figure 3).

KEGG enrichment analysis

According to the KEGG analysis, a total of eight enrichment pathways were screened (Figure 4). The color corresponds to the size of the adjusted P -value. This suggests that liquiritin can be used to treat AMI through chemical carcinogenesis-receptor activation, and peroxisome. Peroxisome is closely related to inflammatory response, which may be the main mechanism.

Molecular docking results

VINA score of liquiritin was higher than that of the COX-2 inhibitor (NS-398) (Table 1 and Figure 5), which showed that liquiritin has a high binding affinity. The results indicated that liquiritin may act on COX-2.

In vivo experimental results

Effect of liquiritin on cardiac function in AMI rats

The results showed that EF and FS were decreased ($P<0.01$), LVIDd, LVIDs, LVEDV, and LVESV were increased in the model group ($P<0.01$). Compared with the model group, the levels of the EF, FS, LVIDd, LVIDs, LVEDV, and LVESV were reversed in the liquiritin group ($P<0.01$) (Figure 6). Liquiritin could significantly improve cardiac impairment in AMI rats.

Effect of liquiritin on pathological changes of AMI rats

HE staining results showed that the myocardium of rats in the sham group was structurally intact, with myofibrils aligned neatly. Meanwhile, rats in the model group had more disorganized myocardial fibers, myocardial fiber breaks, and inflammatory cell infiltration. Liquiritin resulted in aligned myocardium and less inflammatory cell infiltration in AMI model rats, improving myocardial injury caused by AMI (Figure 7).

Effect of liquiritin on serum myocardial enzyme level in AMI rats

Serum levels of BNP, CK, cTn-I, and LDH were significantly higher in the model group ($P<0.01$), which was significantly ameliorated in the liquiritin and Captopril groups ($P<0.01$) (Figure 8). The results indicated that liquiritin was able to significantly reduce the serum levels of cardiac enzymes in AMI rats.

Effect of liquiritin on the expression of key genes in the COX-2/NLRP3 pathway

As shown in Figure 9, COX-2, NLRP3, Caspase-1, ASC, and GSDMD mRNA expressions were increased in the

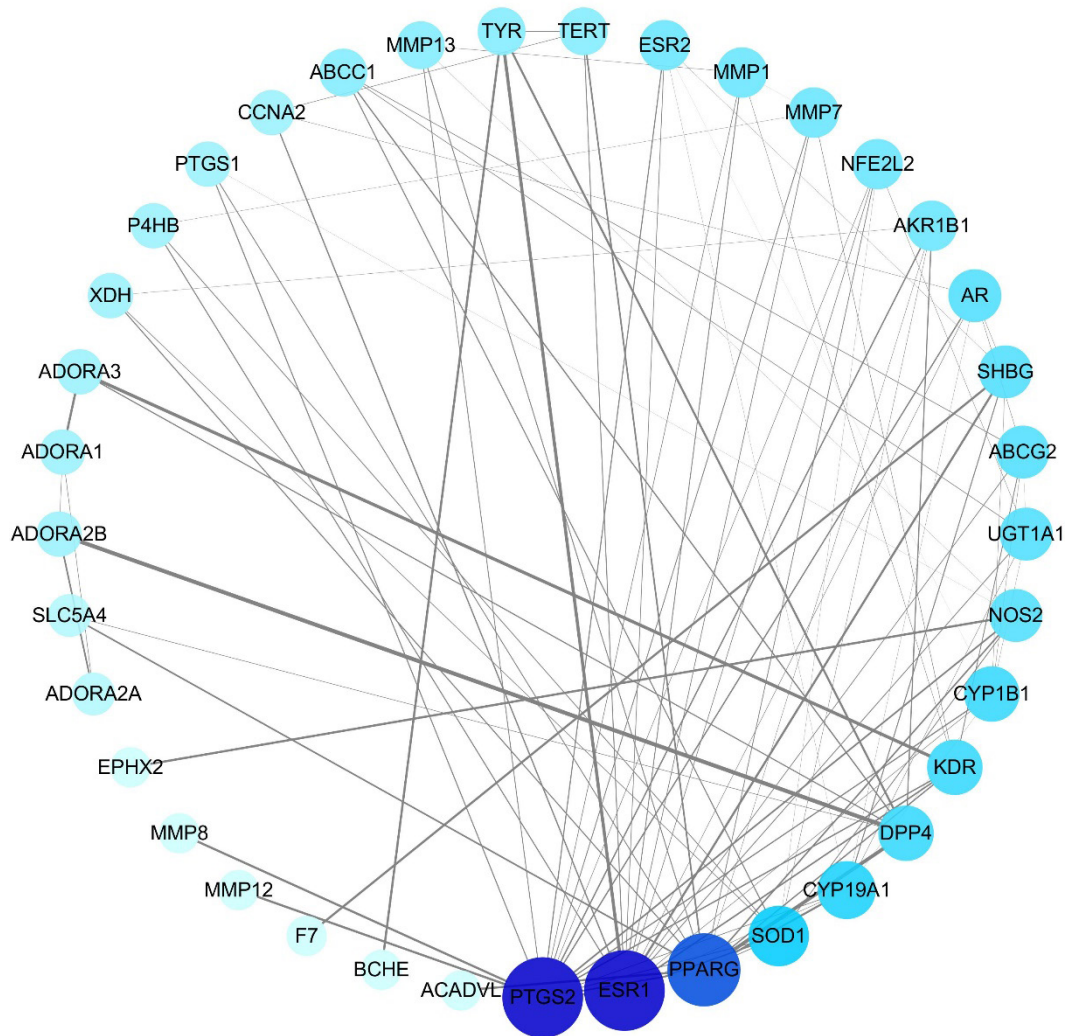


Figure 2. PPI network diagram. The graph shows the interrelationships among 40 common targets.

model group ($P < 0.01$). Liquiritin could significantly reverse the mRNA expressions ($P < 0.01$).

Effect of liquiritin on the protein expression of COX-2 and NLRP3

The protein expression of COX-2, NLRP3, ASC, Cleaved-Caspase-1, GSDMD, and GSDMD-N were increased in the model group ($P < 0.05$, $P < 0.01$). After administration of liquiritin or Captopril, the protein expression was significantly reduced ($P < 0.05$, $P < 0.01$) (Figure 10). Liquiritin could reduce the inflammatory response occurring in the heart of AMI rats by modulating the COX-2/NLRP3 pathway.

Effect of liquiritin on the release of inflammatory factors in AMI rats

The level of IL-1 β and IL-18 were significantly elevated in the model group ($P < 0.01$), whereas the expressions were significantly inhibited in the liquiritin or Captopril

groups ($P < 0.05$, $P < 0.01$) (Figure 11). Therefore, liquiritin could inhibit inflammatory injury in AMI rats.

Discussion

AMI is a common CVDs that occurs when circulation to an area of the heart is blocked, and myocardial tissue is necrotic. Accompanied by increasing risk factors such as hypertension, dyslipidemia, and diabetes mellitus, the incidence of AMI has shown a rising trend, thus posing a serious threat to the health and quality of life of patients with AMI (Lee *et al.*, 2018). Currently, AMI is usually treated by reperfusion of the ischemic myocardium to save the dying myocardium, and clinical drugs used to treat AMI include thrombolytic and antiplatelet agents, anticoagulants, nitrates, and calcium receptor blockers (Xiao *et al.*, 2022). However, the use of these drugs can produce certain side effects such as headaches, decreased

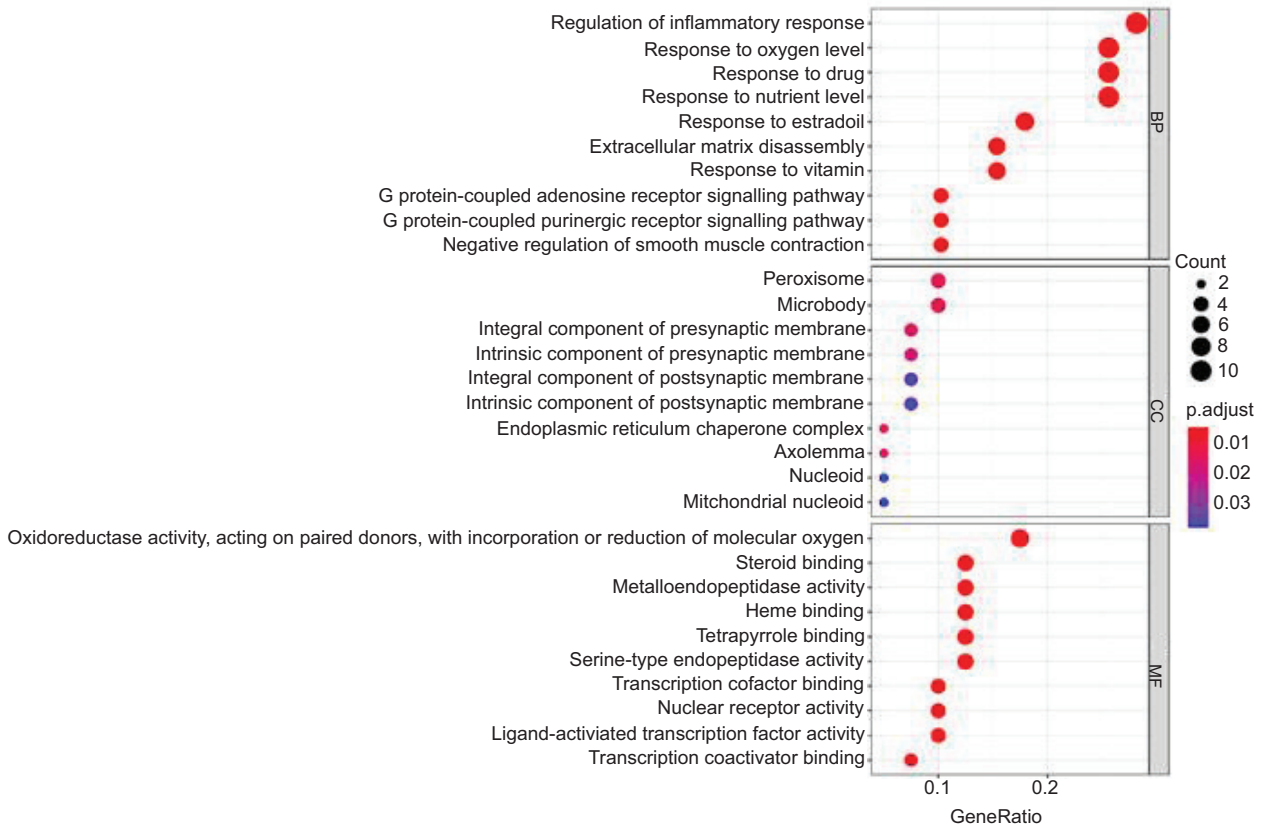


Figure 3. GO enrichment analysis. BP diagram, CC diagram, and MF diagram. BP, CC, and MF column colors indicate enrichment significance based on adjusted *P*-value.

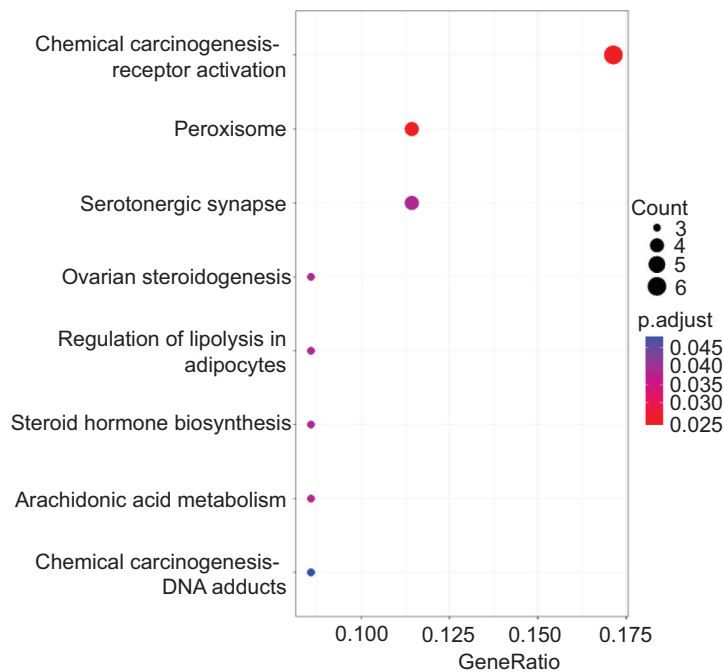


Figure 4. KEGG enrichment analysis. The horizontal axis represents importance according to the corrected *P*-value.

blood pressure, gastrointestinal disorders, impaired liver and kidney function, and electrolyte disorders (Rubinfeld *et al.*, 2022). Despite tremendous progress in modern medical diagnostic and therapeutic techniques and the development of AMI drugs, there is a lack of safe and effective drugs due to the complexity of their pathogenesis.

Table 1. Docking of liquiritin with COX-2

Chemicals	Vina score	Cavity score	Center (x, y, z)	Size (x, y, z)
Liquiritin	-9.2	17137	20, 15, 29	35, 35, 35
NS-398	-7.6	20, 15, 29	35, 35, 35	

Network pharmacology has been widely used to guide the treatment of coronary heart disease by analyzing the synergistic relationship of multiple components, targets, and pathways among drugs, diseases, and targets (Wang *et al.*, 2022). This study predicted liquiritin in treating AMI was associated with COX-2 and its inflammatory cascade response by network pharmacology. Molecular docking is a powerful computational tool for predicting binding modes and binding free energies of proteins and natural products, which can be used to screen possible targets of natural products and provide a theoretical basis for elucidating the mechanism (Pinzi and Rastelli, 2019.). Liquiritin has a high binding affinity with COX-2, which suggests that liquiritin may act on

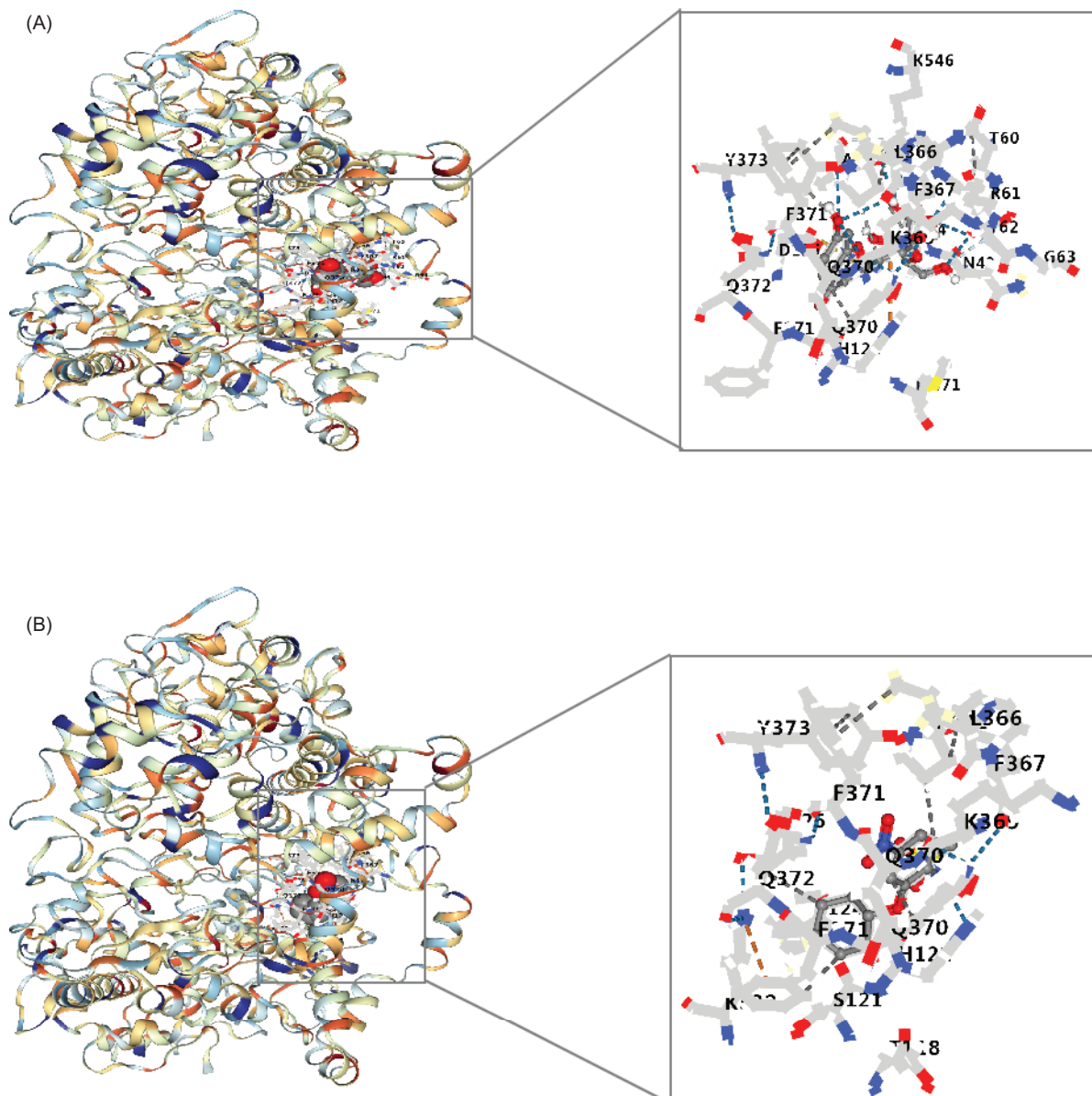


Figure 5. The 3D pictures of liquiritin with COX-2. (A) Liquiritin-COX-2 complex, (B) NS-398- COX-2 complex.

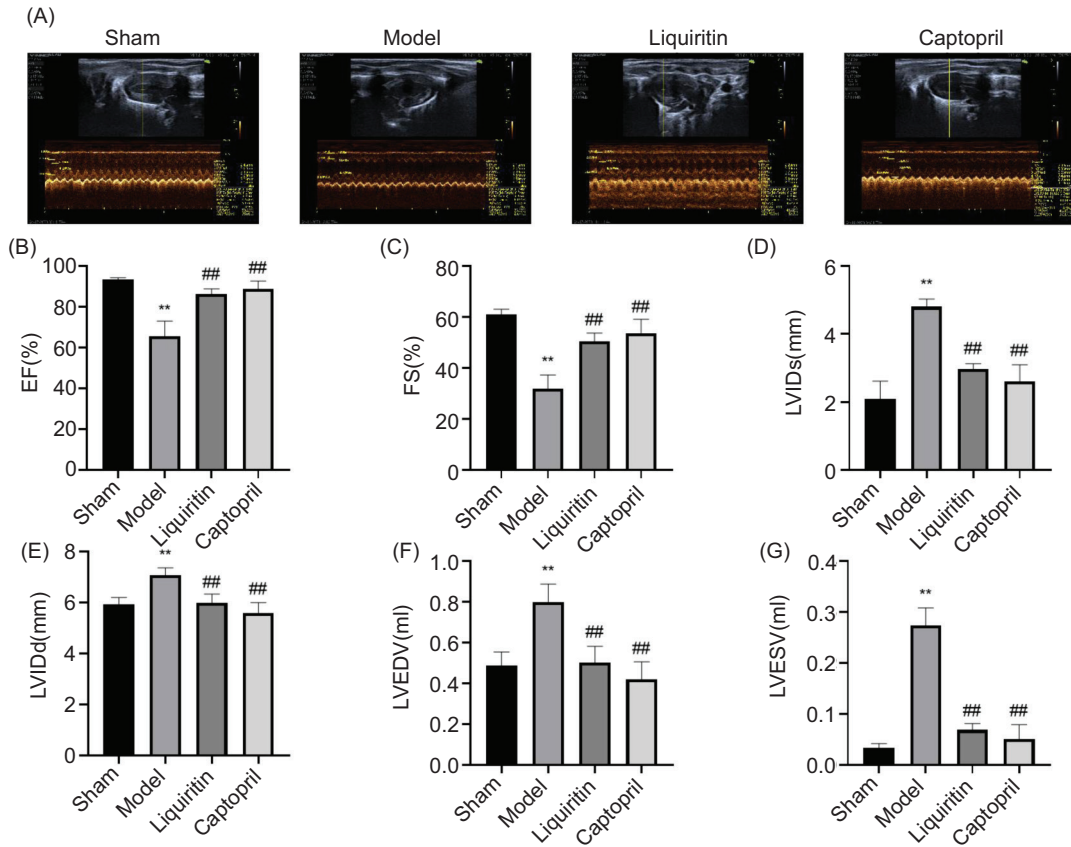


Figure 6. Effect of liquiritin on cardiac function in AML rats. (A) Echocardiogram of each group. (B) EF. (C) FS. (D) LVIDs. (E) LVIDd. (F) LVEDV. (G) LVESV. Compared with the sham group, ** $P < 0.01$, Compared with the model group, ## $P < 0.01$.

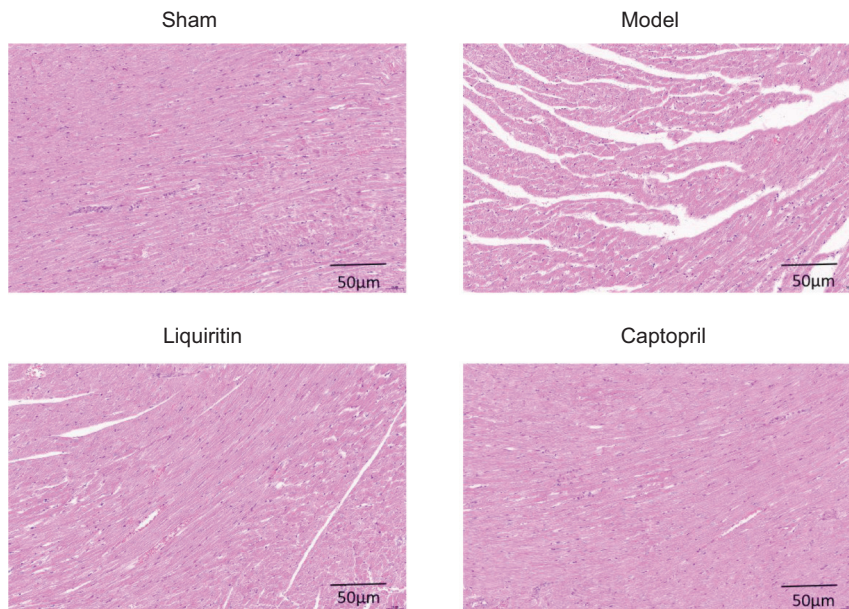


Figure 7. Effect of liquiritin on pathological changes of AML rats.

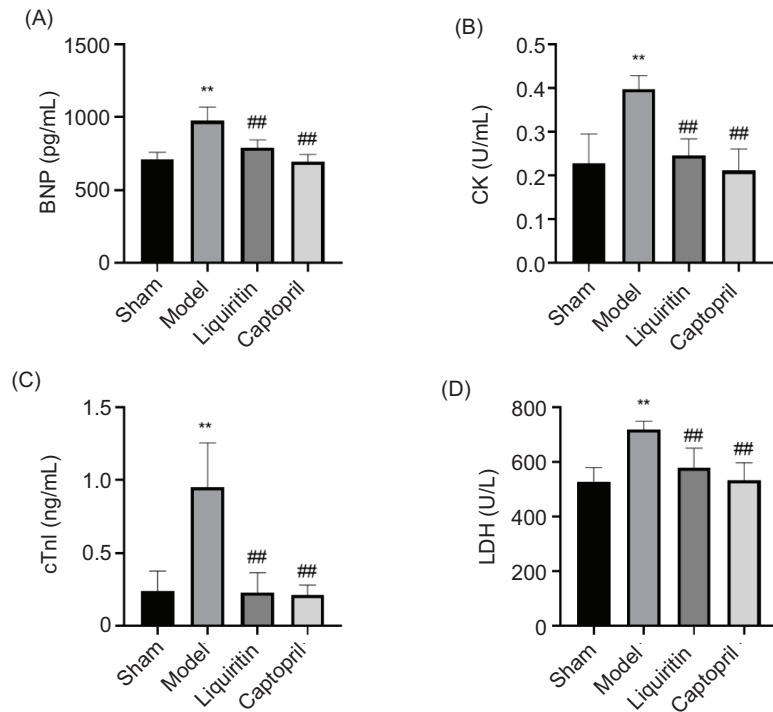


Figure 8. Effect of liquiritin on serum myocardial enzyme level in AMI rats. (A) BNP. (B) CK. (C) cTnI. (D) LDH. Compared with the sham group, ** $P<0.01$, Compared with the model group, ## $P<0.01$.

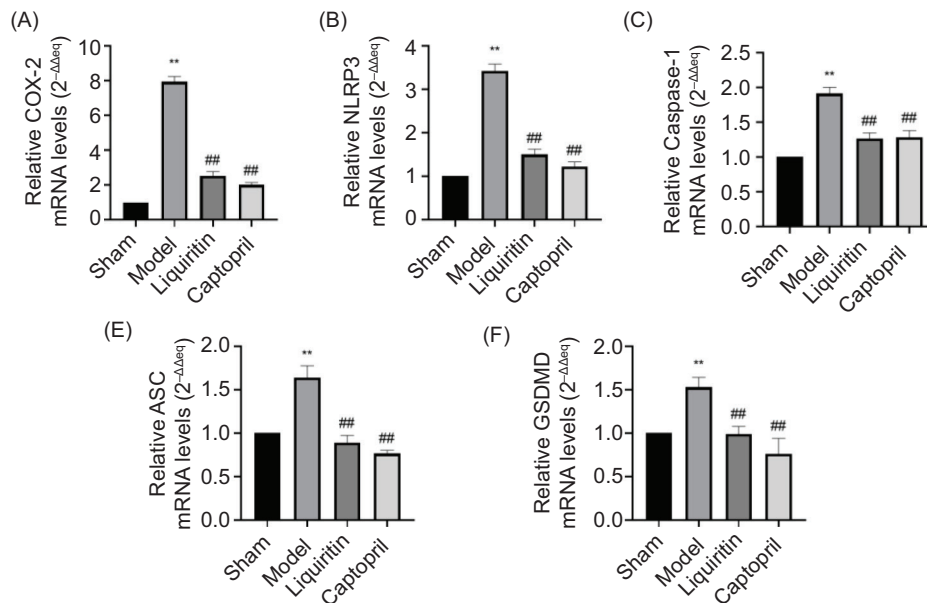


Figure 9. Effect of liquiritin on the expression of key genes in the COX-2/NLRP3 pathway (A) COX-2 mRNA. (B) NLRP3 mRNA. (C) Caspase-1 mRNA. (D) ASC mRNA. (E) GSDMD mRNA. Compared with the sham group, ** $P<0.01$, Compared with the model group, ## $P<0.01$.

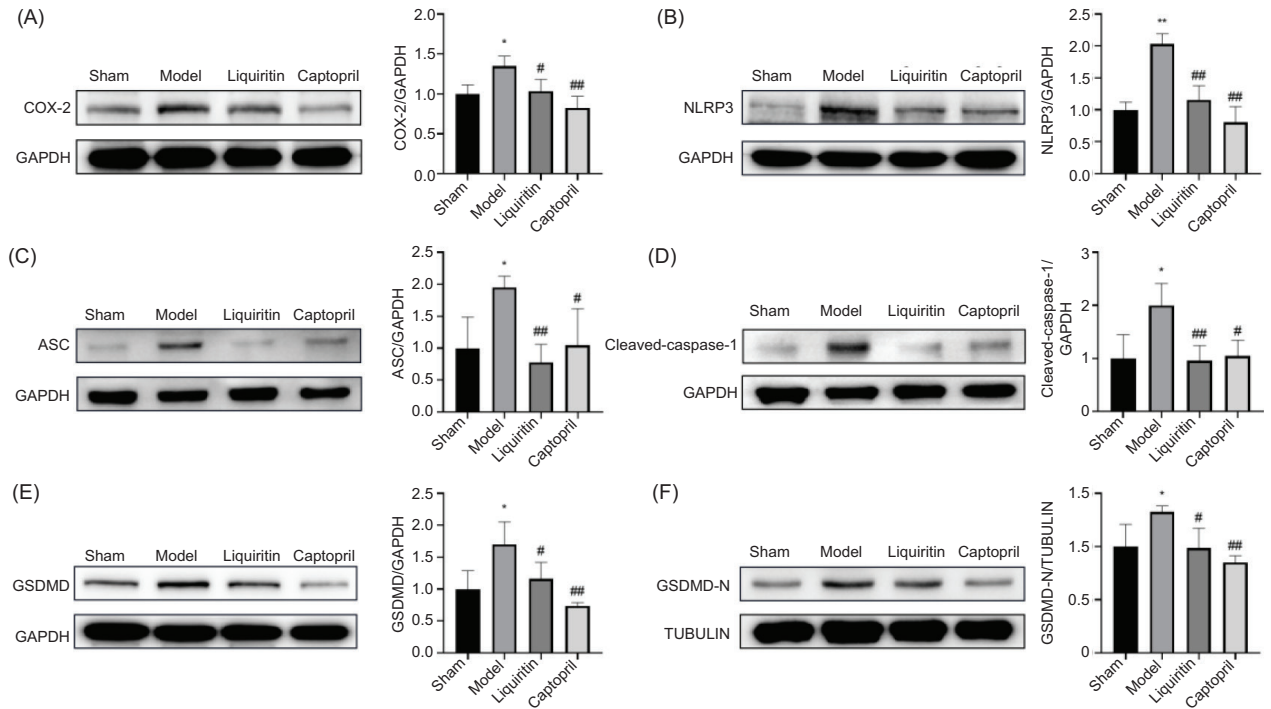


Figure 10. Effect of liquiritin on the protein expression of COX-2 and NLRP3. (A) COX-2. (B) NLRP3. (C) ASC. (D) Cleaved-Caspase-1. (E) GSDMD. (F) GSDMD-N. Compared with the sham group, * $P < 0.05$, ** $P < 0.01$. Compared with the model group, # $P < 0.05$, ## $P < 0.01$.

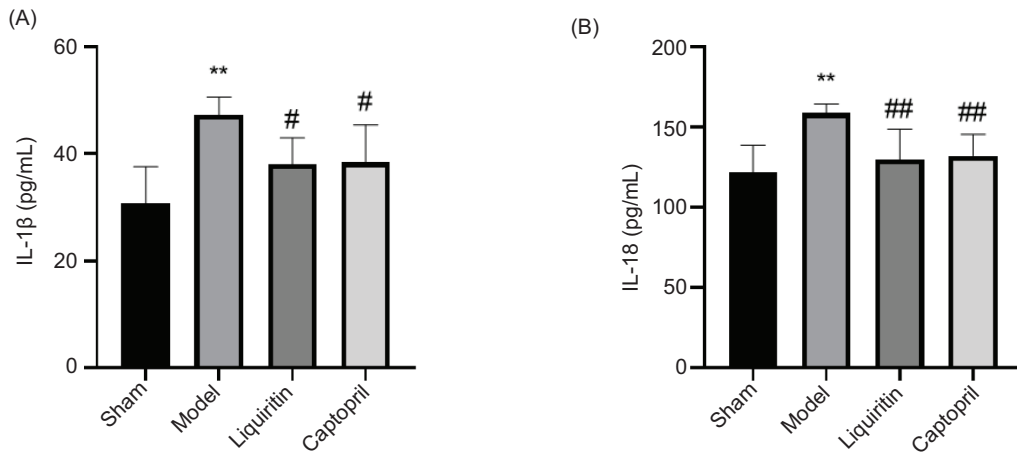


Figure 11. Effect of liquiritin on the release of inflammatory factors in AMI rats. (A) IL-1 β . (B) IL-18. Compared with the sham group, ** $P < 0.01$, Compared with the model group, # $P < 0.05$, ## $P < 0.01$.

COX-2. Therefore, COX-2 can be the focus of this paper to study liquiritin against AMI.

COX-2 is a key enzyme that catalyzes the conversion of arachidonic acid to prostaglandins, and the upregulation of COX-2 levels is related to the pathogenesis of many inflammatory processes (Alhouayek and Muccioli, 2014). COX-2 has also been shown to positively regulate the expression of NLRP3 and IL-1 β in macrophages

(Yang *et al.*, 2020). Silencing the COX-2 gene or using COX-2 inhibitors significantly reduced the activation of NLRP3 inflammasome, which ameliorated cystatinase-1-dependent pyroptosis (Zhuang *et al.*, 2017). In addition, inhibition of the COX-2/NLRP3 signaling pathway was able to attenuate the inflammatory response induced by myocardial fibroblast pyroptosis, resulting in attenuation of myocardial ischemia/reperfusion injury in mice (Zhang *et al.*, 2021). These evidences suggested that

COX-2/NLRP3 signaling pathway-mediated pyroptosis was closely related to the development of VR after AMI and is one of the important signaling pathways. The specific pathological process is that COX-2 expression was increased under pathological stimuli, followed by activation of the NLRP3 inflammasome (including NLRP3, ASC, and caspase-1). Caspase-1 activation cleaves the GSDMD into C-terminal (GSDMD-C) and N-terminal (GSDMD-N) ends, the latter of which mediates pyroptosis. Activated caspase-1 also cleaves inactive pro-IL-1 β and pro-IL-18 cleavage into mature IL-1 β and IL-18, which were released into the extracellular space through the GSDMD-N pore in the cell membrane, inducing an inflammatory cascade response (Zhang *et al.*, 2018).

Liquiritin has a good advantage in cardioprotection, including reducing ROS in H9c2 cells, attenuating mitochondrial Ca²⁺ overload, decreasing H9c2 cell death, and protecting cardiac mitochondria from hypoxia/reoxygenation (HR) injury (Thu *et al.*, 2021). In addition, Liquiritin regulated the ATE1/TAK1-JNK1/2 pathway to exert a protective effect against Ang II-induced cardiomyocyte hypertrophy (Mo *et al.*, 2022). However, the mechanism of liquiritin in preventing and treating AMI is unclear. Therefore, this paper combines prediction and experimental validation to elucidate the mechanism of liquiritin comprehensively.

Based on the network pharmacology and molecular docking results, COX-2 and its associated inflammatory pathways are the key targets and pathways for liquiritin against AMI. In the AMI model rats, echocardiography was used to assess the cardiac function of the rats. AMI model rats showed significant cardiac function abnormalities, such as a significant decrease in EF and FS and an increase in LVIDd, LVIDs, LVEDV, and LVESV, which appeared to improve significantly after administration of liquiritin or captopril. LDH is a well-known marker to assess cellular activity and indicate cardiomyocyte injury. CK and cTn-I as preferred markers for the diagnosis of AMI. As a quantitative marker of heart failure, BNP reflects left ventricular systolic dysfunction, left ventricular diastolic dysfunction, valvular dysfunction and right ventricular dysfunction. LDH, CK, cTn-I, and BNP were significantly up-regulated in AMI model rats. Intervention of liquiritin or captopril reversed myocardial injury. mRNA levels of COX-2, NLRP3, Caspase-1, ASC, and GSDMD were significantly elevated in the model group, liquiritin could reduce the mRNA level. The key protein levels of COX-2 and NLRP3 were significantly elevated in the model group, liquiritin could reverse the results. Serum level of IL-1 β and IL-18 were also significantly elevated, and liquiritin significantly reduced the expression of IL-1 β and IL-18. These results indicated that liquiritin significantly attenuates inflammation in VR after AMI.

Conclusion

We confirmed that liquiritin significantly exerted cardioprotective effects by inhibiting cardiac inflammation in AMI model rats. The mechanism may be related to the COX-2/NLRP3 signaling pathway. However, *in vivo*, experiments were used to study liquiritin against VR after AMI in this study, and further study will focus on the *in vitro* experiments and elucidation of the mechanism using silencing of the COX-2 gene or COX-2 inhibitors.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgment

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References

- Aiyasiding X., Liao H.H., Feng H., Zhang N., Lin Z., Ding W., et al. (2022). Liquiritin attenuates pathological cardiac hypertrophy by activating the PKA/LKB1/AMPK pathway. *Frontiers in Pharmacology*, 13, 870699. <https://doi.org/10.3389/fphar.2022.870699>
- Alhouayek M., Muccioli G.G. (2014). COX-2-derived endocannabinoid metabolites as novel inflammatory mediators. *Trends in Pharmacological Sciences*, 35(6), 284–292. <https://doi.org/10.1016/j.tips.2014.03.001>
- Frantz S., Hundertmark M.J., Schulz-Menger J., Bengel E.M., Bauersachs J. (2022). Left ventricular remodeling post-myocardial infarction: pathophysiology, imaging, and novel therapies. *European Heart Journal*, 43(27), 2549–2561. <https://doi.org/10.1093/eurheartj/ehac223>
- Gfeller D., Grosdidier A., Wirth M., Daina A., Michielin O., Zoete V. (2014). Swiss Target Prediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Research*, 42, W32–38. <https://doi.org/10.1093/nar/gku293>
- Gong F.F., Vaitenas L., Malaisrie S.C., Maganti K. (2021). Mechanical complications of acute myocardial infarction: a review. *JAMA Cardiology*, 6(3), 341–349. <https://doi.org/10.1001/jamacardio.2020.3690>
- Han X., Yang Y., Zhang M., Li L., Xue Y., Jia Q., et al. (2022). Liquiritin protects against cardiac fibrosis after myocardial infarction by inhibiting CCL5 expression and the NF- κ B signaling pathway. *Drug Design, Development and Therapy*, 16, 4111–4125. <https://doi.org/10.2147/DDDT.S386805>
- Lee Y.M., Kim R.B., Lee H.J., Kim K., Shin M.H., Park H.K., et al. (2018). Relationships among medication adherence, lifestyle modification, and health-related quality of life in patients with

- acute myocardial infarction: a cross-sectional study. *Health and Quality of Life Outcomes*, 16(1), 100. <https://doi.org/10.1186/s12955-018-0921-z>
- Li W., Yuan G., Pan Y., Wang C., Chen H. (2017). Network pharmacology studies on the bioactive compounds and action mechanisms of natural products for the treatment of diabetes mellitus: a review. *Frontiers in Pharmacology*, 8, 74. <https://doi.org/10.3389/fphar.2017.00074>
- Liu J., Sun L., Chen D., Huo X., Tian X., Li J., et al. (2022). Prdx6-induced inhibition of ferroptosis in epithelial cells contributes to liquiritin-exerted alleviation of colitis. *Food and Function*, 13(18), 9470–9480. <https://doi.org/10.1039/D2FO00945E>
- Liu Y., Grimm M., Dai W.T., Hou M.C., Xiao Z.X., Cao Y. (2020). CB-Dock: a web server for cavity detection-guided protein-ligand blind docking. *Acta Pharmacologica Sinica*, 41(1), 138–144. <https://doi.org/10.1038/s41401-019-0228-6>
- Mo J., Zhou P., Chu Z., Zhao Y., Wang X. (2022). Liquiritin attenuates angiotensin II-induced cardiomyocyte hypertrophy via ATE1/TAK1-JNK1/2 pathway. *Evidence-Based Complementary and Alternative Medicine*, 2022, 7861338. <https://doi.org/10.1155/2022/7861338>
- Mou S.Q., Zhou Z.Y., Feng H., Zhang N., Lin Z., Aiyasiding X., et al. (2021). Liquiritin attenuates lipopolysaccharides-induced cardiomyocyte injury via an AMP-activated protein kinase-dependent signaling pathway. *Frontiers in Pharmacology*, 12, 648688. <https://doi.org/10.3389/fphar.2021.648688>
- Orlando B.J., Malkowski M.G. (2016). Crystal structure of rofecoxib bound to human cyclooxygenase-2. *Acta Crystallographica Section F Structural Biology Communications*, 72(10), 772–776. <https://doi.org/10.1107/S2053230X16014230>
- Pilz P.M., Lang M., Hamza O., Szabo P.L., Inci M., Kramer A.M., et al. (2020). Semi-minimal invasive method to induce myocardial infarction in rats and the assessment of cardiac function by an isolated working heart system. *Journal of Visualized Experiments*, 2020, 160. <https://doi.org/10.3791/61033>
- Pinzi L., Rastelli G. (2019). Molecular docking: Shifting paradigms in drug discovery. *International Journal of Molecular Sciences*, 20(18), 4331. <https://doi.org/10.3390/ijms20184331>
- Qin J., Chen J., Peng F., Sun C., Lei Y., Chen G., et al. (2022). Pharmacological activities and pharmacokinetics of liquiritin: a review. *Journal of Ethnopharmacology*, 293, 115257. <https://doi.org/10.1016/j.jep.2022.115257>
- Rao T., Tan Z., Peng J., Guo Y., Chen Y., Zhou H., et al. (2019). The pharmacogenetics of natural products: A pharmacokinetic and pharmacodynamic perspective. *Pharmacological Research*, 146, 104283. <https://doi.org/10.1016/j.phrs.2019.104283>
- Rubinfeld G.D., Berger J.S., Smilowitz N.R. (2022). Acute myocardial infarction following hospitalization for gastrointestinal bleeding: Incidence, predictors, management, and outcomes. *The American Journal of Medicine*, 135(8), e263–e278. <https://doi.org/10.1016/j.amjmed.2022.03.030>
- Stelzer G., Rosen N., Plaschkes I., Zimmerman S., Twik M., Fishilevich S., et al. (2016). The GeneCards suite: from gene data mining to disease genome sequence analyses. *Current Protocols in Bioinformatics*, 54, 1–30. <https://doi.org/10.1002/cpbi.5>
- Thu V.T., Yen N.T.H., Ly N.T.H. (2021). Liquiritin from *Radix Glycyrrhizae* protects cardiac mitochondria from hypoxia/reoxygenation damage. *Journal of Analytical Methods in Chemistry*, 2021, 1857464. <https://doi.org/10.1155/2021/1857464>
- Wahab S., Annadurai S., Abullais S.S., Das G., Ahmad W., Ahmad M.F., et al. (2021). *Glycyrrhiza glabra* (Licorice): A comprehensive review on its phytochemistry, biological activities, clinical evidence and toxicology. *Plants*, 10(12) 2751. <https://doi.org/10.3390/plants10122751>
- Wang X., Wang Z.Y., Zheng J.H., Li S. (2022). TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches. *Chinese Journal of Natural Medicines*, 19(1), 1–11. <https://doi.org/10.4236/cm.2022.131001>
- Xiao L., Liu J., Li G., Zheng Y., Chen M., He S. (2022). Acute myocardial infarction after a local anesthetic procedure in a middle-aged patient. *The American Journal of the Medical Sciences*, 364(1), 106–110. <https://doi.org/10.1016/j.amjms.2022.03.009>
- Yang H.H., Duan J.X., Liu S.K., Xiong J.B., Guan X.X., Zhong W.J., et al. (2020). A COX-2/sEH dual inhibitor PTUPB alleviates lipopolysaccharide-induced acute lung injury in mice by inhibiting NLRP3 inflammasome activation. *Theranostics*, 10(11), 4749–4761. <https://doi.org/10.7150/thno.43108>
- Yang H.Y., Liu M.L., Luo P., Yao X.S., Zhou H. (2022). Network pharmacology provides a systematic approach to understanding the treatment of ischemic heart diseases with traditional Chinese medicine. *Phytomedicine*, 104, 154268. <https://doi.org/10.1016/j.phymed.2022.154268>
- Yang R., Yuan B.C., Ma Y.S., Zhou S., Liu Y. (2017). The anti-inflammatory activity of licorice, a widely used Chinese herb. *Pharmaceutical Biology*, 55(1), 5–18. <https://doi.org/10.1080/13880209.2016.1225775>
- Zhang L., Fan Y., Su H., Wu L., Huang Y., Zhao L., et al. (2018). Chlorogenic acid methyl ester exerts strong anti-inflammatory effects via inhibiting the COX-2/NLRP3/NF- κ B pathway. *Food and Function*, 9(12), 6155–6164. <https://doi.org/10.1039/C8FO01281D>
- Zhang M., Lei Y.S., Meng X.W., Liu H.Y., Li L.G., Zhang J., et al. (2021). Igaratimod alleviates myocardial ischemia/reperfusion injury through inhibiting inflammatory response induced by cardiac fibroblast pyroptosis via COX2/NLRP3 signaling pathway. *Frontiers in Cell and Developmental Biology*, 9, 746317. <https://doi.org/10.3389/fcell.2021.746317>
- Zhang Y., Zhang L., Zhang Y., Xu J.J., Sun L.L., Li S.Z. (2016). The protective role of liquiritin in high fructose-induced myocardial fibrosis via inhibiting NF- κ B and MAPK signaling pathway. *Biomedicine and Pharmacotherapy*, 84, 1337–1349. <https://doi.org/10.1016/j.biopha.2016.10.036>
- Zhao L., Zhang H., Li N., Chen J., Xu H., Wang Y., et al. (2023). Network pharmacology, a promising approach to reveal the pharmacology mechanism of Chinese medicine formula. *Journal of Ethnopharmacology*, 309, 116306. <https://doi.org/10.1016/j.jep.2023.116306>
- Zhou J., Liu H., Zhang T., Wang Z., Zhang J., Lu Y., et al. (2023). MORN4 protects cardiomyocytes against ischemic injury via MFN2-mediated mitochondrial dynamics and mitophagy. *Free Radical Biology and Medicine*, 196, 156–170. <https://doi.org/10.1016/j.freeradbiomed.2023.01.016>

Zhou P, Shen A.L., Liu P.P., Wang S.S., Wang L. (2022). Molecular docking and in vivo studies of liquiritin against acute myocardial infarction via TLR4/MyD88/NF- κ B signaling. *Italian Journal of Food Science*, 34(2), 1–9. <https://doi.org/10.15586/ijfs.v34i2.2188>

Zhuang Y., Zhao F., Liang J., Deng X., Zhang Y., Ding G., et al. (2017). Activation of COX-2/mPGES-1/PGE2 cascade via NLRP3 inflammasome contributes to albumin-induced proximal tubule cell injury. *Cellular Physiology and Biochemistry*, 42(2), 797–807. <https://doi.org/10.1159/000478070>