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Study of 7-Hydroxyflavone against Oxidative Stress in Myocardial Ischemia/Reperfusion Injury Based on Network Pharmacology and Bioinformatics

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Abstract

Subjective: This study aimed to investigate the therapeutic mechanisms of 7-hydroxyflavone (7-HF) in treating myocardial ischemia/reperfusion injury (MI/RI) via network pharmacology, molecular docking, target validation, and experiments at the animal level. **Methods:** Firstly, the genes of 7-HF were acquired from PharmMapper, TCMSP, and SwissTargetPrediction. At the same time, MI/RI-related genes were obtained from OMIM, GeneCards, and TTD online platforms. Subsequently, string platform and Cytoscape 3.9.2 were used to construct protein-protein interaction network diagrams and 7-HF-targets-signaling pathways-MI/RI network. Then, the Metascape platform was used to conduct functional enrichment analyses. Next, AutoDock Vina and Pymol were used to perform molecular docking. The hub targets were validated in the GSE66360. Lastly, SOD, MDA, transmission electron microscope, quantitative real-time PCR, and western blot were used to validate in MI/RI rats. **Results:** 139 genes of 7-HF, 4832 genes of MI/RI were obtained. The 47 interact genes between 7-HF and MI/RI targets for MI/RI were likely to act through multiple pathways. And NQO1 was a critical target in the above process. In an animal experiment, 7-HF could relieve the injured interfibrillar mitochondria and myocardial fibers, decrease the expression of MDA and SOD, and increase the expression of Nrf2, NQO1 and HO-1 in the mRNA and protein level in the MI/RI rats. **Conclusion:** This study preliminarily demonstrated that 7-HF

could provide cardioprotection by inhibiting the oxidative stress and up-regulating Nrf2/NQO1/HO-1 signaling pathway based on network pharmacology, molecular docking, target validation, and animal experiments.

Keywords

7-Hydroxyflavone, Myocardial Ischemia/Reperfusion Injury, Oxidative Stress, Network Pharmacology

1. Introduction

Acute myocardial infarction (AMI) remains a health-threatening and disastrous disease resulting from the rupture of unstable plaque and has become the major cause of death all over the world [1]-[2]. Myocardial ischemia/reperfusion injury (MI/RI) is an important reason why AMI is difficult to treat. The pathological mechanisms of MI/RI include oxidative stress, inflammation, apoptosis, etc. Thus, the detailed mechanism of MI/RI still needs to be further investigated [3]. The development of more effective MI/RI drugs will be the focus of cardio-protection. Therefore, it is urgent for us to explore the drugs to treat MI/RI.

7-hydroxyflavone (7-HF), isolated and identified from the traditional Tibetan medicine, *Oxytropis falcata* Bunge, exists widely in plants and fruits [4]. Many studies have demonstrated a wide range of pharmacological effects of 7-HF, including antiplatelet [5], antioxidant [6], inflammation inhibition [7], renal protection [8], etc. Our previous study has demonstrated that 7-HF inhibits the MAPK/NF-κB signaling pathway to depress the expression of inflammatory factors for the treatment of MI/RI. Studies have shown that flavonoids have antioxidant effects in MI/RI. However, whether 7-HF could play a protective role in the treatment of MI/RI by modulating oxidative stress-related pathways is still unclear.

Recently, network pharmacology and molecular docking have been recognized as novel and effective methods for investigating the mode of action of traditional Tibetan medicine, focusing on the construction of a network of multi-target, multi-pathway treatments [9] [10]. The two methods can provide a promising scientific basis for 7-HF to treat MI/RI by regulating oxidative stress-related pathways. Consequently, in this study, network pharmacology and molecular docking were used to predict the potential oxidative stress-related pathways. Then, the screened targets were validated in the gene chip GSE66360. Finally, the pretreatment of 7-HF on the MI/RI rat model was used to testify to the underlying mechanism of 7-HF against MI/RI (**Figure 1**).

2. Materials and Methods

2.1. Targets of 7-HF and MI/RI Collection

PharmMapper (http://www.lilab-ecust.cn/pharmmapper/), Traditional Chinese Medicine Systems Pharmacology database and analysis platform (TCMSP,

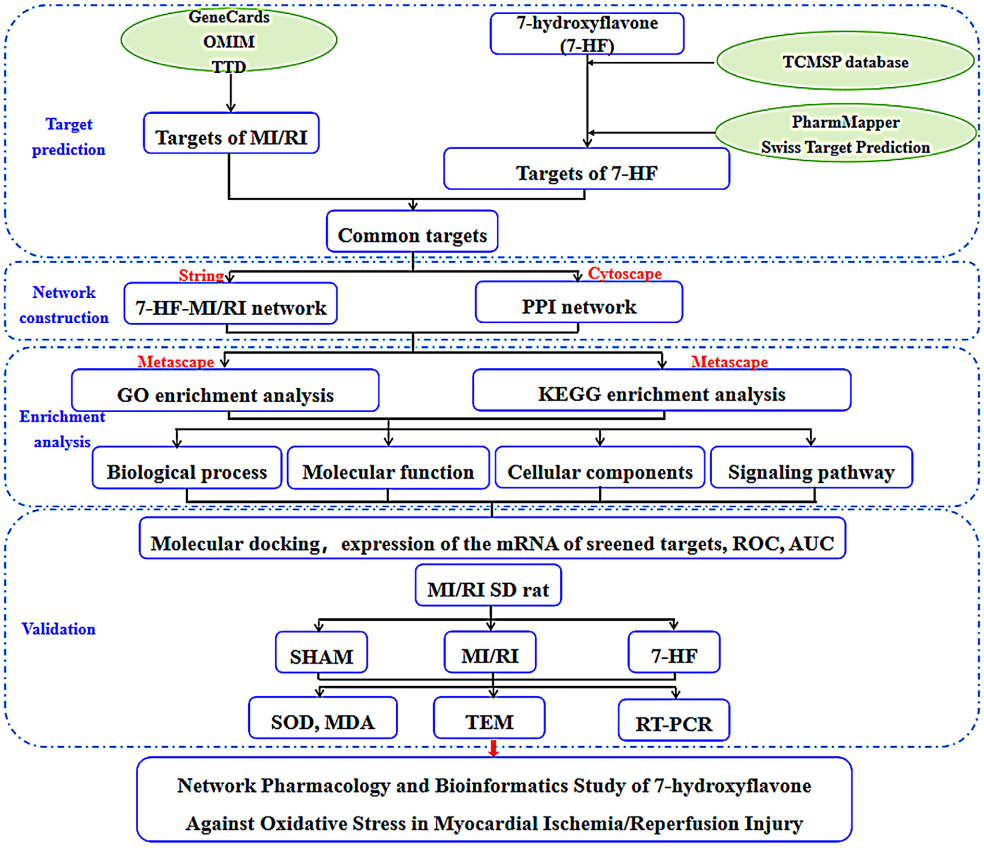

Figure 1. The flowchart of this study.

https://old.tcmsp-e.com/tcmsp.php), and SwissTargetPrediction (http://www.swisstargetprediction.ch/) were used to acquire the targets of 7-HF [11]. OMIM (http://omim.org/), GeneCards (https://www.genecards.org), and TTD (http://db.idrblab.net/ttd/) were used to obtain the targets of MI/RI [11].

2.2. Protein-Protein Interaction (PPI) Network Construction

The mutual targets between 7-HF and MI/RI were imported into the String database (https://cn.string-db.org/). And the confidence scores were set as ≥0.4. The species was selected as "Homo sapiens". The results from the String database were imputed into Cytoscape 3.9.2 to further analyze the network. MCC algorithm in the CytoHubba plug-in was used to construct the hub targets network according to the previous study [12].

2.3. Functional Enrichment Analyses

The mutual targets were imputed into the Metascape database (https://metascape.org/gp/index.html#/main/step1). The results of GO and KEGG enrichment analyses were generated from the above database [3].

2.4. 7-HF-Targets-Signaling Pathways-MI/RI Network Construction

The relationship table of 7-HF, targets, signaling pathways, and MI/RI was imported

into Cytoscape 3.9.2 software to construct a "7-HF-target-signaling pathway-MI/RI" network diagram.

2.5. Molecular Docking

ChemBioDraw (Version 18.0) was used to draw the 2D structure of 7-HF and convert it to a 3D structure. Then, the most structurally similar proteins and ligands of the macromolecular target receptors were obtained from the RCSB PDB online platform (https://www.rcsb.org/). PyMOL (Version 1.7.x) was used to remove water and extract the original ligands to obtain the processed proteins and original ligands. AutoDock Vina (Version 1.5.6) converted the "pdb" format of proteins and corresponding ligands into "pdbqt" format. The activity pocket between the protein and the ligand was obtained, and the relevant parameters were also obtained. Finally, PyMOL (Version 1.7.x) was used for molecular docking to calculate the binding affinity and visualize these results.

2.6. Screened Targets in MI/RI Data Set GSE66360 Validation

Data set GSE66360, collected from the Affymetrix Human Genome (U133 Plus 2.0 Array HG-U133_Plus_2), was used to validate the targets acquired from 4.6. The receiver operator characteristic curves (ROC) and the area under the curves (AUC) were used to assess the diagnostic efficacy of the above targets.

2.7. MI/RI Rats Model Construction

All study protocols were performed and were approved by the Ethics Committee of the Department of Cardiology, The First Affiliated Hospital, Hengyang Medical School, University of South China (No. 2024110306002). 7-HF (CAS: 6665-86-7, H0852) was acquired from TCI Chemical Industry Development Co. Sprague Dawley (SD) male rats (n = 36, weight = 160 - 180 g) purchased from HFK Bioscience Co., were grouped into 3 groups: 1) sham group (SHAM), 2) MI/RI group (MI/RI), and 3) MI/RI-7-HF group (7-HF). The SHAM and MI/RI groups were administered with an intraperitoneal injection of 0.9% saline for 7 days. 7-HF group was injected intraperitoneally with 10 mg/kg 7-HF for 7 days [4]. All operations were conducted 12 hours after the final administration. A sham operation was carried out without ligating the left anterior descending branch of the coronary artery (LAD). Rats in the MI/RI group and 7-HF group were ligated LAD for 45 min and then underwent the perfusion for 120 min. After the operation, the hearts were collected for subsequent analysis.

2.8. Transmission Electron Microscope (TEM)

Cardiac tissue was first pretreated with 3% glutaraldehyde, then postfixed with 1% osmium tetroxide, then dehydrated with serial acetone, infiltrated with Epox 812, and finally embedded. The samples were stained sequentially with methylene blue, uranyl acetate, and lead citrate. The images of cardiac ultrastructure were acquired by transmission electron microscopy (JEM-1400-FLASH, Tokyo, Japan).

2.9. Superoxide Dismutase (SOD) and Malondialdehyde (MDA) Test

SOD and MDA were assayed based on the direction of SOD and MDA (Nanjing Jiancheng Bioengineering Institute).

2.10. Quantitative Real-Time PCR (RT-PCR)

TRIzol reagent, first-strand cDNA synthesis kit, SYBR green supermix were purchased from Tiangen Co. Hearts were harvested after perfusion. And total RNA was acquired from heart tissue dealt with TRIzol regent. The cDNA was generated from 2 000 ng of RNA in a 20 μL system. RT-PCR was carried out by real-time PCR system (ABI7500, Bio-rad, CA, USA). The primer sequences could be mentioned in Table 1. According to the $2^{-\Delta\Delta Ct}$ method, the expression of each mRNA level was calculated.

Table 1. Primer information.

Primer name	Primer sequence
Nrf2 Forward	CCTTCCTCTGCTGCCATTAGTC
Nrf2 Reverse	GAACTCCACCGTGCCTTACG
NQO1 Forward	GCCTACACGTATGCCACCAT
NQO1 Reverse	TGGACACCCTGCAGAGAGTA
HO-1 Forward	CTAAGACCGCCTTCCTGCTC
HO-1 Reverse	GCCTCTGGCGAAGAAACTCT
β-Actin Forward	GCGCAAGTACTCTGTGTGGA
β-Actin Reverse	CATCGTACTCCTGCTTGCTG

2.11. Western Blot (WB)

WB was conducted as described in the previously reported method [4]. Total proteins from heart tissues were extracted using a whole-cell lysis kit (Wanlei Bio, WLA019a, China). Protein density was measured with the BCA Protein Detection Kit (Abbkine, KTD3001, China). 5% skimmed milk (Yamei, No. 025B1050, China) combined with 1 × TBST was for blocking the PVDF membrane, and the incubation was performed overnight at 4˚C with anti-NRF2, anti-NQO1, and anti-HO-1 antibodies. The PVDF membrane was cleansed three times with 1 × TBST buffer. HRP-incorporated goat anti-rabbit antibody was incubated with the washed membrane. After washing, the color was visualized using the enhanced chemiluminescence (ECL) kit (Wanlei Bio, WLA006a, China). BIO RAD was performed for the image.

2.12. Statistical Analysis

Cytoscape (Version 3.9.2) was used for analysis in the part of Network pharmacol-

ogy. R Studio (Version 1.4.1717) was performed for analysis in the part of bioinformatics. $p < 0.05$ and $|\log FC| \geq 1.5$ were used as the filtering criteria. Functional enrichment analyses were completed via Metascape. PyMOL (Version 1.7.x) and AutoDock Vina (Version 1.5.6) were conducted for Molecular docking. In the animal experiment, one-way ANOVA was conducted with GraphPad Prism, version 8.3.1 (GraphPad Software Inc., CA), to compare the two groups. In the analysis of the results of WB, Image J, version 1.52v (National Institutes of Health, USA) was used. All quantitative data were shown as mean ± standard deviation. A p value < 0.05 was regarded as statistically significant.

3. Results

3.1. Targets of 7-HF Collection

The 7-HF were entered into the TCMSP, SwissTargetPrediction, and PharmMapper databases to obtain 0, 0, and 140 potential targets of action, respectively. These targets were merged and then normalized by the UniProt database to obtain 139 targets.

3.2. Targets of MI/RI Collection

The MI/RI were entered into OMIM, GeneCards, and TTD databases to obtain 15, 4820, and 37 MI/RI related targets, respectively. These targets were merged and normalized by the UniProt database to obtain 4832 targets.

3.3. Mutual Targets between 7-HF and MI/RI Acquisition

There were 47 mutual targets between 7-HF targets and MI/RI targets. The interaction targets included *ZPR1*, *WARS1*, *VPS4B*, *VEGFB*, *VDR*, *TMPO*, *THBS1*, *SRP54*, *PTGS2*, *PTGDS*, *PRKCB*, *POLB*, *PELO*, *NQO1*, *NNMT*, *NFATC1*, *MUC1*, *MT-CYB*, *MB*, *MATK*, *LGALS3*, *LDHAL6B*, *IFNGR1*, *HSD11B1*, *HBB*, *HBA1*, *GZMB*, *GYG1*, *GOT1*, *GAD1*, *FAIM*, *FABP2*, *DPP3*, *DLD*, *CTH*, *CST3*, *CKM*, *CHAT*, *CCND1*, *CAT*, *CANT1*, *CA2*, *ASAP2*, *AR*, *ALB*, *ACTA1*, and *ACHE*.

3.4. Protein-Protein Interaction (PPI) Network Construction

The 47 mutual target genes were imported into the STRING database, the "Multiple proteins" was selected, the organism was set to "Homo sapiens", the unlinked target proteins were hidden, and the PPI network was constructed. The PPI network consists of 47 nodes and 82 edges (**Figure 2(a)**). PPI enrichment p-value was 1.11e−16. The average node degree was 3.49. The average local clustering coefficient was 0.508. In this study, we imported the "TSV" table from the STRING database into Cytoscape 3.9.2, applied "Analyze network" in the tool, selected the undirected analysis network graph to calculate the degree, and then used the MCC algorithm in the CytoHubba plug-in to construct the hub target network diagram (**Figure 2(b)**). The top 10 hub targets in order of degree were *ALB*, *CAT*, *PTGS2*, *CCND1*, *NQO1*, *AR*, *MUC1*, *GOT1*, *LDHAL6B*, and *MB*.

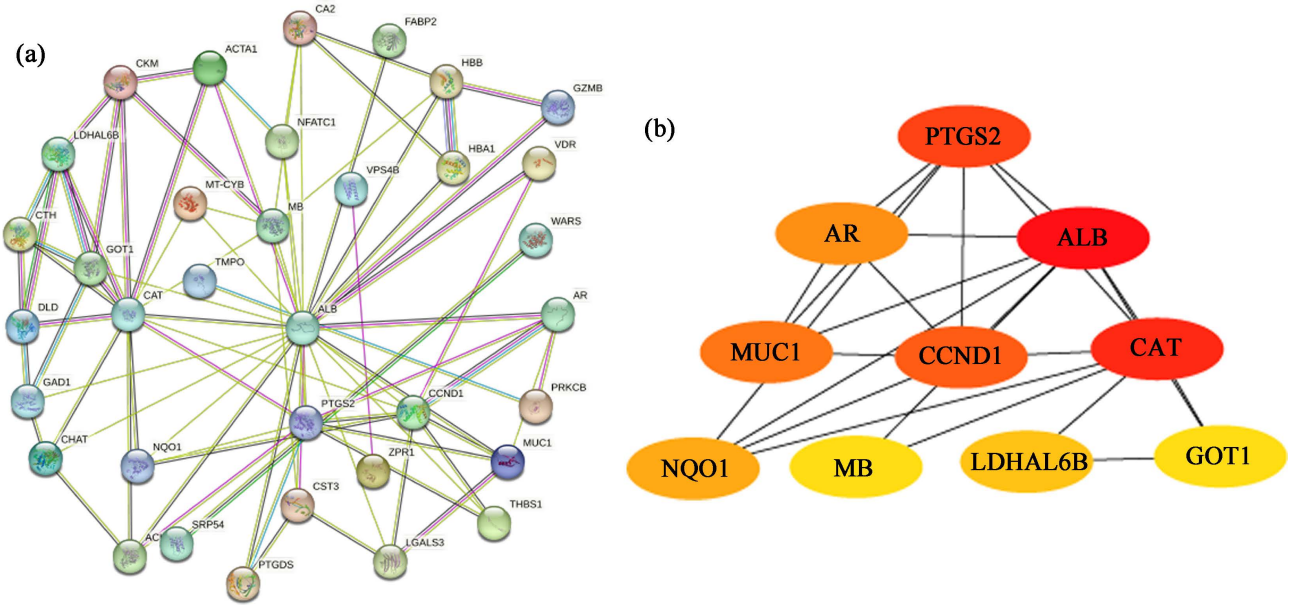

Figure 2. Network diagram: (a) 47 mutual target genes in the PPI network diagram; (b) The top ten target genes in the hub network diagram by MCC algorithm in the CytoHubba plug-in.

3.5. Functional Enrichment Analyses

A total of 47 mutual target genes were imported into the Metascape online platform for enrichment analysis, and a total of 173 Gene ontology (GO) enrichment analysis items, and 26 KEGG signaling pathways were obtained ($p < 0.05$). 133 GO biological process (BP) items, 14 GO cellular component (CC) items and 26 GO molecular function (MF) items were included in the GO enrichment analysis. GO BP was enriched in response to hypoxia, response to decreased oxygen levels, positive regulation of angiogenesis, etc. ($p < 0.05$, **Figure 3(a)**). GO CC was enriched in oxidoreductase, ficolin-1-rich granule lumen, tertiary granule lumen, etc. ($p < 0.05$, **Figure 3(b)**). GO MF was enriched in antioxidant activity, oxygen binding and oxygen carrier activity ($p < 0.05$, **Figure 3(c)**). The mutual targets were enriched in the advanced glycation end products (AGE)/receptor of advanced glycation end products (RAGE) signaling pathway, Wnt signaling pathway and MAPK signaling pathway ($p < 0.05$, **Figure 3(d)**).

3.6. 7-HF-Targets-Signaling Pathways-MI/RI Network Construction

We imported the prepared property files into Cytoscape to construct the "7-HF-Target-Signaling Pathway-MI/RI" network diagram. The red quadrilateral represented 7-HF. The purple triangle represented MI/RI. The green V represented Rap1, AGE-RAGE, MAPK, and Wnt signaling pathways. The orange hexagon represented PRKCB, THBS1, CCND1, VEGFB, and NFATC1, which acted on two or more signaling pathways of 7-HF for the treatment of MI/RI in common. The yellowish oval indicated that 42 target genes acted in one signaling pathway of 7-HF for treatment of MI/RI (**Figure 4**).

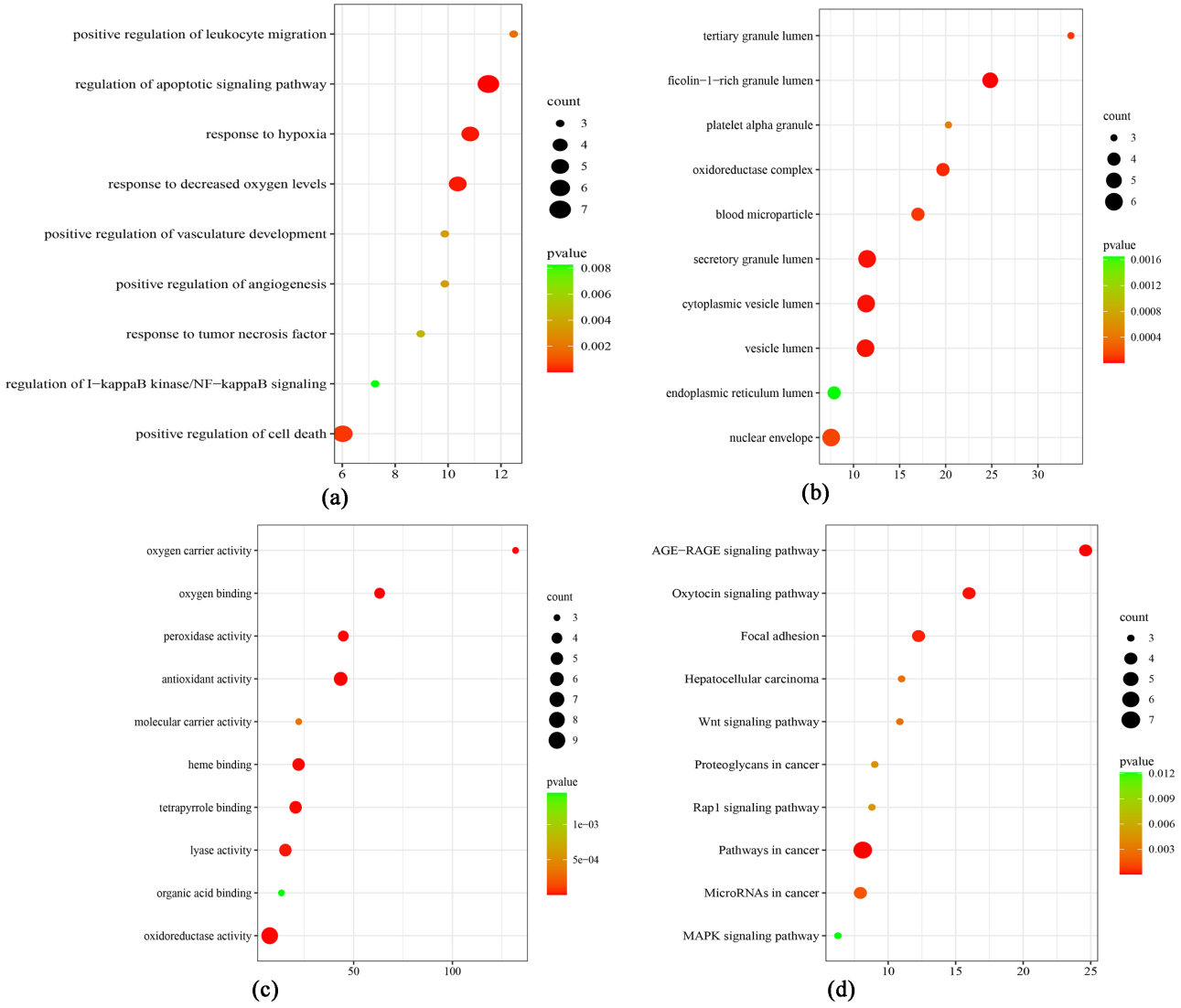

Figure 3. Enrichment analysis: (a) GO biological process for 47 targets; (b) GO cellular components for 47 targets; (c) GO molecular function for 47 targets; (d) Signaling pathway for 47 targets.

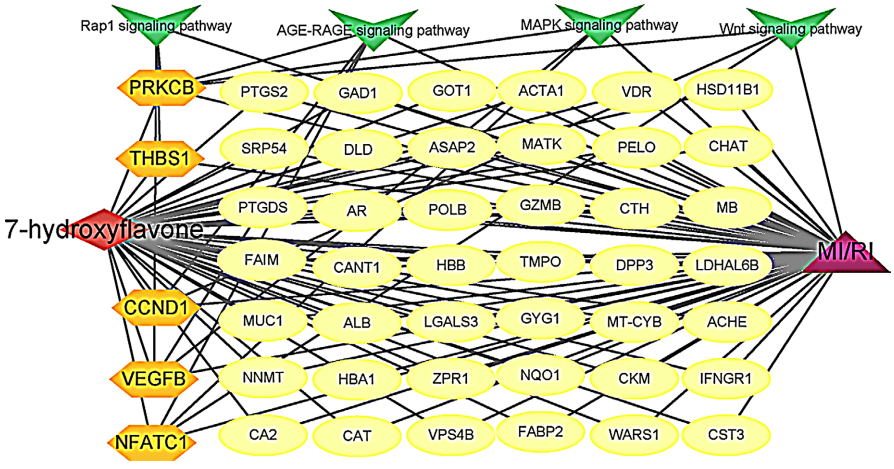

Figure 4. 7-HF-Targets-Siganling pathways-MI/RI network diagram.

3.7. Molecular Docking

In the results of enrichment analysis, oxidative stress played an important role in the treatment of MI/RI. NQO1 was a recognized hub biomarker from the above study. Nrf2/NQO1/HO-1 signaling pathway played a significant role in the treatment of MI/RI in the previous study. Whether Nrf2/NQO1/HO-1 signaling pathway participated in 7-HF for treating MI/RI remains unclear. Thus, molecular docking was used to simulate the binding ability between 7-HF and hub target proteins including Nrf2, NQO1, and HO-1. The results showed that 7-HF could have more stable binding to the above proteins (**Figures 5(a)-(c)**).

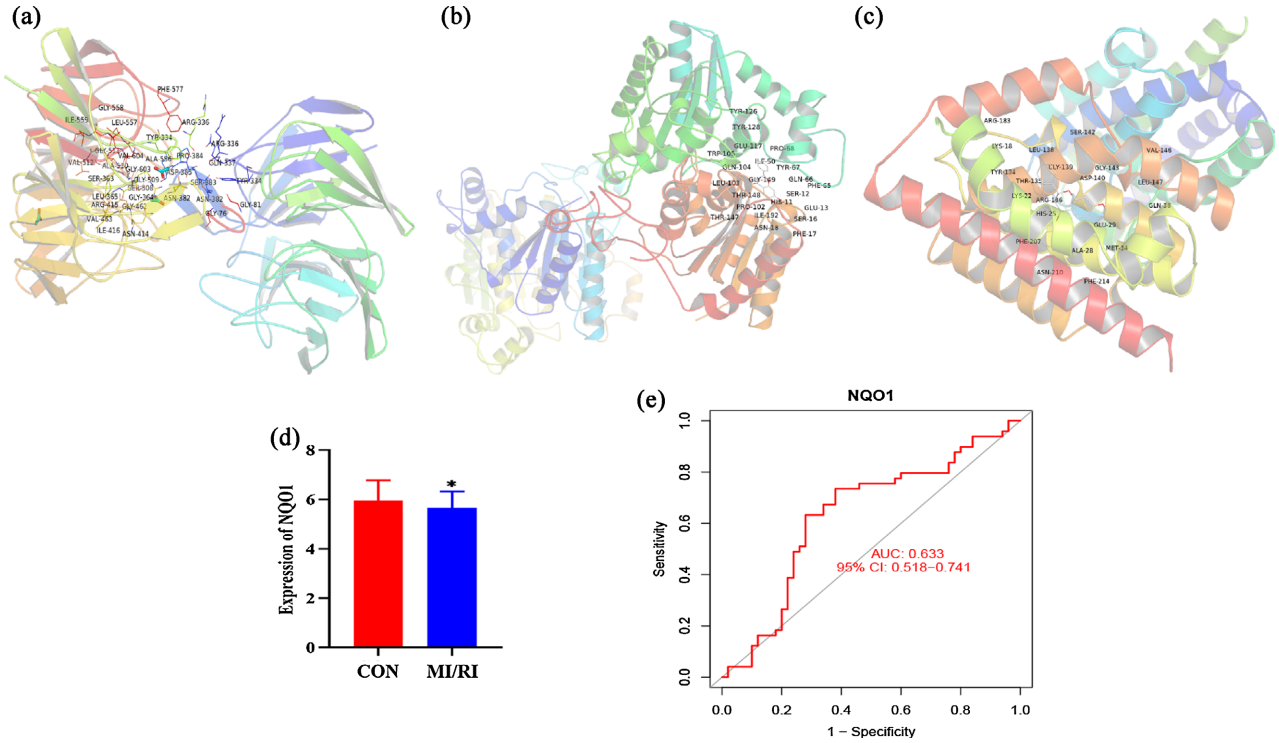

Figure 5. Molecular docking and validation in gene chip GSE66360: (a) Molecular docking between 7-HF and Nrf2; (b) Molecular docking between 7-HF and NQO1; (c) Molecular docking between 7-HF and HO-1; (d) The mRNA expression of NQO1 in the GSE66360; (e) The ROC and AUC of NQO1 in the GSE66360.

3.8. Screened Targets in MI/RI Data Set GSE66360 Validation

From the above results, Nrf2, NQO1, and HO-1 were important targets. Thus, how many of these targets were expressed in MI/RI patients? And we testified these targets in the data set GSE66360. Compared to CON, the expression of NQO1 was much lower in the MI/RI group (**Figure 5(d)**). And NQO1 distinguished between CON and MI/RI. The AUC of NQO1 was 0.633 (0.518, 0.741) (**Figure 5(e)**). The other two targets were not differentially expressed in this dataset.

3.9. TEM

Interfibrillar mitochondria (IFM) and myocardial fibers were normal in the SHAM group, whereas mitochondria were impaired in the MI/RI group. After the

pretreatment of 7-HF in the HF group, injured IFM were repaired (Figures 6(a)-(c)).

SOD and MDA Test

Compared to the SHAM group, an increase in the serum of MDA, and a decrease in the serum of SOD, were noticed in the MI/RI group ($p < 0.05$; Figure 7(a) & Figure 7(b)). Compared to the MI/RI group, the pretreatment of 7-HF could significantly relieve the mentioned above alterations in the 7-HF group ($p < 0.05$; Figure 7(a) & Figure 7(b)).

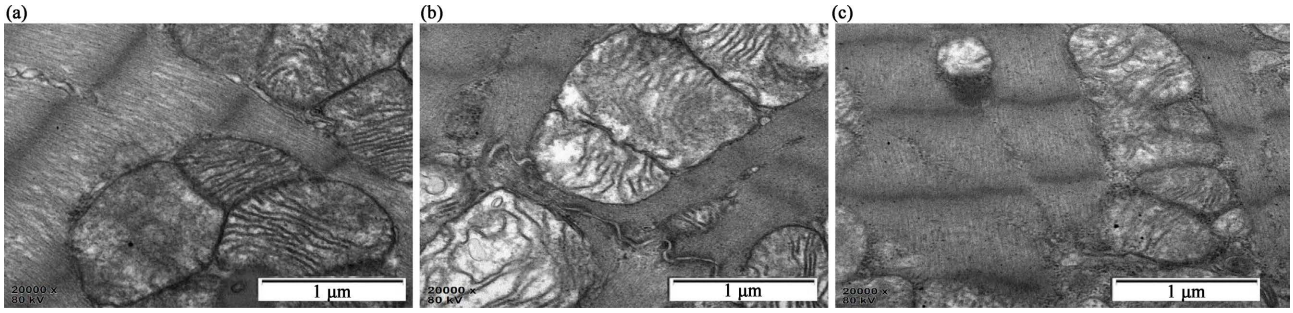

Figure 6. The results of TEM: (a)-(c) Effect of 7-HF on the ultrastructural changes (20,000×) in the SHAM, MI/RI, 7-HF groups, respectively.

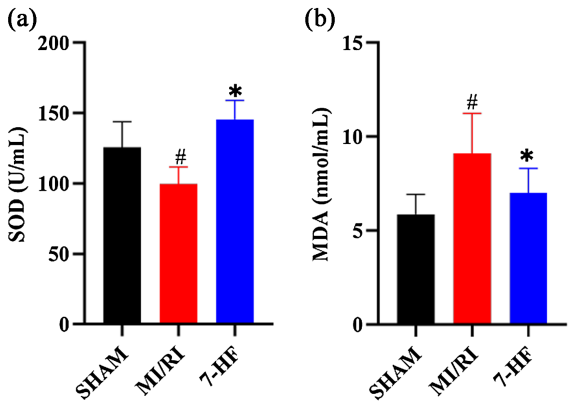

Figure 7. The expression of the serum of SOD and MDA: (a) The expression of the serum of SOD (n = 12); (b) The expression of the serum of MDA (n = 12).

3.10. RT-PCR

The mRNA expression levels in the Nrf2/NQO1/HO-1 signaling pathway were used to investigate the mechanism of 7-HF on MI/RI. The results showed that the expression levels of Nrf2, NQO1, and HO-1 were significantly lower in the MI/RI group compared with the SHAM group ($p < 0.05$; Figures 8(a)-(c)). Compared with the MI/RI group, the expression levels of Nrf2, NQO1, and HO-1 were increased in the 7-HF group ($p < 0.05$; Figures 8(a)-(c)).

3.11. WB

The protein expression levels in the Nrf2/NQO1/HO-1 signaling pathway were used to explore the mechanism of 7-HF on MI/RI. The results demonstrated that

the expression levels of Nrf2, NQO1, and HO-1 were lower in the MI/RI group compared with the SHAM group ($p < 0.05$; Figures 9(b)-(d)). Compared with the MI/RI group, the expression levels of Nrf2, NQO1, and HO-1 were increased in the 7-HF group ($p < 0.05$; Figures 9(b)-(d)).

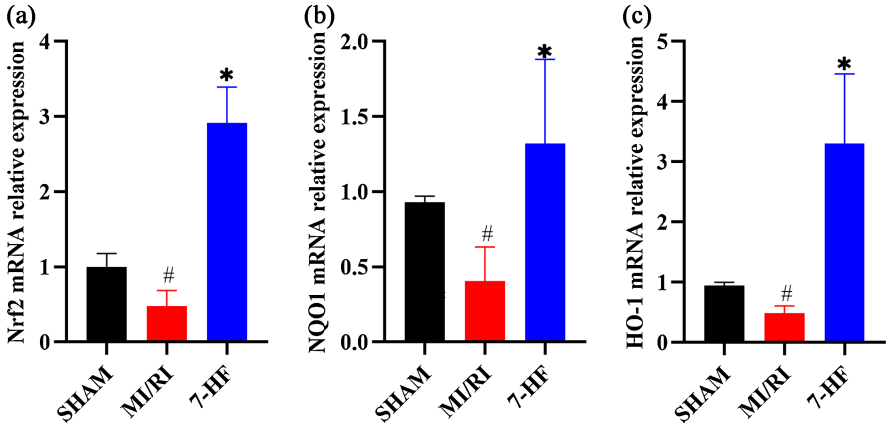

Figure 8. The results of RT-PCR: (a) Nrf2 mRNA relative expression; (b) NQO1 mRNA relative expression; (c) HO-1 mRNA relative expression (n = 3).

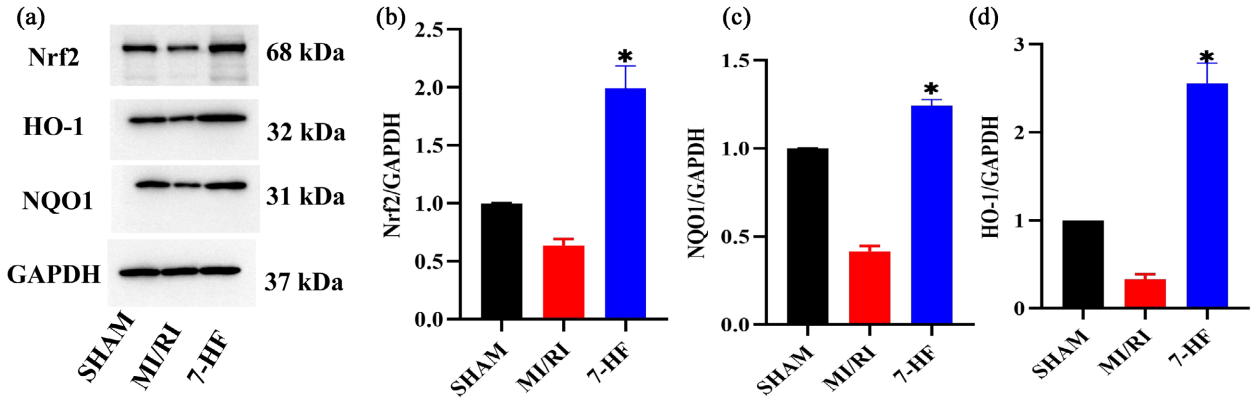

Figure 9. The protein expression of Nrf2, NQO1, and HO-1: (a) Nrf2 protein relative expression; (b) NQO1 protein relative expression; (c) HO-1 protein relative expression (n = 3).

4. Discussion

In this study, network pharmacology, molecular docking, target validation, and animal experiments were used to investigate the targets and mechanisms of 7-HF for MI/RI. We have found the results as follows: a total of 47 target genes of 7-HF for the treatment of MI/RI was obtained; 7-HF for MI/RI might regulate MAPK, AGE/RAGE, and Wnt signaling pathways to treat MI/RI; molecular docking, RT-PCR, and WB both confirmed that 7-HF alleviated MI/RI through activation of Nrf2/NQO-1/HO-1 signaling pathway.

The application of Cytoscape is beneficial for revealing the interactions between proteins in PPI networks. The cytoHubba, as one of the important plug-ins, provides 11 topological analysis algorithms [13]. Of them, the MCC algorithm is more accurate in analyzing proteins in PPI networks. Therefore, the MCC algorithm

was used to explore the hub targets in the PPI network in depth. A total of 10 important targets were screened, among which the top three target genes were *ALB*, *CAT*, and *PTGS2* based on degree ranking. *ALB* encoded plasma albumin, whose structural domain antibodies were combined with glucagon-like peptide-1 to protect the heart from MI/RI. This drug has a longer duration of action and will be an important strategy for alleviating MI/RI during AMI [14]. *CAT* encoded catalase. Studies have shown that ferulic acid, resveratrol, and allicin attenuated MI/RI by inhibiting catalase [15]-[17]. *PTGS2* encoded cyclooxygenase-2, and its interaction with endoplasmic reticulum stress exacerbated MI/RI [18]. Berberine induced the expression of miR-26b-5p and inhibited PTGS2/MAPK signaling pathway to exert anti-MI/RI effects [19]. From the above evidence, it is clear that *ALB*, *CAT*, and *PTGS2* will be important directions for the development of novel drugs to treat MI/RI.

GO enrichment analysis revealed 47 intersecting genes enriched in BP, CC, MF, and mechanisms related to oxidative stress, inflammation, apoptosis, etc. Previous studies have shown that 7-HF exerted anti-platelet effects by inhibiting platelet glycoprotein IIb/IIIa [5]. 7-HF regulated ERK/Nrf2/HO-1 signaling pathway to protect renal tubular epithelial cells from oxidant stress [8]. 7-HF inhibited NF-κB signaling pathway to reduce the inflammatory response of RAW264.7 [20]. The results mentioned above were consistent with our results.

KEGG enrichment analysis revealed that these targets were mainly related to MAPK, Wnt, and AGE-RAGE signaling pathways. Mangostin regulated AGE-RAGE/MAPK signaling pathway to inhibit myocardial oxidative stress, inflammatory response and apoptosis to attenuate MI/RI in streptozotocin-induced diabetes mellitus rats [21]. Simvastatin-loaded nanocarriers could alleviate MI/RI by inhibiting MAPK/NF-κB signaling pathway [22]. Blocking the Wnt signaling pathway could inhibit the inflammatory response and ameliorate MI/RI [23]. The evidences also provided research references for clinical new drug development and precision treatment of MI/RI.

Based on the obtained hub targets and signaling pathways, "7-HF-target-signaling pathway-MI/RI" regulatory network diagram was constructed, which initially revealed the role of 7-HF in treating MI/RI through multiple targets, signaling pathways, and BP. Molecular docking was used to confirm that 7-HF could be combined with Nrf2, NQO1, and HO-1. Subsequently, NQO1 was also validated in the GSE66360. ROC and AUC both proved that NQO1 was a significant target in MI/RI.

Oxidative stress was an important cause of MI/RI [24]. Nrf2, an important receptor during cellular oxidation, entered the nucleus and regulates the expression of SOD, HO-1, and NQO1 under the stimulation of ischemia and hypoxia [25]. SOD was the first line of defense against oxidative stress, and MDA was positively correlated with lipid peroxidation. NQO1 was a flavinase with antioxidant activity. HO-1 was a protective enzyme with antioxidant activity mediated by Nrf2. The Nrf2/NQO1/HO-1 signaling pathway was involved in oxidative and antioxidant

processes and played an important role in organ protection [26]. Sun *et al.* have found that aloin activated the Nrf2/HO-1 signaling pathway to inhibit oxidative stress and inflammatory responses to protect H9c2 cardiomyocytes from H/R-induced injury [27]. Lan *et al.* have demonstrated that melatonin upregulated the Nrf2/HO-1 signaling pathway to attenuate oxidative stress to protect the heart from MI/RI [28]. In this study, we used 7-HF to pretreat MI/RI rats and found that 7-HF could increase the mRNA expression of Nrf2, NQO1, and HO-1 to alleviate MI/RI. It cannot be ruled out that there is an accompanying relationship. Detailed and in-depth exploration will be further developed.

Although our study shed new light on the mechanisms of 7-HF for MI/RI treatment, it still had limitations. Western blotting, transfection, or overexpression techniques were required to further elucidate the mechanism of 7-HF for MI/RI treatment.

5. Conclusion

In summary, this study focuses on the pretreatment with 7-HF could provide cardioprotection by inhibiting the oxidative stress and up-regulating Nrf2/NQO1/HO-1 signaling pathway based on network pharmacology, molecular docking, target validation, and animal experiment.

Consent for Publication

All authors have agreed to publish this manuscript.

Data Availability Statement

The original contributions shown in the study are selected in the article/supplementary material. Appropriate inquiries can be made with the corresponding author.

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Authors' Contributions

Z.T. and Q.Z. in the study design. Q.Z., Y.O., Z.T., H.C., and Y.L. in the collection. Q.Z., Y.O., Z.T., H.C., and Y.L. analysis and interpretation of data. Z.T. and Q.Z. in the writing of the manuscript. Z.T. and Q.Z. administered and coordinated the whole study project. All authors have read and agreed to the published version of the manuscript.

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Ethical Approval

All studies have been approved by the Ethics Committee of the Department of Cardiology, The First Affiliated Hospital, Hengyang Medical School, University of South China. Ethics Approval No. 2024110306002. This study was approved by the First Affiliated Hospital, Hengyang Medical School, University of South China, Hunan Province, China.

Statement of Human and Animal Rights

All of the experiment procedures involving animals were conducted in accordance with the First Affiliated Hospital, Hengyang Medical School, University of South China, Hunan Province, China.

Statement of Informed Consent

There are no human subjects in this article, and informed consent is not applicable.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

AMI	Acute myocardial infarction
AGE	Advanced glycation end products
BP	Biological process
CC	Cellular component
GO	Gene ontology
IFM	Interfibrillar mitochondria
lad	Left anterior descending branch of the coronary artery
MDA	Malondialdehyde
MI/RI	Myocardial ischemia/reperfusion injury
MF	Molecular function
7-HF	7-hydroxyflavone
PPI	Protein-protein Interaction
RAGE	Receptor of advanced glycation end products
RT-PCR	Quantitative real-time PCR
WB	Western blot
SOD	Superoxide dismutase
TEM	Transmission electron microscope