

Review

The Investigation of the Molecular Mechanism of *Morinda officinalis* How in the Treatment of Heart Failure

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Abstract

Heart failure (HF) is a cardiovascular disease with an extremely high mortality rate. However, *Morinda officinalis* How (MO) has not been studied for cardiovascular purposes at this time, the aim of this study was to find new mechanism for the MO of treatment of HF through a bioinformatics and experimental validation. The present study also aimed to establish a link between the basic and clinical applications of this medicinal herb. MO compounds and targets were obtained by traditional Chinese medicine systems pharmacology (TCMSP) and Pubchem. Subsequently, HF targets were acquired from DisGeNET and the interactions of all the targets and other human proteins were obtained via String so as to establish a component-target interaction network by Cytoscape 3.7.2. All the targets of clusters were inserted into Database for Annotation, Visualization and Integrated Discovery (DAVID) to perform GO (gene ontology) enrichment analysis. Molecular docking was adopted to predict the targets of MO relevant to the treatment of HF and to further explore the associated pharmacological mechanisms. Subsequently, a series of *in vitro* experiments, including histopathological staining, immunohistochemical and immunofluorescence analyses were conducted for further verification. Moreover, western blot analysis and *in vivo* experiments were performed. The results indicated that MO alleviated apoptosis, regulated cholesterol metabolism and transport function, and reduced inflammation, which resulted in the successful treatment of HF. Beta-sitosterol, Asperuloside tetraacetate and americanin A were the key bioactive components of MO. ALB, AKT1, INS, STAT3, IL-6, TNF, CCND1, CTNNB1, CAT, and TP53 were the core potential targets, which were significantly associated with multiple pathways, namely the FoxO signaling pathway, the AMPK signaling pathway, and the HIF-1 signaling pathway. *In vivo* experiments validated that MO may protect against heart failure or treat this disease by increasing the levels of autophagy via the FoxO3 signaling pathway in rats. The present study suggested that a combination of network pharmacology prediction with experimental validation may offer a useful tool to characterize the molecular mechanism of action of the traditional Chinese medicine (TCM) MO in the treatment of HF.

Keywords: traditional Chinese medicine; mechanism of action; heart failure; *Morinda officinalis* How

1. Introduction

Heart failure (HF) is the leading cause of cardiovascular death, it is not considered a single disease based on a specific etiology and causes a clinical syndrome that includes symptoms, such as dyspnea and weariness. Markers are high jugular venous pressure, tachycardia, and peripheral edema [1]. The prevalence of heart failure has been projected to increase to 46% between 2012 and 2030 [2], causing a serious financial and social burden in the world. At present, the remedy of heart failure is still a conservative cure containing organic chemicals. However, the continuous use of modern medicine may result in dizziness and fainting due to excessively low blood pressure, which can be life-threatening in severe circumstances. Using traditional Chinese medicine (TCM) for to treatment cardiovas-

cular diseases has last for thousands of years. It has been used in combination with various clinical drug regimens and diagnostic guidelines, which can greatly promote the development of local medical care. TCM has been widely utilized to treatment heart failure [3], which reduces the side effects caused by excessive modern medicine. Therefore, TCM is likely to become mainstream drugs in the future. *Morinda officinalis* (MO) is a traditional Chinese medicinal herb used in southeastern China that has shown various pharmacological activities. The Nrf2 is a crucial transcription factor that controls the intracellular antioxidant response and is an arising focus for the treatment and prevention of illnesses caused by oxidative stress [4]. MO could reduce H₂O₂-induced oxidative stress, increases Nrf2 production detoxification mechanism to activate antioxi-



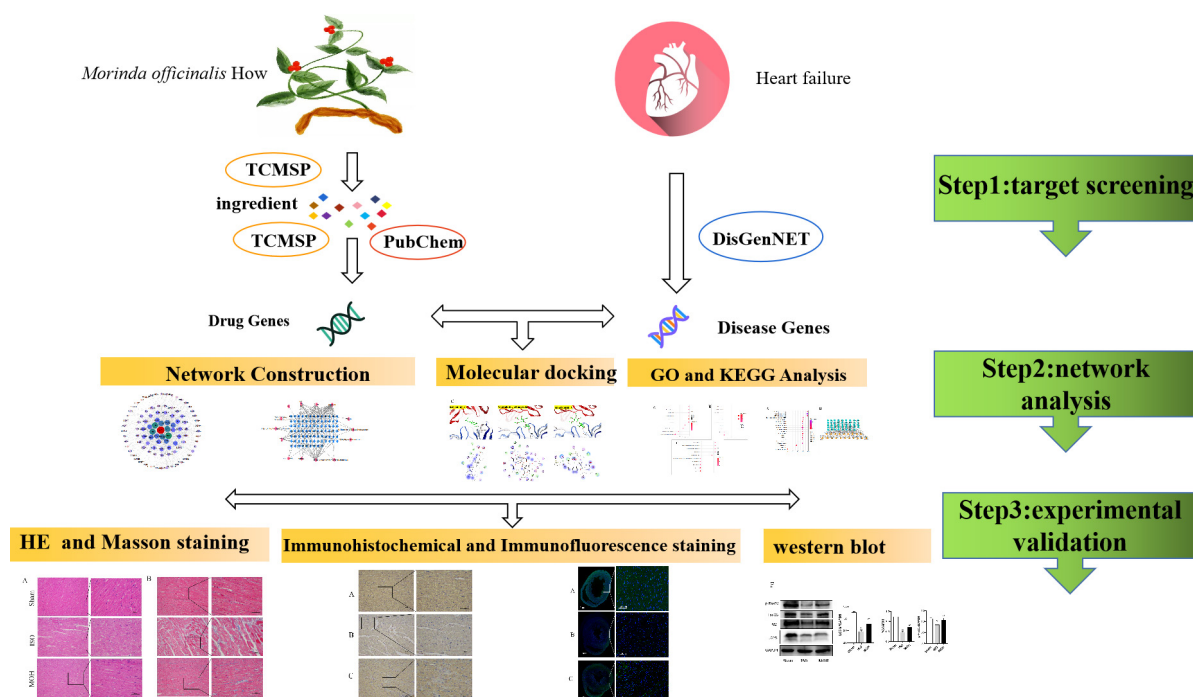


Fig. 1. Detailed design flow chart of the current study.

dant enzymes and superoxide dismutase (SOD) and Catalase (CAT) activities [5,6]. Moreover, it promotes Bcl-2 expression and inhibits Bax expression, leading to the inhibition of apoptosis in hypoxic-hypoxic mastitic rat cardiomyocytes [7], which demonstrates protective effects on cardiovascular vessels and heart tissues [8]. Autophagy has been highlighted as a protective or harmful response. Autophagy usually promotes cell survival, although it can also cause cell death under specific circumstances [9]. The appropriate regulation of autophagy can modulate cardiac remodeling [10], and reduce cardiomyocyte hypertrophy and apoptosis [11,12], which delays the process of heart failure. Network pharmacology, which merges pharmacology with information networks, is gaining considerable attention. TCM network pharmacology is a systematic strategy used to identify relationships between various diseases and TCM formulae based on molecular networks. They can also demonstrate multi-target medications that integrate target profile prediction of herbal substances with various analytical methodologies. This application aims to provide reliable data support and direction to develop the medicinal value of TCM. A limited number of studies have been performed on the mechanism of cardiovascular pathology of MO. The present study uses a network pharmacology approach for target prediction of the MO active compounds. The interactions of all the targets and other human proteins were obtained by using STRING. MO active compounds and targets were obtained by TCMSP and PubChem. Subsequently, HF targets were acquired from DisGenNET. Screening of potential targets of MO for HF was performed by a Venn diagram; the interactions of all the tar-

gets and the protein-protein interaction (PPI) networks were performed via STRING. Subsequently, enrichment analysis was carried out on the basis of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms, and a drug-target-pathway network was built. Furthermore, to elucidate the mode of the interaction with the compound at the molecular level, molecular docking was used to confirm the associations between MO and its targets. Finally, *in vitro* experiments were also performed to further verify the novel mechanism of action of MO responsible for treating HF with a particular reference to the activation of autophagy. The detailed study design of the current study is shown in (Fig. 1).

2. Materials and Methods

2.1 bioinformatics

2.1.1 Acquisition of Active Compounds and Targets

All components of the MO were searched from the traditional Chinese medicine systems pharmacology (TCMSP) database (<http://tcmspw.com/>). The exploration of the pharmacokinetic characteristics was performed using the TCMSP database, which is a unique pharmacological platform for TCMs or compounds. It can offer systematic information on the Absorption Distribution Metabolism and Excretion (ADME) characteristics of a drug with potential biological function such as, oral bioavailability (OB) and drug-likeness (DL). The values set as cut-off for the evaluation of the bioactive components were $OB \geq 30\%$ and $DL \geq 0.18\%$ [13]. Since 2004, PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) has functioned as a public archive for micromolecule and RNAi filtering results, mak-

ing its information available to the public. Researchers from academia, industry, and government agencies from all over the world can submit data to PubChem [14]. Subsequently, the introduction of related compounds into the PubChem data can be directly associated with specific gene targets. The STRING database (<http://www.string-db.org>) was used to set the screening species category to “Homo sapiens”. The targets collected in TCMSP and PubChem were imported into the “List Of Names” dialog; “MAP-PING” was selected to download the collection of the symbol gene.

2.1.2 Collecting Disease Targets

DisGeNET (<http://www.disgenet.org/>) serves a wide range of users and purposes by providing one of the most comprehensive repositories on the genetic origins of human illnesses [15], we collected HF-related targets with “heart failure” as the search term, and screened the targets with $\text{Score_gda} \geq 0.7$.

2.1.3 Clustering of MO-HF Related Target Genes

Based on the Venny 2.1.0 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>) was analyzed to intersect the MO active component targets with the HF targets, and obtain information on potential targets for MO for HF. And then, the target information of the active component compounds of MO and the target information of Heart failure were categorized and saved, and a Venn diagram was created.

2.1.4 Protein-Protein Interaction (PPI) Data

Establishment of STRING Network and Module Construction of heart failure targets. The database STRING has collected and integrate a large number of organisms PPI networks, including physical and functional relationship [16,17]. The PPI network construction data were exported from STRING. The term “multiple proteins” was selected in the module area, overlapping genes were input, and “Homo sapiens” was used as the study object with a confidence level of >0.4 . The successfully built PPI network file “Cytoscape.tsv” was imported into Cytoscape 3.7.2 [18]. Finally, the PPI data was based on the “degree” value. Then, to highlight the better prediction of key proteins in the PPI network, we base the Maximal Clique Centrality (MCC) algorithm, the top 10 genes with center targets for FGHP against CHD were identified by the molecular complex detection using the CytoHubba plugin [19].

2.1.5 Network Construction

Based on above results, a compound-target-disease network composed of compounds, Compounds, genes, and proteins are nodes, while the interactions between compounds and targets are denoted as edges [20]. The molecular mechanism of MO in the treatment of HF can be further expressed to generate a network map of targeting HF and an interactive map of core gene-related pathways.

2.1.6 Analyses of GO and KEGG Pathways

The DAVID functional annotation bioinformatics microarray analysis bioinformatics resources (<https://david.ncifcrf.gov/>) are a set of integrated biological knowledge repository and analytic website targeted at systematically retrieving biological meaning from humongous gene/protein lists [21]. The core targets collected were imported into the GO enrichment and KEGG pathway analyses were performed with $p < 0.05$ as the inspection condition.

2.1.7. Molecular Docking

Based on the aforementioned study, the integration of the core targets of MO-HF was performed by selecting the key targets from the Cytohubba plug-in gene network map and conducting molecular docking analysis with active compounds. The 3D structures of the core active ingredients were obtained by PubChem database search, the file was saved in SDF format, and MOE software was used to convert it to PDB by clicking “quickprep”. The protein crystal structures of the kernel targets were obtained from the PDB database (<http://www.rcsb.org/>), and PyMOL software was used to remove waters and ligands. Finally, the molecular structure protein was docked to the ligand using MOE software.

2.2 Experimental Validation

2.2.1 Reagents and Instrument

MO pellet herbs were produced in Guangdong Party Pharmaceutical Co., Ltd (Foshan, Kwangtung, China) purchased from Hainan Hospital of Traditional Chinese Medicine; ISO (Isoprenaline Hydrochloride) was purchased from Macklin Co. (Shanghai, China). The antibodies against FoxO3, p-FoxO3, P62, and GAPDH were from Proteintech, and LC3B was from Cell Signaling Technology (Danvers, MA, USA), whereas HRP-labeled Goat Anti-Rabbit IgG, HRP-labeled Goat Anti-Mouse IgG and BeyoECL Star from Beyotime (Shanghai, China). Hematoxylin-eosin and DAPI staining solutions were from Thermo Fisher Scientific (Waltham, MA, USA). A fully automated chemiluminescence image analysis system (978978, Tanon, Shanghai, China); Embedding Machine (EG1150C, Leica, Wetzlar, ED); tabletop high-speed frozen centrifuge (5424R, Eppendorf, Hamburg, Germany) and a multifunctional Enzyme Labeler (Synergy-HTX, BioTek, Vermont, USA).

2.2.2 Laboratory Animals

A total of 18 SPF Wistar male rats (200 ± 10 g) were purchased from Changsha Tianqin Biotechnology Co. (Changsha, China). Animal Certificate Number: SYXK (HQ) 2017-0013. The animals were raised in special pathogen-free (SPF) experimental centres. The animal room temperature was set at $20\text{--}25^\circ\text{C}$. The relative humidity was adjusted from 45 to 50%. The animals were main-

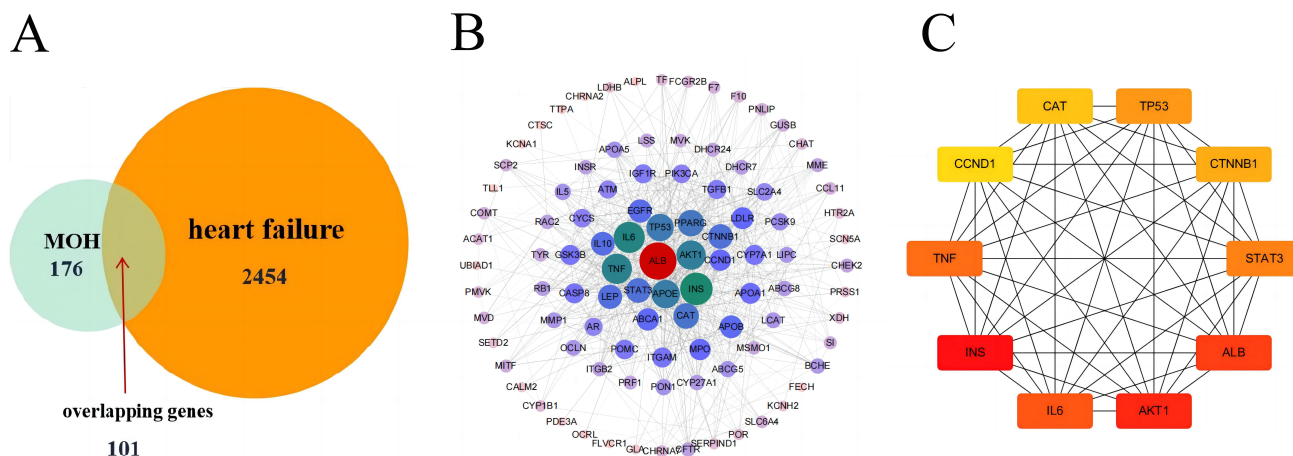


Fig. 2. Active Compounds and Targets of MO. (A) Venny diagram of *Morinda officinalis* How and heart failure targets. (B) *Morinda officinalis* How-Heart failure core target PPI network. (C) The MCC algorithm was used to analyze the top 10 hub genes network of MO for HF treatment.

tained under normal light conditions and were allowed free access to food and water. Animal studies showed that serum ADMA levels were significantly increased in isoproterenol (ISO)-induced acute myocardial ischemia and cardiac heart failure (CHF) models in rats [22,23]. Therefore, the experiment establishment of the induced CHF rat model was performed by ISO. SPF rats were randomly divided into three groups as follows: MO pellet herbs poured into boiling distilled water, ISO and MO groups were given ISO hypodermic injection at a dose of 5 mg/kg/d, MO group intervention was given the dose of 3 g/kg/d on the same day (MO pellet herbs was converted using regular human doses), gavage drug administration. While equal doses of normal saline were injected into the Sham group, all operations last for 10 days. The experimental procedure adhered to the applicable animal ethics guidelines.

2.2.3 Histological Analysis

SpecificpathogenFree (SPF) rats were anesthetized with 1% pentobarbital sodium intraperitoneally and perfused with 4% paraformaldehyde after rapid perfusion of heart with prechilled sodium chloride solution via the aorta to fix. Subsequently, it was dehydrated and paraffin-embedded blocks were prepared. The cardiac histology and myocardial fibrosis were observed using haematoxylin and eosin (H & E) and Masson staining heart slices.

2.2.4 Immunohistochemical Staining

Microwave heating in citrate buffer for 20 min was used to perform antigen unmasking. At 4 °C overnight, the sections were immunostained with a primary antiFoxO3 antibody. The sections were stained with diaminobenzidine following incubation with the secondary antibody. The purpose of this study was to investigate the mechanism of action of MO in the treatment of heart failure by assessing the protein expression of FoxO3 in the myocardium.

2.2.5 Immunofluorescence Staining

The heart tissue sections were incubated with blocking buffer at room temperature for 30 min and antiFoxO3 was added (1:100). The samples were incubated overnight. The antibody was discarded and the samples were washed three times with PBS the following day. Subsequently, they were incubated with a goat antirabbit antibody (1:500) for 1 h at 37 °C and washed three times with PBS. Subsequently, they were covered with 75% glycerol containing 1% DAPI. Finally, they were examined on a fluorescence microscope.

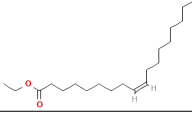
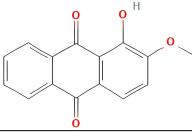
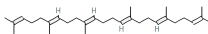
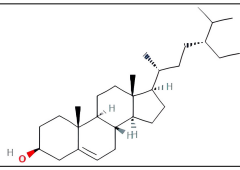
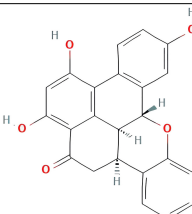
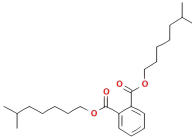
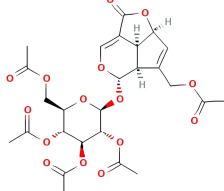
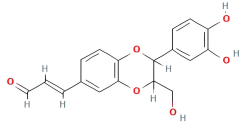
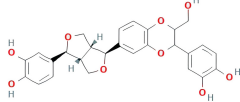
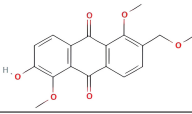
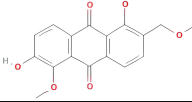
2.2.6 Western Blotting Assay

The proteins were extracted from the left ventricular myocardial tissue of each group of rats. Total protein was extracted and analyzed by western blot analysis. Protein separation was performed using SDS-PAGE and subsequently, the samples were transferred on polyvinylidene fluoride (PVDF) membranes. The membranes were blocked in a western blot blocking buffer for 15 min at room temperature and incubated with a primary antibody. The bands were visualized using horseradish peroxidase-coupled secondary antibody and BeyoECL Star. The final analysis was performed in a chemiluminescence imaging system.

2.3 Statistical Analysis

The data are shown as mean \pm standard deviation. ANOVA was used to examine the differences between groups using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). A p value < 0.05 was considered to indicate a statistically significant difference.

Table 1. 18 compounds from *Morinda officinalis* How, screening condition OB \geq 30%, DL \geq 0.18%.

Molecule Name	Chemical structures	OB (30%)	DL (0.18%)
Ethyl oleate (NF)		32.39738821	0.19061
Alizarin-2-methyl ether		32.80877385	0.20971
Supraene		33.54594264	0.42161
3beta-24S(R)-butyl-5-alkenyl-cholesterol	NA	35.35248934	0.82221
sitosterol	NA	36.91390583	0.7512
beta-sitosterol		36.91390583	0.7512
3beta,20(R),5-alkenyl-stigmastol	NA	36.91390583	0.75074
Ohioensin-A		38.13467065	0.75842
Diop		43.59332547	0.39247
Asperuloside tetraacetate		45.47262233	0.81584
americanin A		46.70571232	0.34901
isoprincepin		49.12131675	0.77375
(2R,3S)-(+)-3',5-Dihydroxy-4,7-dimethoxydihydroflavonol	NA	77.23781866	0.33461
1,5,7-trihydroxy-6-methoxy-2-methoxymethylanthracenequinone	NA	80.4229501	0.37789
1-hydroxy-6-hydroxymethylanthracenequinone	NA	81.76548177	0.2115
2-hydroxy-1,5-dimethoxy-6-(methoxymethyl)-9,10-anthraquinone		95.85173977	0.37249
1,6-dihydroxy-5-methoxy-2-(methoxymethyl)-9,10-anthraquinone		104.5393992	0.33917
2-hydroxy-1,8-dimethoxy-7-methoxymethylanthracenequinone	NA	112.3026489	0.37164

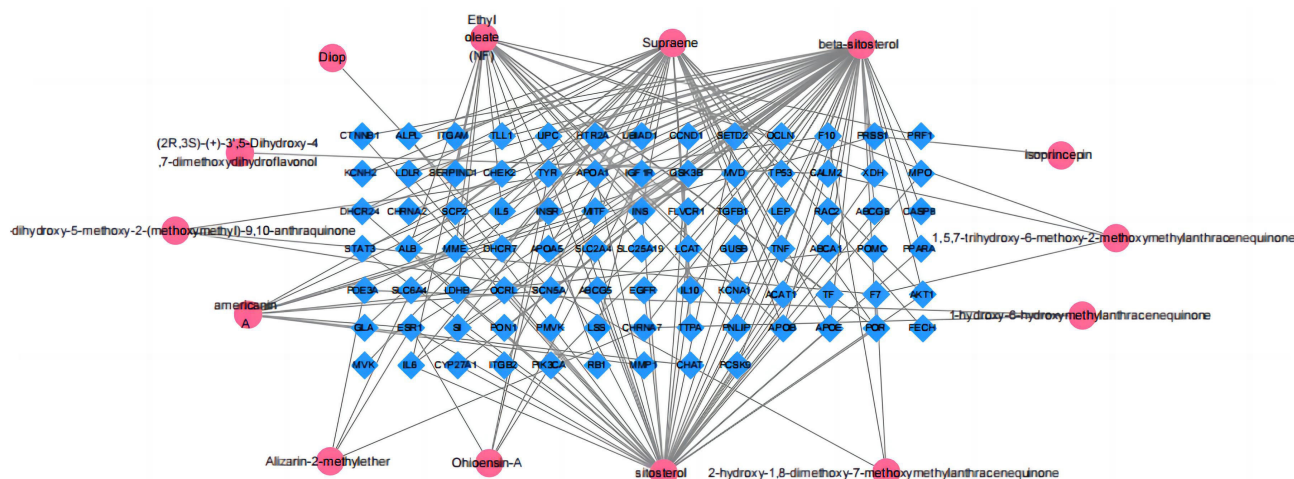


Fig. 3. *Morinda officinalis* How active ingredients-potential targets network.

3. Results

3.1 Active Compounds and Targets of MO

By using the aforementioned database, the potential active ingredients of MO were screened and the basic information of the relative active compound of MO is shown in (Table 1). A total of 176 drug targets and 2454 HF-related targets were mapped by applying a Venn diagram. A total of 101 overlapped targets were obtained (Fig. 2A).

3.2 Construction and Analysis of PPI and Core Targets Network

In Cytoscape 3.7.2 software, the PPI network of the 101 targets was established (Fig. 2B). A total of 101 nodes and 832 edges were involved in the PPI network. These targets with higher values of “Degree” were classified as the core targets for HF (Fig. 2C).

3.3 Target Genes Linked to Target Components

Firstly, the no-target and repeat active compounds were removed, resulting in 14 compounds. The “active compounds-disease targets” network was built in Cytoscape 3.7.2 using overlapping targets and related active components (Fig. 3). A total of 101 nodes and 175 edges were found in this network. The compounds with the dense connectivity maybe the main compound components of MO used in the treatment of HF.

3.4 GO and Pathway Enrichment Analyses of Core Targets

According to GO and KEGG enrichment analyses, the mechanism of action of MO in the treatment of heart failure was mainly related to the role of nitric oxide biosynthesis in regulating the synthesis of secondary metabolites in cells. Biological process (BP) was mainly involved in the positive regulation of transcription, DNA-template synthesis, positive regulation of protein kinase B signaling and regulation of cholesterol metabolism and transport; the cel-

lular component (CC) mode mainly involved extracellular space, lipoprotein particles, and receptor complexes in the lumen of the endoplasmic reticulum lumen; the molecular function categories (MF) mainly involved binding of enzymes, cholesterol, and phospholipids. The graphs were formed using the bioinformatics (<http://www.bioinformatics.com.cn/>) platform (Fig. 4A–C). The top 20 pathways and the number of targets involved were screened (Fig. 5A), including cancer (prostate, colorectal, and pancreatic), inflammation (nonalcoholic fatty liver, hepatitis B), apoptosis, receptor-conduction (FoxO, AMPK, Rap1, and HIF-1 signaling pathway, and blood glucose (insulin resistance). The results indicated that the mechanism of action of MO in the treatment of HF involved AKT1, STAT3, IL-6, and other targets, which affected cancer, inflammation, regulation of blood glucose levels, and receptor functions. The target-pathway network comprised 64 nodes and 202 edges (Fig. 5B).

3.5 Validation of Molecular Docking

MOE software was used to dock the MO active components (beta-sitosterol, americanin A, Asperuloside tetraacetate) with the core targets of ALB (PDB code: 6O69), AKT1(PDB code: ZUZW), STAT3 (PDB code: 4Z1A), IL6 (PDB code: 4O9H). These receptor proteins play a significant role in the KEGG signaling pathways and in the critical nodes of the PPI network and clusters. Most of the docking interactions between MO active ingredients and protein are hydrophobic interactions and hydrogen bonding effects. The lower the intermolecular binding energy, the better the docking strength. When the binding energy was less than 5 kcal/mol, the receptors and ligands had optimal binding properties. The core targets and their corresponding compounds demonstrated binding energies of almost – 5 kcal/mol, which suggested that the core targets and their matching molecules exhibited a high affinity (Fig. 6A–C, Table 2). By using molecular docking, it was shown that

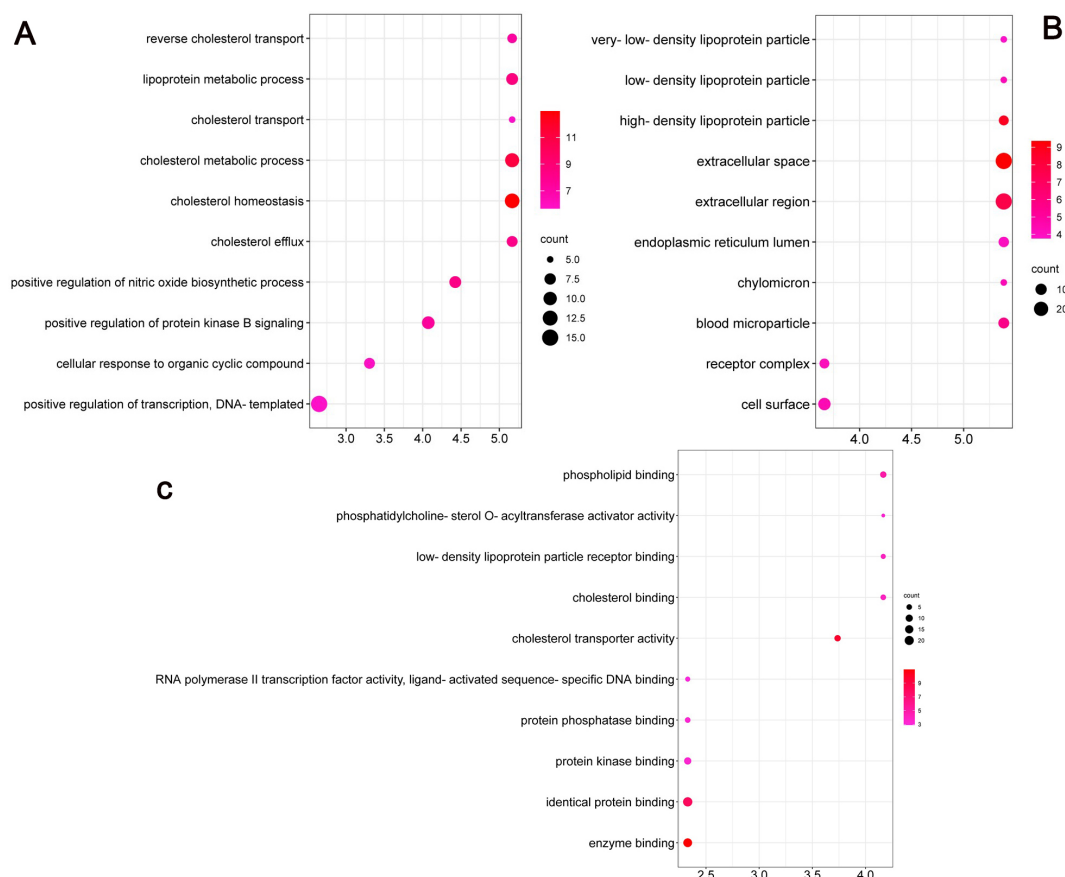


Fig. 4. GO enrichment analysis of the antiheart failure targets of *Morinda officinalis* How. (A) Biological Processes, (B) Cellular Components and (C) Molecular Function.

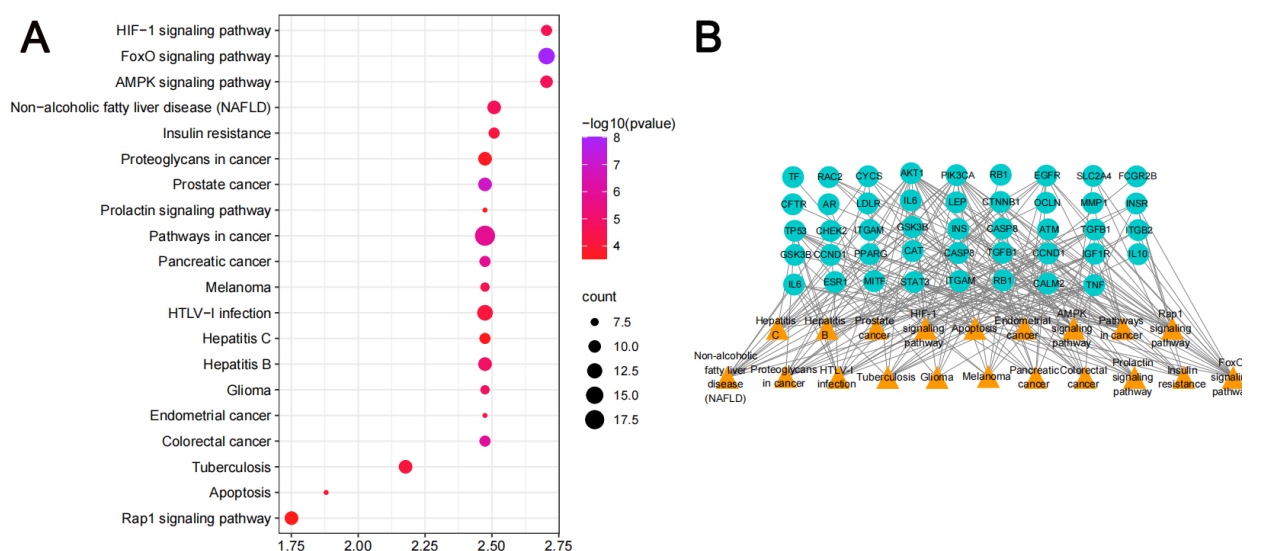


Fig. 5. KEGG pathway analyses of the antiheart failure targets of *Morinda officinalis* How. (A) KEGG enrichment analysis (B) The target-pathway network implicated in the mechanism of *Morinda officinalis* How in heart failure treatment.

the core targets of MO responsible for its action on HF and the relevant active constituents had a strong binding ability, which confirmed the reliability of the network pharmacological model used.

3.6 Experimental Validation

H & E and Masson staining were used to estimate whether MO could be used to treat interstitial fibrosis in HF rats. The results indicated that the cardiomyocytes in

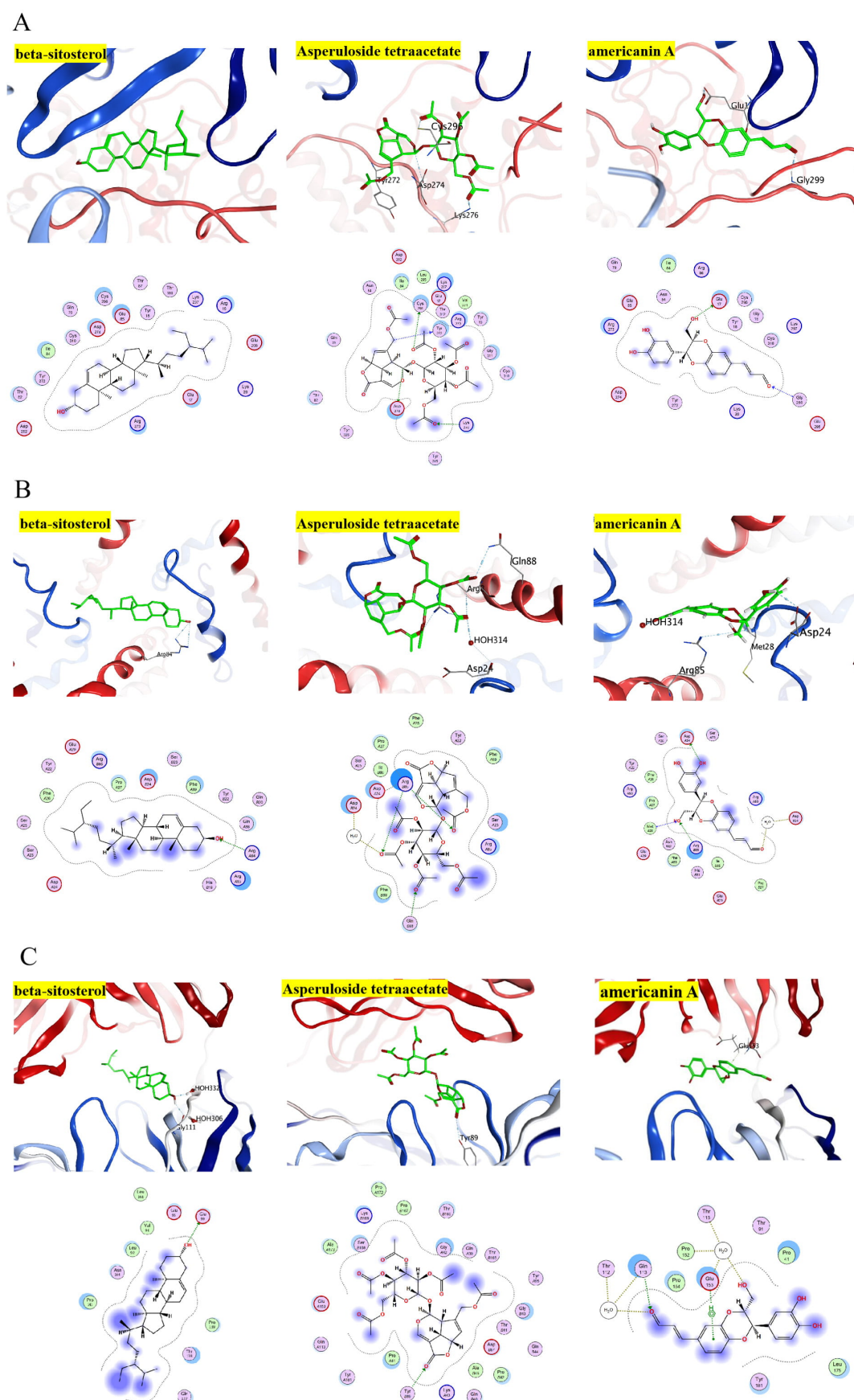


Fig. 6. Diagrammatic 3D and 2D representation that the molecular docking model. (A) The docking result of compounds with AKT. (B) The docking result of compounds with STAT3. (C) The docking result of compounds with IL-6.

Table 2. *Morinda officinalis* How molecular docking energy scoring results (kcal/mol).

Ligand receptors	beta-sitosterol	Asperuloside tetraacetate	americanin A
AKT	-6.4641	-7.0476	-6.6326
STAT3	-6.3519	-6.5650	-5.0980
IL-6	-5.3850	-6.9994	-5.2379

the Sham group were normal in shape without inflammatory infiltration. The cardiac muscle fibers were ordered neatly. In the ISO group, the myocardial cells were associated with interstitial edema, which is a disorder of the cardiac muscle fibers; they were also arranged in a disordered manner and were accompanied by a high number of infiltrating inflammatory cells. Such histological changes were obviously alleviated in the MO groups compared with the ISO group (Fig. 7A,B).

KEGG enrichment analysis indicated that the FoxO signaling pathway was widely active in the cells investigated and could regulate the cell cycle, metabolism, autophagy, and other functions (Fig. 7C), to investigate whether MO could modulate the FoxO3 signaling pathway for the treatment of HF, the role of this pathway was investigated in myocardial tissues. The results indicated that the FoxO3 of the ISO group exhibited a dramatic reduction compared with the Sham group. Moreover, the FoxO3 of the MO group was apparently increased in comparison with the ISO group (Fig. 7D,E). Based on network pharmacology analysis and literature research, the exploration of the underlying mechanism of MO was assessed in the treatment of HF. FoxO3 is a master regulator of protein catabolism in the heart, orchestrating an atrophy response via both autophagy-lysosomal and proteasomal degradation pathways [24]. The expression levels of LC3B, P62 (autophagy markers), p-FoxO3 proteins were analyzed in tissues following ISO injury. The results demonstrated that the expression levels of LC3B and P62 in the ISO group indicated an apparent decrease compared with those of the Sham group, whereas significant recovery of these protein levels were achieved following MO group intervention. In addition, the expression levels of p-FoxO3 in the ISO group were reduced compared with the Sham group, which were significantly recovered by MO treatment. These results were in accordance with the immunohistochemical and immunofluorescence analysis (Fig. 7F).

4. Discussion

Adjuvant therapy with TCM may be helpful to patients with HF, and its mechanism of action may be related to the regulation of angiogenesis, apoptosis, oxidative stress, and to the attenuation of inflammation [25]. Network pharmacology can provide additional insight into a better understanding of the pharmacological mechanisms of TCM and may also offer a reference for interdisciplinary

research [26]. The active ingredient of MO, beta-sitosterol (BS) causing an upregulation of cellular glutathione redox cycling, restored the red blood cell membrane mobility, and reduced oxidative damage to cardiac muscle cells [27]. During inflammation, BS can be imported by calcium uptake in activated neutrophils and inhibits IL-1 β and TNF- α levels [28,29]; asperuloside tetraacetate (ASP) was the first iridoid isolated from the root of *Rubia-tinctorum* L which was beneficial in reducing LPS-induced cell injury through regulation of TLR4 overexpression and MAPK pathway signaling. Endoplasmic reticulum (ER) stress is involved in the development and progression of HF [30,31]. Nevertheless, ASP can target GRP78; it induces ER stress-regulated apoptotic cell death [32]. In addition, ASP has pharmacological effects that are beneficial to treatment HF, such as antihypertensive, antiobesity, and antioxidant activities [33]. Americanin A (AA) is a seed isolated from *Phytolacca Americana*. Excessive mitochondrial ROS plays a deleterious role in HF [34]. A study reported that AA could attenuate ROS and inhibit apoptosis by upregulating Nrf2 levels [35]. In addition, AA also has demonstrated apparent anti-inflammatory and antioxidant activities. These findings demonstrate that the main active compounds of MO exhibit cardioprotective effects mainly by regulating oxidative stress, inhibiting apoptosis, and reducing inflammation. Consequently, BS, ASP, and AA may be the active compounds of MO that can improve or treat HF.

The top 10 important key targets related to the development of HF were identified based on CytoHubba analysis, including AKT1, ALB, INS, IL-6, STAT3, TNF, CCND1, CTNNB1, TP53, and CAT. Loss of the Akt protein could lead to early contractile dysfunction, which could in turn lead to heart failure [36]. By activating the Akt protein, cardiac insulin sensitivity is improved, which prevents the reduction of myocardial contraction caused by aging [37]. In conclusion, AKT regulates cardiac growth, heart aging, glucose metabolism, and lipid metabolism disorders. STAT3 is a transduction protein and activity factor that plays a key function that affects the heart from myocardial infarction, hypertrophy, and cardiomyopathy [38]. Likewise, previous studies have shown damage angiogenesis and aggravated HF via suppressing STAT3 [39,40]. IL-6 is an inflammatory cytokine, which exerts different effects on the cardiovascular system. During the development of ventricular hypertrophy, IL-6 knockout mice alleviated LV hypertrophy, and well preservation of LV function, reducing myocardial fibrosis after TAC, leading to the reduction of fibrosis and apoptosis [41]. Molecular docking results also strongly suggest that active MO compounds can effectively treat HF by binding to the above core genes. The extracellular and intracellular signals play a crucial role in cardiac homeostasis [42]. KEGG pathway analysis indicated that the targets influenced by HF included primarily the cancer-related, receptor-conduction, and insulin signaling pathways. HIF-1 is a key regulator of the hypoxia re-

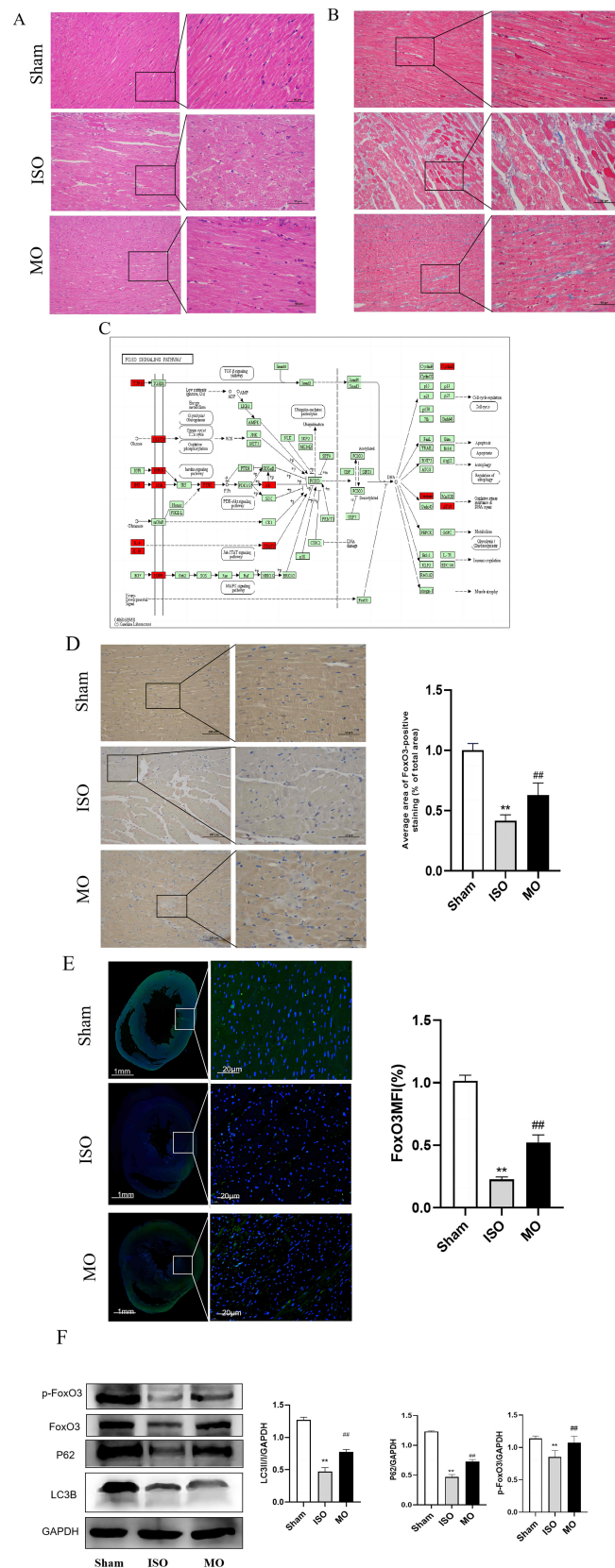


Fig. 7. *In vivo* study. (A,B) Pathological changes of myocardial tissue in rat. H&E and masson staining. (C) The regulation of FoxO signaling pathway in heart failure. (D,E) FoxO3 expression in rat myocardial tissue, Immunohistochemical and immunofluorescence staining. ** $p < 0.01$ compared with Sham group; ## $p < 0.01$ compared with ISO group. (F) Expression levels of autophagy-related proteins, ** $p < 0.01$ compared with Sham group; ## $p < 0.01$ compared with ISO group.

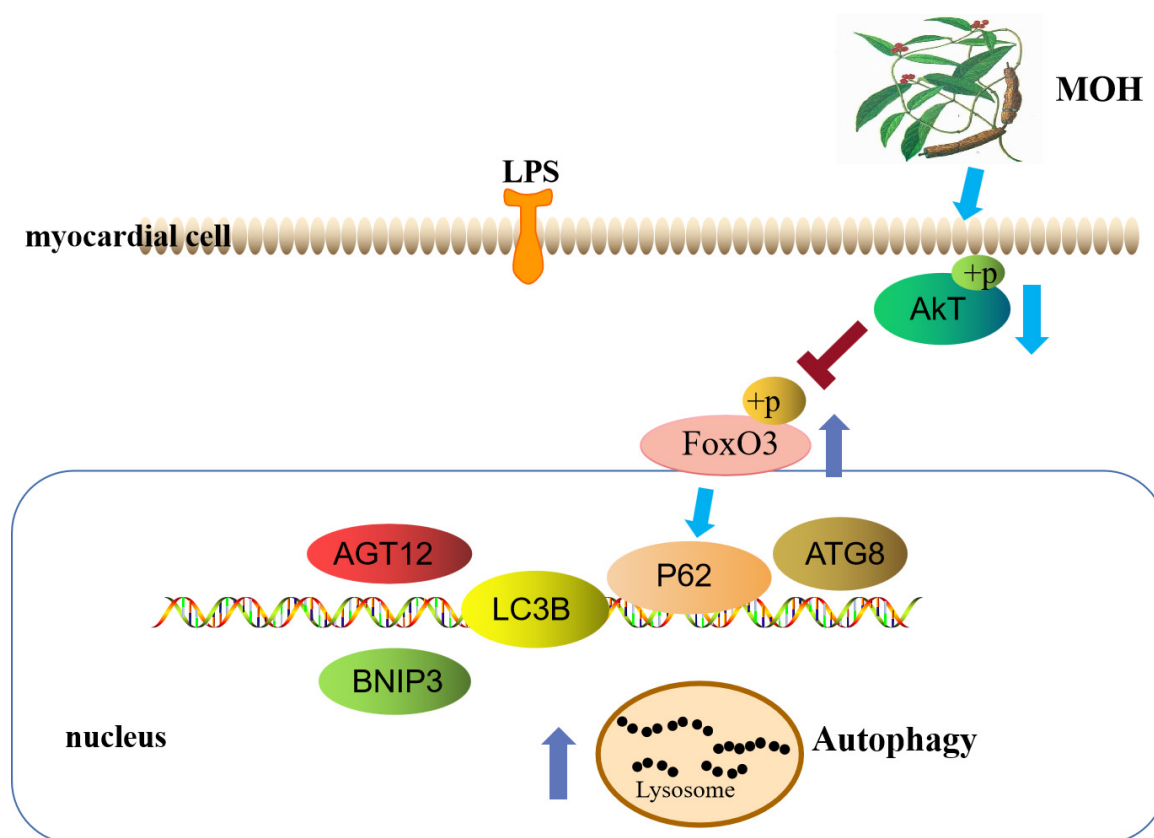


Fig. 8. *Morinda officinalis* How activates autophagy through FoxO3 Signaling Pathways to inhibit heart failure.

sponse, stimulating the transcription of target genes [43], regulating mitochondrial ROS to prevent excessive postischemic cardiac fibroblasts activation and proliferation [44]. Energy deficiency in cardiomyocytes is a dominant cause of heart failure. AMPK can sense the energy state of the cell and orchestrate a global metabolic response to energy deprivation [45]. FoxO has the most primitive antistress signaling molecule, and as a transcription factor that regulates the transcription of several target genes. In cardiomyocytes, it is mainly involved in cellular resistance to oxidative stress, cellular autophagy, mitochondrial regulation, and inflammatory response. These processes can regulate the function of heart cells following HF [46]. There are many isoforms of the Forkhead Box O (FoxO) gene in mammals, FoxO3 is the most widely expressed isotype in the heart [47]. Under starvation or myocardial ischemia conditions, FoxO3 increases the levels of autophagy. The increased FoxO3 activity following starvation or myocardial ischemia reduces cardiac apoptosis and fibrosis [48], and prevents ISO-induced myocardial fibrosis by increasing autophagy. Therefore, the regulation of FoxO3 can improve myocardial remodeling in atrial fibrillation [49].

In vivo studies combined with network pharmacology to provide more information and discuss the therapeutic applications of FoxO3 in the treatment of HF. In the present study, we found the expression levels of the autophagy markers downregulation in cardiac tissues of the ISO group.

However, the autophagy markers significantly increased following MO pretreatment. The histopathology showed that decreased inflammatory cell infiltration and improved cellular fibrosis, indicating that MO may increase the immune function of myocardial cells; the associated mechanism of action may be mediated via the regulation of FoxO3 (Fig. 8), which in turn increases autophagy. These findings are consistent with the aforementioned research study.

5. Conclusions

In conclusion, our results preliminarily revealed the pharmacological effects of MO against HF through bioinformatics and experimental investigations. The results showed that MO is a potential source that can be used in HF treatment, and it will contribute to validating the herbal medicine for treating cardiovascular disease. The data provided a theoretical basis for the subsequent studies that can be conducted to explore the mechanism of action of MO in the treatment of HF. However, the present study did not assess the clinical indicators which MO treated cardiac function. Therefore, follow-up experiments are further required to confirm these preliminary findings.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

YL and HZ—conceived and designed the study and experiments. AW and SD—drafted the experiments and manuscript. YG—analyzed interpreted the data. YY and ZY—help with the experiment and data collection. YL were responsible for supervision of the work. All authors reviewed the final manuscript. AW, SD and YG—have contributed equally to this work and share the first authorship.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Tanai E, Frantz S. Pathophysiology of Heart Failure. *Comprehensive Physiology*. 2015; 6: 187–214.
- [2] Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, *et al.* Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation*. 2019; 139: e56–e528.
- [3] Fu S, Zhang J, Gao X, Xia Y, Ferrelli R, Fauci A, *et al.* Clinical practice of traditional Chinese medicines for chronic heart failure. *Heart Asia*. 2010; 2: 24–27.
- [4] Satta S, Mahmoud AM, Wilkinson FL, Yvonne Alexander M, White SJ. The Role of Nrf2 in Cardiovascular Function and Disease. *Oxidative Medicine and Cellular Longevity*. 2017; 9237–9263.
- [5] Chang MS, Kim WN, Yang WM, Kim HY, Oh JH, Park SK. Cytoprotective effects of *Morinda officinalis* against hydrogen peroxide-induced oxidative stress in Leydig TM3 cells. *Asian Journal of Andrology*. 2008; 10: 667–674.
- [6] Syed AM, Ram C, Murty US, Sahu BD. A review on herbal Nrf2 activators with preclinical evidence in cardiovascular diseases. *Phytotherapy Research*. 2021; 35: 5068–5102.
- [7] He Y, Ma XQ, Fu RF. Effects of n-butanol extract of *Morinda officinalis* How on apoptosis of myocardial cells in neonatal rats undergoing anoxia/reoxygenation. *China Pharmacy*. 2009; 20: 16081610.
- [8] Zhang J, Xin H, Xu Y, Shen Y, He Y, Hsien-Yeh, *et al.* *Morinda officinalis* How. – a comprehensive review of traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*. 2018; 213: 230–255.
- [9] Chaanine AH, Hajjar RJ. AKT signalling in the failing heart. *European Journal of Heart Failure*. 2011; 13: 825–829.
- [10] Dorn LE, Lawrence W, Petrosino JM, Xu X, Hund TJ, Whitson BA, *et al.* Microfibrillar-Associated Protein 4 Regulates Stress-Induced Cardiac Remodeling. *Circulation Research*. 2021; 128: 723–737.
- [11] Fitzwalter BE, Thorburn A. FOXO3 links autophagy to apoptosis. *Autophagy*. 2018; 14: 1467–1468.
- [12] Laurent A, Bissier M, Lucas A, Tortosa F, Roumieux M, De Régibus A, *et al.* Exchange protein directly activated by cAMP 1 promotes autophagy during cardiomyocyte hypertrophy. *Cardiovascular Research*. 2015; 105: 55–64.
- [13] Zhang W, Tao Q, Guo Z, Fu Y, Chen X, Shar PA, *et al.* Systems Pharmacology Dissection of the Integrated Treatment for Cardiovascular and Gastrointestinal Disorders by Traditional Chinese Medicine. *Scientific Reports*. 2016; 6: 32400.
- [14] Wang Y, Bryant SH, Cheng T, Wang J, Gindulyte A, Shoemaker BA, *et al.* PubChem BioAssay: 2017 update. *Nucleic Acids Research*. 2017; 45: D955–D963.
- [15] Piñero J, Bravo A, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, Centeno E, *et al.* DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Research*. 2017; 45: D833–D839.
- [16] Liu Z, Luo Z, Meng Q, Zhong P, Hu Y, Shen X. Network pharmacology-based investigation on the mechanisms of action of *Morinda officinalis* How. in the treatment of osteoporosis. *Computers in Biology and Medicine*. 2020; 127: 104074.
- [17] Wittkopp PJ, Haerum BK, Clark AG. Evolutionary changes in cis and trans gene regulation. *Nature*. 2004; 430: 85–88.
- [18] Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data. *Journal of Proteome Research*. 2019; 18: 623–632.
- [19] Tang D, Zhao X, Zhang L, Wang Z, Wang C. Identification of hub genes to regulate breast cancer metastasis to brain by bioinformatics analyses. *Journal of Cellular Biochemistry*. 2019; 120: 9522–9531.
- [20] Smoot ME, Ono K, Ruscheinski J, Wang P, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics*. 2011; 27: 431–432.
- [21] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*. 2009; 4: 44–57.
- [22] Chen W, Liang J, Fu Y, Jin Y, Yan R, Chi J, *et al.* Cardioprotection of cortistatin against isoproterenol-induced myocardial injury in rats. *Annals of Translational Medicine*. 2020; 8: 309–309.
- [23] Li X, Wang X, Guo Y, Deng N, Zheng P, Xu Q, *et al.* Regulation of endothelial nitric oxide synthase and asymmetric dimethylarginine by matrine attenuates isoproterenol-induced acute myocardial injury in rats. *Journal of Pharmacy and Pharmacology*. 2012; 64: 1107–1118.
- [24] Cao DJ, Jiang N, Blagg A, Johnstone JL, Gondalia R, Oh M, *et al.* Mechanical Unloading Activates FoxO3 to Trigger Bnip3-Dependent Cardiomyocyte Atrophy. *Journal of the American Heart Association*. 2013; 2: e000016.
- [25] Wang Y, Wang Q, Li C, Lu L, Zhang Q, Zhu R, *et al.* A Review of Chinese Herbal Medicine for the Treatment of Chronic Heart Failure. *Current Pharmaceutical Design*. 2018; 23: 5115–5124.
- [26] Zhang R, Zhu X, Bai H, Ning K. Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment. *Frontiers in Pharmacology*. 2019; 21: 123.
- [27] Wong HS, Chen N, Leong PK, Ko KM. B-Sitosterol Enhances Cellular Glutathione Redox Cycling by Reactive Oxygen Species Generated from Mitochondrial Respiration: Protection against Oxidant Injury in H9c2 Cells and Rat Hearts. *Phytotherapy Research*. 2014; 28: 999–1006.
- [28] Liz R, Zanatta L, dos Reis GO, Horst H, Pizzolatti MG, Silva

- FRMB, *et al.* Acute effect of β -sitosterol on calcium uptake mediates anti-inflammatory effect in murine activated neutrophils. *Journal of Pharmacy and Pharmacology*. 2012; 65: 115–122.
- [29] Zhang F, Liu Z, He X, Li Z, Shi B, Cai F. B-Sitosterol-loaded solid lipid nanoparticles ameliorate complete Freund's adjuvant-induced arthritis in rats: involvement of NF- κ B and HO-1/Nrf-2 pathway. *Drug Delivery*. 2020; 27: 1329–1341.
- [30] He J, Lu X, Wei T, Dong Y, Cai Z, Tang L, *et al.* Asperuloside and Asperulosidic Acid Exert an Anti-Inflammatory Effect via Suppression of the NF- κ B and MAPK Signaling Pathways in LPS-Induced RAW 264.7 Macrophages. *International Journal of Molecular Sciences*. 2018; 19: 2027.
- [31] Wang S, Binder P, Fang Q, Wang Z, Xiao W, Liu W, *et al.* Endoplasmic reticulum stress in the heart: insights into mechanisms and drug targets. *British Journal of Pharmacology*. 2018; 175: 1293–1304.
- [32] Rong C, Wei W, Yu-Hong T. Asperuloside exhibits a novel anti-leukemic activity by triggering ER stress-regulated apoptosis via targeting GRP78. *Biomedicine & Pharmacotherapy*. 2020; 125: 109819.
- [33] Manzione MG, Martorell M, Sharopov F, Bhat NG, Kumar NVA, Fokou PVT, *et al.* Phytochemical and pharmacological properties of asperuloside, a systematic review. *European Journal of Pharmacology*. 2020; 883: 173344.
- [34] Bou-Teen D, Kaludercic N, Weissman D, Turan B, Maack C, Di Lisa F, *et al.* Mitochondrial ROS and mitochondria-targeted antioxidants in the aged heart. *Free Radical Biology and Medicine*. 2021; 167: 109–124.
- [35] Jung C, Hong JY, Bae SY, Kang SS, Park HJ, Lee SK. Antitumor Activity of Americanin A Isolated from the Seeds of *Phytolacca americana* by Regulating the ATM/ATR Signaling Pathway and the Skp2-p27 Axis in Human Colon Cancer Cells. *Journal of Natural Products*. 2015; 78: 2983–2993.
- [36] Ock S, Lee WS, Kim HM, Park K, Kim Y, Kook H, *et al.* Connexin43 and zonula occludens-1 are targets of Akt in cardiomyocytes that correlate with cardiac contractile dysfunction in Akt deficient hearts. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2018; 1864: 1183–1191.
- [37] Granado M, Amor S, Martín-Carro B, Guerra-Menéndez L, Tejera-Muñoz A, González-Hedström D, *et al.* Caloric restriction attenuates aging-induced cardiac insulin resistance in male Wistar rats through activation of PI3K/Akt pathway. *Nutrition, Metabolism and Cardiovascular Diseases*. 2019; 29: 97–105.
- [38] Harhous Z, Booz GW, Ovize M, Bidaux G, Kurdi M. An Update on the Multifaceted Roles of STAT3 in the Heart. *Frontiers in Cardiovascular Medicine*. 2019; 6: 150.
- [39] Ni Y, Deng J, Bai H, Liu C, Liu X, Wang X. CaMKII inhibitor KN-93 impaired angiogenesis and aggravated cardiac remodeling and heart failure via inhibiting NOX2/mtROS/p-VEGFR2 and STAT3 pathways. *Journal of Cellular and Molecular Medicine*. 2022; 26: 312–325.
- [40] Jacoby JJ, Kalinowski A, Liu M, Zhang SS, Gao Q, Chai G, *et al.* Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to inflammation, cardiac fibrosis, and heart failure with advanced age. *Proceedings of the National Academy of Sciences*. 2003; 100: 12929–12934.
- [41] Zhao L, Cheng G, Jin R, Afzal MR, Samanta A, Xuan Y, *et al.* Deletion of Interleukin-6 Attenuates Pressure Overload-Induced Left Ventricular Hypertrophy and Dysfunction. *Circulation Research*. 2016; 118: 1918–1929.
- [42] Frangogiannis NG. The Extracellular Matrix in Ischemic and Nonischemic Heart Failure. *Circulation Research*. 2019; 125: 117–146.
- [43] Baumann J, Tsao C, Huang S, Gassmann M, Ogunshola OO. Astrocyte-specific hypoxia-inducible factor 1 (HIF-1) does not disrupt the endothelial barrier during hypoxia in vitro. *Fluids and Barriers of the CNS*. 2021; 18: 13.
- [44] Janbandhu V, Tallapragada V, Patrick R, Li Y, Abeygunawardena D, Humphreys DT, *et al.* Hif-1 α suppresses ROS-induced proliferation of cardiac fibroblasts following myocardial infarction. *Cell Stem Cell*. 2022; 29: 281–297.
- [45] Beauloye C, Bertrand L, Horman S, Hue L. AMPK activation, a preventive therapeutic target in the transition from cardiac injury to heart failure. *Cardiovascular Research*. 2011; 90: 224–233.
- [46] Xin Z, Ma Z, Jiang S, Wang D, Fan C, Di S, *et al.* FOXOs in the impaired heart: New therapeutic targets for cardiac diseases. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2017; 1863: 486–498.
- [47] Schips TG, Wietelmann A, Höhn K, Schimanski S, Walther P, Braun T, *et al.* FoxO3 induces reversible cardiac atrophy and autophagy in a transgenic mouse model. *Cardiovascular Research*. 2011; 91: 587–597.
- [48] Wang C, Xu W, Zhang Y, Zhang F, Huang K. PARP1 promote autophagy in cardiomyocytes via modulating FoxO3a transcription. *Cell Death & Disease*. 2018; 9: 1047.
- [49] Hu J, Wang X, Cui X, Kuang W, Li D, Wang J. Quercetin prevents isoprenaline-induced myocardial fibrosis by promoting autophagy via regulating miR-223-3p/FOXO3. *Cell Cycle*. 2021; 20: 1253–1269.