Systems pharmacology dissection of the anti-myocardial ischemia-reperfusion injury mechanism for Ganjiang Fuzi decoction

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Research

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Systems pharmacology dissection of the anti-myocardial ischemia-reperfusion injury mechanism for Ganjiang Fuzi decoction

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Abstract

Background: As the commonest form of ischemic heart diseases, the Myocardial Ischemia-Reperfusion injury (MI/RI) accounts for almost 50 percent of all deaths. The prevention and treatment of MI/RI while reducing the mortality of myocardial infarction has become a raging topic of research in the cardiovascular field. At present, there are no effective drugs for the treatment of MI/RI. Hence, it becomes imperative to identify or develop efficient lead compounds for treating MI/RI. It has been reported that the Ganjiang Fuzi Decoction (GFD) could be used for the effective treatment of MI/RI due to its promotion of vasodilation and vascular endothelial cell proliferation besides reducing the oxidative damage.

Methods: The network pharmacological methods were used in this study, for analyzing the biological processes and the molecular mechanisms of the GFD for MI/RI treatment. *In vitro* and *in vivo* experiments were performed for verification of the results of the network pharmacological predictions.

Results: Around 16 active components of GFD were discovered against MI/RI, where aconitine, 6-ginger, mesaconitine, and hypaconitine were the leading ones with regard to the degree value. Moreover, it was found that

88 MI/RI-related targets mainly involved six aspects, apoptosis, oxidative stress, inflammation, mitochondrial energy metabolism, and vasodilation. *In vitro* studies indicated the ability of the GFD to increase the survival rate, decrease the apoptosis rate, reduce oxidative damage, and increase the expression of HIF-1α, VEGF, and eNOS in hypoxia/reoxygenation(H/R) injured Rat Vascular Endothelial Cells (RVEC). The *in vivo* studies illustrated the capacity of the GFD to reduce the myocardial tissue damage and the infarction area, while increasing the expression of HIF-1α, VEGF, and eNOS in the MI/RI rats.

Conclusions: The results of this study confirmed the anti-MI/RI role of the GFD through the activation of the HIF-1α signaling pathway, promotion of vascular proliferation and dilation, and the reduction in oxidative damage. The findings of this study would further provide experimental evidence for the application of the GFD in the treatment of MI/RI.

Keywords Fuzi, Ganjiang, myocardial ischemia-reperfusion injury, vasodilation, Hypoxia injury

Introduction

Cardiovascular diseases have long been causing deaths to the largest number of humans. Acute Myocardial Infarction (AMI), one of the world's most common major cardiovascular diseases is characterized by high mortality and disability [1, 2]. Despite the significant progress made in the treatment of AMI in recent years, its mortality rate remains relatively high [3]. The main reason for this happens to be the treatment method of thrombolysis and Percutaneous Coronary Intervention (PCI). These treatments could open the blocked blood vessels and save dying hearts [4]. Nevertheless, while reducing the size of the acute myocardial infarction, the sudden opening of a coronary artery could cause myocardial ischemia-reperfusion injury (MI / RI)[5, 6]. The MI/RI poses a new difficulty and

challenge in the treatment of AMI. Presently, there are four recognized manifestations of MI/RI, reperfusion-induced arrhythmia, myocardial arrhythmia, vascular occlusion, and fatal myocardial reperfusion injury. The first two cases are reversible, however, the last two are irreversible [7]. Clinically, the PCI, the antiplatelet, and the anticoagulants are mainly used to treat the latter two types of injuries, while this could maintain the patency of the relevant coronary arteries [8]. Nonetheless, the efficacy of surgery for MI/RI has not been found to be significant. Hence, the necessity to develop a new strategy at the earliest for the treatment of MI/RI.

Traditional Chinese Medicine (TCM) is a medical system which different from the Western systems of medicine. It has been widely used by the Chinese people for the treatment of diseases and illnesses since several centuries. TCM has been found to play a vital role in the treatment of MI/RI. Several TCM remedies have been used for treating MI/RI and the efficacies have been remarkable. The Buyang Huanwu Decoction has shown the properties to reduce oxidative damage in MI/RI rats [9]. The Gualou Xiebai Banxia Decoction was found to inhibit the expression of the PINK1/Parkin pathway, reducing the mitochondrial autophagy and plays a protective role in the myocardium [10]. The Qishen Yiqi Drop Pills activated the p38/JNK/ERK pathway and inhibited the expression of inflammation-related cytokines TNF-α, IL-1β, and IL-6 against MI/RI [11]. The Ganjiang Fuzi Decoction (GFD) was recorded in the *Sheng Nong's Herbal Classic* to have great efficacy for warming yang and activating pulse, besides being used commonly for treating MI/RI [12, 13]. Studies have revealed the GFD having a protective impact on the oxidative stress injury caused by the myocardial ischemia-reperfusion in the rats [14]. Nevertheless, the fundamental molecular action mechanisms of the GFD have not yet been systematically explored. The bioactive compounds, the

potential targets, and the related pathways of GFD remain relatively unknown.

With the advent of bioinformatics, network pharmacology has become a powerful tool for the exploration of TCM [15, 16]. Network pharmacology integrated systems biology and multi-aspect pharmacological thinking, besides integrating the biological network and the drug action network, transcending the limitations of the single-objective thinking and realizing the comprehensive network analysis of drug effects from the perspective of a multi-objective research strategy [17]. Network pharmacology enabled the visual network analysis of active ingredients, central targets, signaling pathways, and diseases. Moreover, it could fully explain the complex relationship between drugs and diseases. Hence, network pharmacology could be used for the effective exploration of the multi-component, multi-target, and multi-pathway of TCM, complementary to the concept of TCM [18]. Network pharmacology has been used in this study for the prediction of the biological process and the mechanism of GFD against MI/RI. The network pharmacology prediction would be subsequently validated by in vitro and in vivo experiments. A detailed flow chart of this study is as illustrated in Figure 1.**Materials and methods**

The *Aconitum abietetorum W.T.Wang, L.Q.Li* (No. 51078020190385YC), and the *Zingiber officinale Roscoe* (No. 51078020191511YC) were purchased from the Shaanxi Xingshengde Pharmaceutical Co. Ltd. (Tongchuan, China) and identified by Professor Gang Zhang from Shaanxi University of Chinese Medicine. The samples were deposited at the Herbal Medicine Museum at the same university. The Compound Danshen Dripping Pills (CDDP) were purchased from Tusly Pharmaceutical Co. Ltd. (Tianjin China). The Fetal Bovine Serum (FBS) was purchased from BI (USA). The Phosphate Buffer Saline (PBS) and the

Dulbecco's Modified Eagle Medium (DMEM) were procured from Gibco (USA). The Penicillin streptomycin mixture, 2, 3, 5, Triphenyl-2H-Tetrazolium (TTC), and the Cell Counting Kit-8 (CCK-8) were obtained from the Shanghai Biyuntian Co. Ltd. (Shanghai, China). Dimethyl Sulfoxide (DMSO) and Trypsin was also procured from Gibco (USA). The assay kits for Malondialdehyde (MDA), apoptosis, and Superoxide Dismutase (SOD) were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The antibodies against HIF-1α, eNOS, VEGF, and β-actin were obtained from Cell Signaling Technology Inc. (Boston, USA). The 6-gingerol, aconitine, mesaconitine, and hypaconitine were purchased from the Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China). Their purities were≥98.0%. Acetonitrile and ammonium bicarbonate were purchased from the Boster Biological Technology Co. Ltd. (Wuhan, China).

Preparation of GFD and quality control

Around 350 g of Fuzi and 233 g of Ganjiang were prepared for the study. Both components were totally immersed in water for half an hour, before being boiled twice for 1.5 hours each time. The filtrates were collected using 4-layer gauzes, combined, and concentrated to 6 g/mL to separate the extract. The supernatant was obtained following the centrifugation of the solution at 3000 r/min, sterilized with 0.22 μm aqueous microporous membrane, and sealed. The GFD quality was then controlled by the LC-MS method. The LC-MS components were separated using Waters Bridge C18 (4.6 mm×150 mm, 5 μm) and a C18 guard. The flow rate was set at 0.2 mL/min, min−1. The column temperature was maintained at 35°C and the wavelength was set at 237 nm. The mobile phases were (A) acetonitrile and (B) ammonium bicarbonate solution (10mmol / L, pH = 10), with gradient elution of 0-6 min (A: B = 30:70), 6-

12 min (A: B = 65:35) 13-18 min (A: B = 55:45), 0-6 min (A: B = 45:55), 19-24 min (A: B = 35:65), and baseline (A: B = 30:70). Quality control of the anti-MI/RI active components in GFD was conducted by the predictive results of network pharmacology and the quality control was as has been illustrated in Figure 1.

Active components composition of GFD

Initially, the Traditional Chinese Medicine Integrated Database (TCMSP, https://tcmspw.com/tcmsp.php) was used to determine the active components of Fuzi and Ganjiang. The screening criteria were Oral Bioavailability (OB) ≥30% and the Drug-Likeness (DL) ≥0.18 [19]. Moreover, to ensure convincing results, the active ingredients of Fuzi and Ganjiang were also collected using the available literature. All components that met the screening criteria were used for the network pharmacology analysis.

Target prediction

The TCMSP and the Comparative Toxicogenomic Database (CTD, http://ctdbase.org/) were used for obtaining the targets of Fuzi and Ganjiang. As the target information in the TCMSP was full of names, it was imported into the Universal Protein (Uniprot) database (https://www.uniprot.org/) for correction. Using 'myocardial infarction' as the keyword, the MI/RI targets in the DisGeNET database (https://www.disgenet.org/) were restricted to 'homo sapiens' were obtained. The common targets of GFD and MI/RI were considered to be the targets of GFD anti MI/RI.

GO and KEGG analysis

The Database for Annotation, Visualization, and Integrated Discovery (David) V6.8 (www.david.ncifcrf.gov/) was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The

GFD anti-MI/RI targets were imported into the DAVID database, the functional annotations panel was selected, the species was defined as homosapiens, and the GO and KEGG results were subsequently saved. The results of $P<0.05$ were considered to be significant.

Network construction

The Cytoscape software was used for investigating the relationship between the components and the targets, with the potential mechanism of GFD in MI/RI treatment. Hence, the Cytoscape was used for constructing the Component-Target (C-T) networks and the target-pathway (T-P) networks. In the network, the compounds, the targets, and the pathways were represented by nodes, and their relationships were represented by the edges. With regard to these nodes, it was observed that the larger the degree, the more significant it was.

Experimental validation

A total of 80 Sprague-Dawley male rats were purchased from the Chengdu Da Shuo Experimental Co. Ltd. (Sichuan, China). All animals were kept under conducive conditions at 24°C ± 1°C temperature, 60%–70% relative humidity, and a 12-hour light/dark cycle. The experiment was started once the rats had been subjected to adaptive feeding for one week. The rats were used for two sections of the experiment, (1) 20 rats were used for preparing the drug-containing serum, while (2) 60 rats were used for *in vivo* experiments. All the animal experiments were performed in consonance with the rules of the Animal Care and Use Committee at the Institute of Materia Medica, P.R. China (No. TCM-2020-145-060-C09).

Preparation of drug-containing serum

The 20 rats were randomly further segregated into four groups and there were five rats in each group. Those in the control group, the GFD low-dose group (0.5 clinical equivalent dose), the GFD medium-dose

group (clinical equivalent dose), and the GFD high-dose group (2 clinical equivalent dose) were orally administered with 1.4g/kg, 2.8g/kg, and 5.6g/kg GFD twice daily. Besides, the control group was orally administered with the same volume of 0.5% CMC-Na. The rats were anesthetized by an intraperitoneal injection of 10% chloral hydrate (0.3 mL /100 g) two hours after the final administration. The serum was collected and inactivated in a water bath at a temperature of 56℃ for 30 minutes. The serum was subsequently stored at -80℃ for later use.

Cell grouping and modeling

The Rat Vascular Endothelial Cells (RVEC) were purchased from the Chinese Tissue Culture Collections (Zhejiang China). The RVEC were cultured in DMEM with 10% FBS. In case of all the experiments, the cells were cultured at 37C° with 5% CO2. Upon reaching an 80% confluence level of the population, they were subjected to the experimental procedures. The optimal concentration of the drug-containing serum for the RVEC was determined before the experiment and was maintained at 15%. The cells were divided into five groups, the control group, the hypoxia/reoxygenation (H/R) group, the low-dose group (GFD1.4g/kg), the medium-dose group (GFD2.8g/kg), and the high-dose group (GFD5.6g/kg). The H/R method was as described hereunder [20]. In all experiments, the cells were treated with serum starvation at rest for 24 hours prior to the treatment. The Treatment groups were treated using various concentrations of the GFD containing serum (1.4g/kg, 2.8g/kg, 5.6g/kg). The control group was treated with the complete medium and the model group was treated with a glucose-free medium. All the groups were subsequently subjected to anoxic treatment for 12 hours in an anoxic apparatus with a mixture of 95%N2-5%CO_2 at a flow rate of 4 L/min. The

cell culture medium was replaced with a complete medium and reoxygenated for 2 hours in a cell incubator which contained 5%CO_2-95%O_2.

Cell morphology and survival rate

The RVEC in the logarithmic phase were seeded at a density of 1 x 10^5 cells/ml in 96-well culture plates, according to the above method for grouping, administration, and H/R. Once the H/R model was created, the morphology and number of cells were determined through observation under an optical microscope (Olympus BX 41, Japan). 10μl CCK-8 was subsequently added to each well and it was incubated for 2 hours. The absorbance was recorded at 450 nm, while the experiment was repeated three times parallelly.

Biochemical and apoptosis testing

After the RVEC was damaged by H/R, the cells were collected from each group. Following the protocols of the manufacturer, the SOD, MDA, and the apoptosis levels were determined by their respective commercial kits. The Optical Densities (OD) of SOD and MDA were measured at 560nm and 532nm. The Flow cytometry was conducted for detecting the rate of apoptosis of the cells (Novo Cyte 452180529501, Thermo, USA).

Establishment and grouping of the myocardial ischemia-reperfusion injury rat model

The 60 rats were randomly segregated into six groups and with 10 rats in each group, the sham group, the MI/RI group, the CDDP group, the GFD low-dose group, the GFD medium-dose group, and the GFD high-dose group. The GFD low-dose group, the GFD medium-dose group, and the GFD high-dose group were administered with 1.4g/kg, 2.8g/kg, and 5.6g/kg GFD orally, once daily. The positive control group (CDDP) received 0.09 g/kg orally once daily, while the sham group and the MI/RI

groups were orally administered with the same volume of 0.5% CMC-Na. The model was made after one week of administration. The rats were subsequently anesthetized with an intraperitoneal injection (IP) of 10% Chloral hydrate (0.3 ml/100 g). The left-thoracotomy, pericardiectomy, and the left anterior descending coronary artery ligation were performed as described earlier [21]. The criteria for the successful modeling were the instant whitening of the apex of the heart and the elevation of the S and the T waves (ST) segments of the electrocardiograph. Following the successful modeling, ischemia occurred for 40 minutes, after which the ligation was opened, and there was reperfusion for 2 hours. After modeling, the serum, the heart tissue, and the abdominal aortic blood vessels were collected for use in subsequent experiments.

Histological analysis

The hearts of around three rats from each group were randomly chosen for the TTC staining. After modeling, the rat hearts were placed at -20° for 15 minutes and the specimens were then evenly sliced into 1mm sections at the ligation line before being stained with 2% TTC at 37° for 20 minutes. Finally, the sections were fixed with 10% formaldehyde solution and photographed (Xiaomi China). The infarct area was found to be white and the non-infarct area was red. A comparison of the infarct area on the same level of the cross-section was done. The infarct area was calculated using the Image J software (Media Cybernetics, Inc., Rockville, MD, USA).

The hearts of four rats were sampled randomly and washed using PBS. The anterior wall of the left ventricle's myocardium was precooled with 4% paraformaldehyde and dehydrated. It was subsequently soaked in xylene and dyed using the conventional paraffin embedding for hematoxylin-eosin

(H-E) staining. The pathological changes to the myocardium were observed under a light microscope (Olympus BX41, Japan).

Biochemical and apoptosis testing

After reperfusion, using chloral hydrate (30 mg/kg) the rats were anesthetized intraperitoneally, with the blood samples being obtained from the abdominal aorta. The samples were left standing at room temperature for around 30 minutes and centrifuged thereafter at 3,000 r/min for 15 minutes. Basing on the protocols of the manufacturer, the SOD, and the MDA levels were detected using the respective commercial kits. The Optical Densities (OD) of SOD and MDA were measured at 560nm and 532nm respectively.

Western blotting

The RIPA Lysis Buffer was used to extract the total protein from each group of the MI/RI rat vascular tissue and each group of the RVEC cells damaged by H/R. Equal amounts of proteins were then subjected to 8% SDS-PAGE for HIF1a (1:500), VEGF (1:1000), eNOS (1:500), and β-actin before transferring to the PVDF membranes. The slides were fixed and incubated with primary antibodies overnight at 4C° with gentle agitation, followed by secondary antibody incubation[22]. Images were captured by GeneGnomeXRQ (Syngene).

Statistical analysis

All data were expressed as the mean ± Standard Deviation (SD). The differences amongst the multiple groups were evaluated using the one-way analysis of variance (ANOVA). The difference between the means was considered statistically significant at $P < 0.05$.

Results

Around 213 components of the GFD were obtained from the TCMSP database. Six of the active components of the GFD were obtained, based

on the OB≥30% and DL≥0.18 screening conditions. To ensure more comprehensive results and to compensate for the deficiency of the theoretical screening, the relevant literature was consulted to supplement the active ingredients [23-26]. Certain components did not fulfill the screening requirements of OB and DL and exhibited poor pharmacokinetic properties. Nonetheless, they were also chosen as active ingredients for further studies due to their high content in GFD. Hence, nine components were added, including aconitine and 6-gingerol.

Potential targets and components analysis

About 171 GFD and 996 MI/RI targets were obtained The common targets of GFD and the MI/RI were considered as the anti-MI/RI targets of the GFD, while 88 anti-MI/RI targets of the GFD were obtained (Figure 2A). For exploring the relationship between the components and the targets, a Component-Target(C-T) network was established (Figure 2B). The degree was used for evaluating the importance of the potential active components. Aconitine, 6-ginger, meso-aconitine, and hyaconitine were the top four-degree values, and they were considered to be the potential therapeutic components of the GFD against MI/RI. Simultaneously, through the LC-MS, the existence of these four components in GFD was confirmed.

GO analysis

The biological process of the GFD anti-MI/RI remained unclear, hence, the targets of the GFD anti-MI/RI were introduced into DAVID to obtain the Biological Processes (BP), The Cell Components (CC), and the Molecular Functions (MF) (Table 1). The BP, CC, and MF with $P < 0.05$ were considered significant. From this research, it was believed that the BP, CC, and the MF ranking in the top ten with $P < 0.05$ were considered to have a high correlation with the MI/RI (Figure 3). The BP was found to be

mostly related to apoptosis, oxidative stress, mitochondria, NO release, and germ cell development. The GFD could have an anti-MI/RI effect by inhibiting the cell apoptosis, reducing inflammation, and promoting vasodilation.

KEGG analysis

To determine the specific mechanism of the GFD resistance to the MI/RI, the KEGG signaling pathway of GFD anti-MI/RI was obtained from DAVID. $P < 0.05$ was considered to be a significant signaling pathway. We collected information on the top ten signaling pathways (Table2). As indicated in Figure 4, thePI3K-Akt signaling pathway, TNF signaling pathway, and the HIF-Iα signaling pathways were considered to be potential GFD anti-MI/RI signaling pathways. The PI3K-Akt signaling pathway was found to be associated with apoptosis [27], with the TNF signaling pathway being associated with inflammation [28], and the HIF-Iα signaling pathway being associated with the oxidative damage and releasing NO to relax the blood vessels [29]. To summarize, it was presumed that the GFD anti-MI/RI could play therapeutic roles in the reduction of myocardial apoptosis, inflammatory injury, and hypoxia injury, along with the promotion of angiogenesis and vasodilation through the PI3K-Akt signaling pathway, the TNF signaling pathway, and the HIF-Iα signaling pathways. This study was intended to investigate the anti-MI/RI mechanism of the GFD from the perspective of the activation of the HIF-Iα signaling pathway for reducing oxidative damage and promoting vascular proliferation and dilation.

***In vitro* experimental validation**

CCK-8 assay and cell morphology observation

Following the H/R injury, the morphology and number of cells in each group were observed under a light microscope (Olympus BX41, Japan).

The cells in the control group grew well, while the adherent growth cell morphology was found to be like the rice grain. The total number of cells in the H/R group was observed to have been significantly reduced, and they were wrinkled and rounded ($p<0.01$). Compared to the H/R group ($p<0.01$), following the pretreatment of the GFD drug-containing serum, the cell status was changed, with an increase in the number of cells. The morphology of the RVEC cells was close to that of the control group. Of all administration groups, the morphology and the quantity of the GFD medium-dose group (2.8g/kg) were most similar to that of the control group (Figure 5).

The survival rate of the control group in this study (Figure 5), was considered to be 100%. The survival rate of the RVEC cells with H/R injury was significantly reduced to 49% ($p<0.01$). Nevertheless, three GFD groups demonstrated significant improvement to the survival rate of H/R-damaged RVEC cells, while the GFD (2.8 g /kg) group was observed to be having the best therapeutic effect (81% survival rate) of all treatment groups ($p<0.01$).

Biochemical and apoptosis testing

The SOD is a scavenger of oxygen free radicals, representing the ability of the body in terms of resisting oxidation. The MDA is a product of oxidative damage, and the more severe the oxidative damage, the greater the MDA content would be [30]. It is pertinent to note that severe oxidative damage could lead to cell apoptosis or necrosis. The apoptotic (35.2% apoptosis rate) rate and the MDA expression of the RVEC cells injured by H/R indicated significant increases (Figure 6A, B), whereas the SOD expression was found to have decreased significantly ($p<0.01$). When the RVEC was pretreated with GFD and then H/R, it was observed that the

GFD could reduce the apoptosis rate and the MDA expression of the RVEC cells that were damaged by H/R and increase the SOD activity. Of all the administration groups ($p<0.05$), the GFD medium-dose group (2.8g/kg) demonstrated the greatest therapeutic effect with an apoptosis rate of 19.72%.

GFD active HIF-1 α /VEGF/eNOS signaling (*In Vitro*)

The network pharmacology predicted that the GFD anti-MI/RI was capable of having a therapeutic role in the reduction of the myocardial apoptosis, the inflammatory injury, and the hypoxia injury, along with the promotion of angiogenesis and vasodilation through the synergistic effects of the PI3K-Akt signaling pathway, the TNF signaling pathway, and the HIF-Iα signaling pathways. Hence, the HIF-1α/VEGF/eNOS was selected as the specific signaling pathway for verification (Figure 6C). The H/R injury inhibited the expression of HIF-1α, VEGF, and eNOS in the RVEC cells ($P < 0.05$). Nonetheless, the GFD activated the HIF-1α/VEGF/eNOS signaling pathway in the H/R-damaged RVEC cells.

***In Vivo* experimental validation**

The effects of GFD on the myocardial tissue injury and the infarct size in the MI/RI rats

The MI/RI rats also displayed an increase in the myocardial infarct size of .2% ($P<0.01$). The myocardial infarct area of the MI/RI rats decreased to various degrees subsequent to the GFD pretreatment (Figure 7A). The infarct area of the CDDP group decreased by up to 11% ($P<0.01$), followed by the infarct area of the GFD (5.6g/kg), which decreased by 23.7% ($P<0.01$).

Similarly, the myocardial fibers were intact in the sham group, almost without breakages, with the fibers arranged neatly (Figure 7B). The myocardial tissue of the MI/RI group displayed obvious disorder and the myocardial fibers were found to be ruptured. The degree of the myocardial tissue damage in the drug group was significantly lower in comparison to that of the MI/RI group, while the CDDP displayed the best therapeutic effect, followed by the GFD (5.6g/kg).

Biochemical and apoptosis testing

As compared to the control group (Figure 8A), the oxidative damage of the MI/RI group was found to have increased, the expression of the SOD decreased, while there was an increase in the expression of the MDA. After the GFD treatment, the expression of SOD in the serum of MI/RI rats increased, whereas, the expression of MDA decreased. The GFD reduced the oxidative damage in MI/RI rats, and the treatment effect of 5.6g/kg was the best of all the three GFD groups ($P < 0.05$).

GFD active HIF-1α/VEGF/eNOS signaling (*In vivo*)

In vitro studies revealed that the GFD could activate the expression of the HIFα/VEGF/eNOS signaling pathway in the RVEC cells after the H/R injury. Hence, *in vivo* experiments also validated this perspective (Figure 8B). Interestingly, *in vivo*, it was observed that the GFD could activate the expression of the HIFα/VEGF/eNOS signaling pathway in the blood vessels of MI/RI rats.

Discussion

Of all the cardiovascular diseases, acute myocardial infarction has been the leading cause of human deaths globally. The restoration of blood supply by percutaneous coronary intervention or thrombolysis was considered as the most effective means of saving the ischemic myocardium, nonetheless, potentially leading to MI/RI [31]. Pertinent to note that as the

society developed, the irregular lifestyles, the resting patterns, and the diets of the young people have led to the probability of an increase in the MI/RI. The MI/RI could also occur during organ transplants. At the same time, the mechanism of MI/RI is complex and diverse, and solving the MI/RI from a single perspective was extremely difficult. Hence, MI/RI has become an urgent problem for medical students to solve. The TCM treatment of the diseases has the characteristics of the multi-pathway, the multi-target, and the multi-mechanism synergy, while it could be used for treating the MI/RI. Recently, the TCM demonstrated unique advantages in the prevention and treatment of MI/RI, besides indicating broad application potentials [32, 33]. Nevertheless, from the perspective of the syndrome differentiation and witreatment of traditional Chinese medicines, drawing on the modern medical research methods, and the technical means was still necessary. Full use should be made of cells, animal models, and clinical research when conducting in-depth research to help explain the material basis and the mechanism of action of the traditional Chinese medicine in the prevention of the MI/RI. An innovative approach was used here. The Network pharmacology was employed for the prediction of the anti-MI/RI mechanisms of the GFD and *in vivo* and *in vitro* experiments conducted.

The network pharmacology predicted the GFD anti-MI/RI to be highly correlated with apoptosis, inflammation, oxidative damage, and the release of NO. The KEGG analysis illustrated the GFD anti-MI/RI to be highly correlated with the PI3K-Akt signaling pathway, the TNF signaling pathway, and the HIF-Iα signaling pathways. The PI3K-Akt signaling pathway was an important anti-MI/RI signaling pathway [34]. The phosphatidylinositol 3-kinase specifically catalyzed the phosphorylation of the phosphatidylinositol-3 hydroxyl group. First, it phosphorylated the PIP2 for the production of PIP3, subsequently, it activated the Akt,

activating the downstream targets and promoting apoptosis [35, 36]. Li et al. discovered that the hydrogen-rich water activated the PI3K-Akt signaling pathway, reduced the ischemia/reperfusion injury of the isolated rat hearts, and inhibited the apoptosis of the myocardial cells[37]. The TNF was found to bind to two types of receptors, TNFR1 and the TNFR2. The TNF signaling pathway was involved in the inflammatory response of the body triggering the activation of several pathways, including the NFKB and the MAPK pathways. The Febuxostat inhibited the inflammation and apoptosis mediated MAPK /NF-κBp65/TNF-α signaling pathway and demonstrated a better protective effect against the IR injury than allopurinol[38]. The Hypoxia was found to be associated with the pathophysiology of several major human diseases, including myocardial and cerebral ischemia and cancer. The Oxygen-sensing and response in the animal cells was a particularly significant process that displayed a crucial role in the pathophysiology of a series of diseases, such as cancer and cardiovascular diseases [39]. This process is mainly regulated by the Hypoxia Inducible Factor (HIF) and its regulator Vonhippel-Lindau tumor suppressor protein (PVHL). The HIF1 was a heterodimer that was composed of two basic helix-ring-helix PAS (per-arnt-sim) proteins, the HIF1-alpha and the HIF1-beta. The HIF1-α could only be found under hypoxia conditions. Under hypoxia, the HIF1-α was not recognized by the PVHL and transferred to the nucleus in order to interact with the cofactors including CBP/ P300 and the Pol II complexes. The HIF1-α bonded with the Hypoxia Response Element (HRE), thereby activating the downstream targets. For example, activation of the VEGF- promoted angiogenesis, activation of LDHA led to participation in the glycolytic pathway, and the activation of eNOS promoted the angiogenesis and vasodilation. Studies have revealed that sevoflurane could significantly improve the myocardial

injury that was induced by I/R in the rats, and the mechanism could be related to the activation of the Akt/ HIF-1α /VEGF signaling pathway for promoting vasodilation and reducing oxidative stress [40-42].

The Fuzi could improve blood circulation, increase the flow of blood to the heart and expand the blood vessels. A Fuzi injection helps in dilating the blood vessels. After the injection of the anesthetized dogs, the femoral artery blood flow and the cerebral blood flow were found to have increased, and the vascular resistance also decreased. The Fuzi has a relaxing effect on the aorta of the isolated rabbits. Moreover, the Fuzi could dilate the microvessels of the auricles of the mice, speed up the blood flow, increase the blood flow, and promote the elimination of microcirculation disorders in the auricles of the mice caused by adrenaline. The gingerol in the Ganjiang had the effect of improving the cardiovascular functions. Studies have confirmed that the Ganjiang could fight blood clots and inhibit the aggregation of the platelets. Earlier studies have indicated that Fuzi and Ganjiang could regulate vasodilation, however, the mechanism of vasodilation remained unclear. The GFD was a TCM commonly used in the treatment of MI/RI. It was unclear whether it could prevent MI/RI by promoting vasodilation. Hence, we used the network pharmacology to predict the BP and KEGG of the GFD against MI/RI. Combined with network pharmacology, it is predicted that GFD could promote the release of vascular NO through the HIF signaling pathway. The intention of this study was to investigate the anti-MI/RI mechanism of the GFD from the perspective of the activation of the HIF-Iα signaling pathway for reducing oxidative damage and promoting vascular proliferation and dilation.

The vascular endothelial cell damage was the initial factor that caused cardiovascular disease and was also an important link that led to the occurrence and the development of several other systemic diseases[43].

The blood supply to the heart comes mainly from the coronary arteries, and the myocardial insufficiency is caused mainly by the stenosis of the coronary artery. Reperfusion aggravated the injury of the endothelial cells, while the damage it caused was irreversible. During reperfusion, the vascular endothelial cells recruited the neutrophils and released a significant quantity of proteolytic enzymes and oxygen free radicals, damaging the cells and the intercellular matrix, while destroying the barrier function of the endodermis [44, 45]. This led to the edema of the endothelial cells and the cardiomyocytes, while the myocardial function declined significantly [46]. Moreover, the reperfusion process also disrupted the vasoconstriction/diastolic balance, increasing the likelihood of vasoconstriction and further exacerbating the reperfusion injury. It was observed that the GFD could activate the HIF-α/VEGF/eNOS signaling pathway to increase the survival rate of the H/R damaged RVEC cells, reduce the rate of apoptosis, and reduce the oxidative damage. *In vivo* studies revealed that the GFD could activate the expression of the HIF-α/VEGF/eNOS signaling pathway to reduce the area of myocardial infarction, myocardial pathological damage, and reduce oxidative damage in M/RI rats.

Earlier studies of the GFD against the MI/RI focused on the heart, this study proved that GFD could promote vasodilation anti-MI/RI. Secondly, the network pharmacology was used to predict the mechanism of the GFD against the MI/RI. It was discovered in the pertinent study that the GFD activated the HIF-1α/VEGF/eNOS signaling pathway and reduced the oxidative damage in the MI/RI rats and the RVEC cells. This was found to be consistent with the network pharmacology results. These were the innovations of this research. Nevertheless, this research also has certain shortcomings, as the complexity of the components of the GFD, and not

verifying if the components were anti-MI/RI through vasodilation. Secondly, the dose dependence was not determined, which could be because of the concentration the gradient established not being large enough. In the research following GFD, we need to pay special attention to these shortcomings.

Conclusions

To summarize, a new method was applied for obtaining the potential components, the biological processes, and the mechanisms of the GFD anti-MI/RI, and they were confirmed by *in vivo* and *in vitro* experiments. The anti-MI/RI therapeutic components of the GFD were predicted by network pharmacology as being aconitine, 6-Ginger, mesaconitine, and hypaconitine, and their presence in the GFD was determined by LC-MS. The GO and KEGG analyses confirmed that the GFD anti-MI/RI could play a therapeutic role in the reduction of apoptosis, inflammatory injury, and hypoxia injury, with the promotion of angiogenesis and vasodilation through the PI3K-Akt signaling pathway, TNF signaling pathway, and the HIF-Iα signaling pathways. In vitro studies confirmed that the GFD activated the HIF-Iα signaling pathway and reduced oxidative damage and apoptosis rates in the RVEC cells. In vivo studies illustrated that the GFD could reduce the myocardial infarction size and the pathological damage, while increasing the expression of the HIF-Iα, VEGF, and the eNOS in the blood vessels of the MI/RI rats. Hence, this study has proven that the GFD could play an anti-MI/RI role through the activation of the HIF-Iα signaling pathway to promote vascular endothelial cell proliferation and vasodilation. This study is expected to provide a foundation for follow-up research and the further development of the GFD.

List of abbreviations

AMI, Acute Myocardial Infarction; BP. Biological Processes; CDDP, Compound Danshen Dripping Pills; CC, Cell Components; CCK8, Cell Counting Kit-8; CTD, Comparative Toxicogenomic Database; C-T, Component-Target; DAVID, Database for Annotation Visualization, and Integrated Discovery; DMEM, Dulbecco's Modified Eagle Medium; DMSO, Dimethyl Sulfoxide; DL, Drug-Likeness; FBS, Fetal Bovine Serum; GFD, Ganjiang Fuzi Decoction; GO, Gene Ontology; H-E, hematoxylin-eosin; H/R, Hypoxia/reoxygenation; HIF, Hypoxia Inducible Factor; HRE, Hypoxia Response Element; KEGG, Kyoto Encyclopedia of Genes and Genomes; MDA, Malondialdehyde; MF, Molecular Functions; MI/RI, Myocardial ischemia-reperfusion injury; OB, Oral Bioavailability; PCI, Percutaneous Coronary Intervention; PBS, Phosphate Buffer Saline; RVEC, Rat Vascular Endothelial Cells; SOD, Superoxide dismutase; ST, S and the T waves; TCM, Traditional Chinese Medicine; TCMSP, Traditional Chinese Medicine Integrated Database; T-P, Target-pathway; TTC, 2, 3, 5, Triphenyl-2H-Tetrazolium Chloride;

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Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available fromthe corresponding author on reasonable request

Ethics approval and consent to participate

The animal study was reviewed and approved by the Ethics Committee of Shaanxi University of Traditional Chinese Medicine

Authors' contributions

Feng Xie, Nan Gou,performed the experiments, analyzed the data and wrote the manuscript. Min Li,Tiantian Zhang, Yue Ma, Ji Peng designed

the study. Min Li revised the article. All authors have read and agreed to the final version of the manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests regarding the publication of this manuscript.

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REFERENCES

1. Isiozor NM , Kunutsor SK , VoutilainenA , et al. Ideal cardiovascular health and risk of acute myocardial infarction among Finnish men[J]. Atherosclerosis, 2019, 289.
2. Ragnhild S , Siri A , Eliassen BM , et al. changes in conventional cardiovascular risk factors and the estimated 10-year risk of acute myocardial infarction or cerebral stroke in sami and non-sami populations in two population-based cross-sectional surveys: the saminor study[J]. BMJ Open, 2019, 9(7)
3. Hausenloy D J , Yellon D M . Myocardial ischemia-reperfusion injury: a neglected therapeutic target[J]. J Clin Inves, 2013, 123(1):92-100.
4. Wang J,, Xie, et al. "Attenuation of Myocardial ischemia reperfusion injury by Geniposide preconditioning in diabetic rats," *Curr Res Trans Med.*
5. Amin, MZ, Narges, et al. "The protective role of curcumin in myocardial ischemia–reperfusion injury," *J cell physiol, vol.* 234, no. 1, pp. 214-222.

6. Li Y, Fei L, Wang J, et al. "Inhibition of miR-217 Protects Against Myocardial Ischemia–Reperfusion Injury Through Inactivating NF-κB and MAPK Pathways," *Cardiovas Eng Techn*.

7. Chiu F , Yang L. "Clinical manifestations and basic mechanisms of myocardial ischemia/reperfusion injury," *Ci ji yi xue za zhi, vol.* 30, no. 4, pp. 209-215.

8. Worner F , Cequier A , BardajíVA, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation.[J]. Eur Hear J, 2012, 33(1):2569-619.

9. Song YZ, Chen YC, and Yong WX. "Effect of Buyang Huanwu Decoction Preconditioning on Antioxidant Function in Rats with Myocardial Ischemia-reperfusion Injury," *Chin J Bac Med Trad Chinese Med, vol.* 26, no. 11, pp. 1621-1625.

10. Li X, *Study on the Mechanism of Mitochondrial Autophagy and PINK1/Parkin Pathway of Trichosanthes Trichosanthes Maxim Decoction on Myocardial Ischemia-reperfusion Injury Rats*, Yunnan Univ Chinese Med, 2020.

11. Li XX. Chen XJ, Wu YB. "Study of Qishen Yiqi Drop Pill on MAPK/p38/JNK/ERK Signaling Pathway of Rat Myocardial Ischemia-reperfusion Injury," *Chinese J Evid Cardiovas Med, vol.* v.12, no. 09, pp. 43-47.

12. Wu PC, Xi LJ. "Study on synergistic and attenuated mechanism of Fuzi and Ganjiang," *J Prac Chinese Med*.

13. Li TM, Wang GX, Gao J. "Experimental study on the effect of warming yang and activating pulse after the combination of Fuzi and Ganjiang," *Chinese J New Dru ClinicPharmacol, vol.* 22, no. 004, pp. 373-375.

14. .Shi Q, Peng F, Zhao YH. "Experimental study of ginger aconite decoction on myocardial cell injury induced by ischemia-reperfusion in rats," *J Guiyang College of Chinese Med, vol.* 36, no. 005, pp. 5-8.

15. An L, Lin Y, Li L, et al. "Integrating Network Pharmacology and Experimental Validation to Investigate the Effects and Mechanism of Astragalus Flavonoids Against Hepatic Fibrosis," *Front Pharmacol, vol.* 11.

16. Wu RM, Jiang B, Li H,et al. Shen. "A network pharmacology approach to discover action mechanisms of Yangxinshi Tablet for improving energy metabolism in chronic ischemic heart failure," *J Ethnopharmacol, vol.* 246, pp. 112227-112227.

17. Chen Y, Yue S, Li W, et al. "Systems Pharmacology Dissection of the Anti-Stroke Mechanism for the Chinese Traditional Medicine Xing-Nao-Jing," *J Pharmacol Sci, vol.* 136, no. 1, p. 16.

18. Jiang M, Su X, Liu J, et al. "Systems Pharmacology-Dissection of the Molecular Mechanisms of Dragon's Blood in Improving Ischemic Stroke Prognosis," *Evid based Compl Alt, vol.* 2020, no. 2, pp. 1-14.

19. Yu J, Li Y, Liu X, et al. "Mitochondrial dynamics modulation as a critical contribution for Shenmai injection in attenuating hypoxia/reoxygenation injury," *J Ethnopharmacol, vol.* 237, pp. 9-19.

20. Fei C,. Sun ZW, Ye LF, et al. "Lycopene protects against apoptosis in hypoxia/reoxygenation-induced H9C2 myocardioblast cells through increased autophagy," *Mol Med Rep, vol.* 11, no. 2.

21. Zhao M , Jin Y. "The PI3K/Akt pathway mediates the protection of SO(2) preconditioning against myocardial ischemia/reperfusion injury in rats," *Acta pharmacol Sin.*

22. Liu XW, Lu MK, Zhong HT, et al "Panax Notoginseng Saponins Attenuate Myocardial Ischemia-Reperfusion Injury Through the HIF-1α/BNIP3 Pathway of Autophagy," *J Cardiovas Pharmacol, vol.* 73, no. 2, pp. 92-99.

23. Wu KH, *Study on chemical constituents and activity of Fuzi*, China Aca Chinese Med Sci, 2013.

24. Mu JL Kao YP, Yuan ZQ. "Chemical composition and pharmacological action of Fuzi," *Shaanxi J Tradit Chinese Med*, no. 12, pp. 92-94.

25. Sun FJ,Li ZL, Qian SH. "Research progress on chemical constituents and pharmacological action of Ganjjiang," *Chinese J Wil Plant Re*, no. 03, pp. 40-43.

690 26. Yuan X, Zhang YY. "Research progress on chemical constituents and
691 pharmacological action of GanJiang," *Shandong J Chem Ind, vol.* 047, no. 014, pp. 41-
692 42.
693 27. .Osaki M, Kase S, Adachi K, et al. "Inhibition of the PI3K-Akt signaling pathway
694 enhances the sensitivity of Fas-mediated apoptosis in human gastric carcinoma cell line,
695 MKN-45," *J Cancer ResClini Oncol, vol.* 130, no. 1, pp. 8-14.
696 28. Oyama R,. Jinnin M, A. Kakimoto, et al. "Circulating microRNA associated with
697 TNF-α signaling pathway in patients with plaque psoriasis," *J Dermatol Sci, vol.* 61,
698 no. 3, pp. 209-211.
699 29. Kimura S , Kitadai Y , Tanaka S , et al. Expression of hypoxia-inducible factor
700 (HIF)-1alpha is associated with vascular endothelial growth factor expression and
701 tumour angiogenesis in human oesophageal squamous cell carcinoma.[J]. Eur J Canc,
702 2004, 40(12):1904-1912.
703 30. Yang Y, Duan W, Jin Z, et al. "JAK2/STAT3 activation by melatonin attenuates
704 the mitochondrial oxidative damage induced by myocardial ischemia/reperfusion
705 injury," *J Pin Res, vol.* 55, no. 3, pp. 275-286.
706 31. .Ferdinandy P, Schulz R, Baxter G.F. "Interaction of Cardiovascular Risk Factors
707 with Myocardial Ischemia/Reperfusion Injury, Preconditioning, and Postconditioning,"
708 *Pharmacol Rev, vol.* 59, no. 4, pp. 418-458.
709 32. Zhao Y, Xu L, Qiao Z, et al. "YiXin-Shu, a ShengMai-San-based traditional
710 Chinese medicine formula, attenuates myocardial ischemia/reperfusion injury by
711 suppressing mitochondrial mediated apoptosis and upregulating liver-X-receptor α,"
712 *Rep, vol.* 6, p. 23025.
713 33. .Liu H, Shang J, Chu F, et al. "Protective Effects of Shen-Yuan-Dan, a Traditional
714 Chinese Medicine, against Myocardial Ischemia/Reperfusion Injury In Vivo and In
715 Vitro," *Evid Based Compl Alte,2013,(2013-12-23), vol.* 2013, no. 5, p. 956397.

34. Zhang P, Zhao S, Lu X, Z. et al. "Metformin enhances the sensitivity of colorectal cancer cells to cisplatin through ROS-mediated PI3K/Akt signaling pathway," *Gene, vol.* 745, p. 144623.

35. .Wu LY, Enkhjargal B,. Xie ZY, et al. "Recombinant OX40 attenuates neuronal apoptosis through OX40-OX40L/PI3K/AKT signaling pathway following subarachnoid hemorrhage in rats," *Exper Neurol, vol.* 326, p. 113179.

36. .Wang M, Yu Z,. Feng H, et al. "In situ biosynthesized gold nanoclusters inhibiting cancer development via the PI3K–AKT signaling pathway," *J Mat Chem B, vol.* 7.

37. Li L, Li X, Zhang Z, et al. "Effects of hydrogen-rich water on the PI3K/AKT signaling pathway in rats with myocardial ischemia-reperfusion injury," *Cur Mol Med, vol.* 19, pp. -.

38. Khan SI, Malhotra RK., Rani N, et al. "Febuxostat Modulates MAPK/NF- κ Bp65/TNF- α Signaling in Cardiac Ischemia-Reperfusion Injury," *Oxid med cell longev, vol.* 2017, pp. 1-13.

39. . Jiang BH,. Zheng JZ, Leung SW, et al. "Transactivation and Inhibitory Domains of Hypoxia-inducible Factor 1α: Modulation of Transcriptional Activity By OxygenTension ," *J BiolChem, vol.* 272.

40. Wu C, Chen J, Chen C, et al. "Wnt/β-catenin coupled with HIF-1α/VEGF signaling pathways involved in galangin neurovascular unit protection from focal cerebral ischemia," *Sci Rep, vol.* 5, p. 16151.

41. Kim BR, Seo SH, Park MS, et al. "sMEK1 inhibits endothelial cell proliferation by attenuating VEGFR-2-dependent-Akt/eNOS/HIF-1α signaling pathways," *Oncotarget, vol.* 6, no. 31, pp. 31830-31843.

42. .Tang X, Zhang Q, ShiS, et al. "Bisphosphonates suppress insulin-like growth factor 1-induced angiogenesis via the HIF-1alpha/VEGF signaling pathways in human breast cancer cells," *InterJ Cancer, vol.* 126, no. 1, pp. 90-103.

43. .Mechtcheriakova D, Wlachos A,. Holzmüller H, et al. "in Endothelial Cells Is Mediated by EGR1 Vascular Endothelial Cell Growth Factor-Induced Tissue Factor Expression," *Blood, vol.* 93, no. 11, pp. 3811-3823.

44. .Kloner RA, Jennings RB. "Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 2," *Circulation, vol.* 104, no. 25, p. 3158.

45. Verma S, Fedak P, Weisel RD., et al. "Fundamentals of Reperfusion Injury for the Clinical Cardiologist," *Circulation, vol.* 105, no. 20, pp. 2332-2336.

46. Perrelli MG,. Pagliaro P, . Penna C. "Ischemia/reperfusion injury and cardioprotective mechanisms: Role of mitochondria and reactive oxygen species," *Wor J Cardiol, vol.* 3, no. 6, pp. 186-200.

Figures

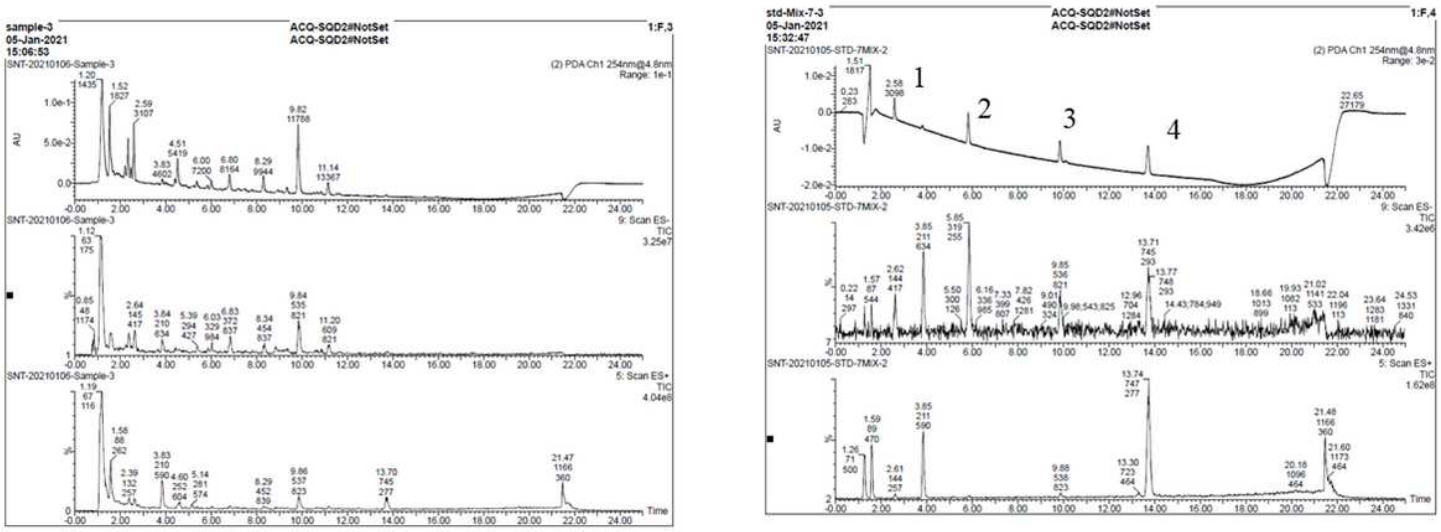

Figure 1

The typical chromatograms of standard compounds. A chromatogram of FG, B chromatogram of standard. 1aconitine. 2 mesaconitine. 3hypaconitine.4 6-ginger.

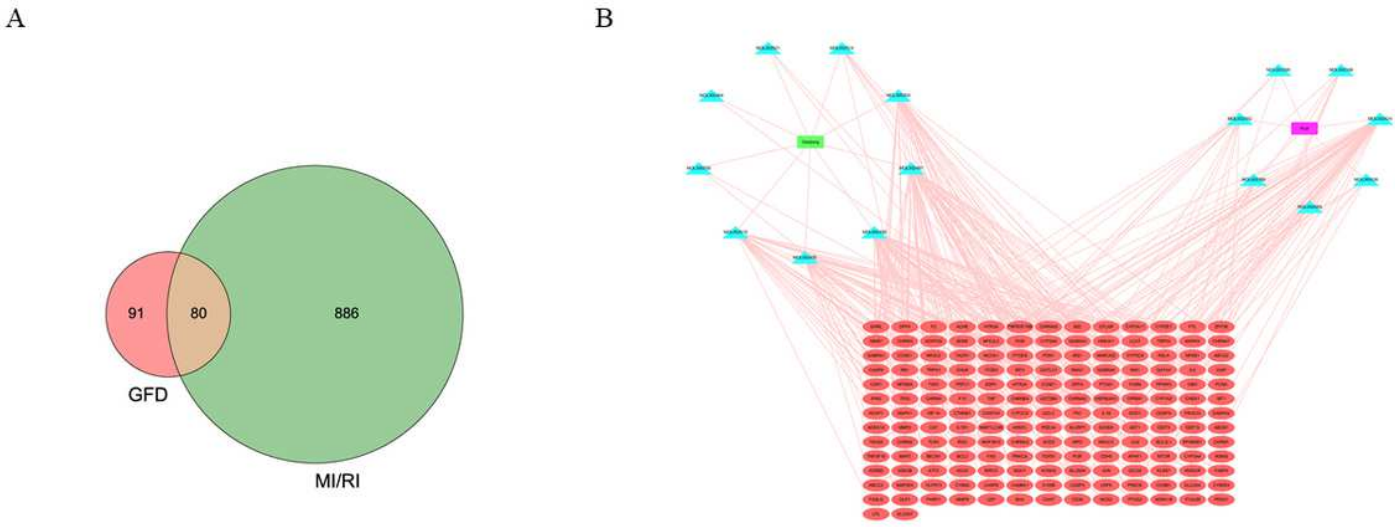

Figure 2

The networks of ganjiang fuzi decoction (GFD) anti- MI/RI. (A) Venn diagram of drug targets and disease proteins. The targets in orange are the common targets of GFD and MI/RI, which is also the anti-MI /RI target of GFD.(B) The component-target network of GFD. The green nodes represent Ganjiang, the purple

nodes represent Fuzi, the blue nodes represent components, and the red nodes represent targets. The more lines between the component and the target, the more important the component is

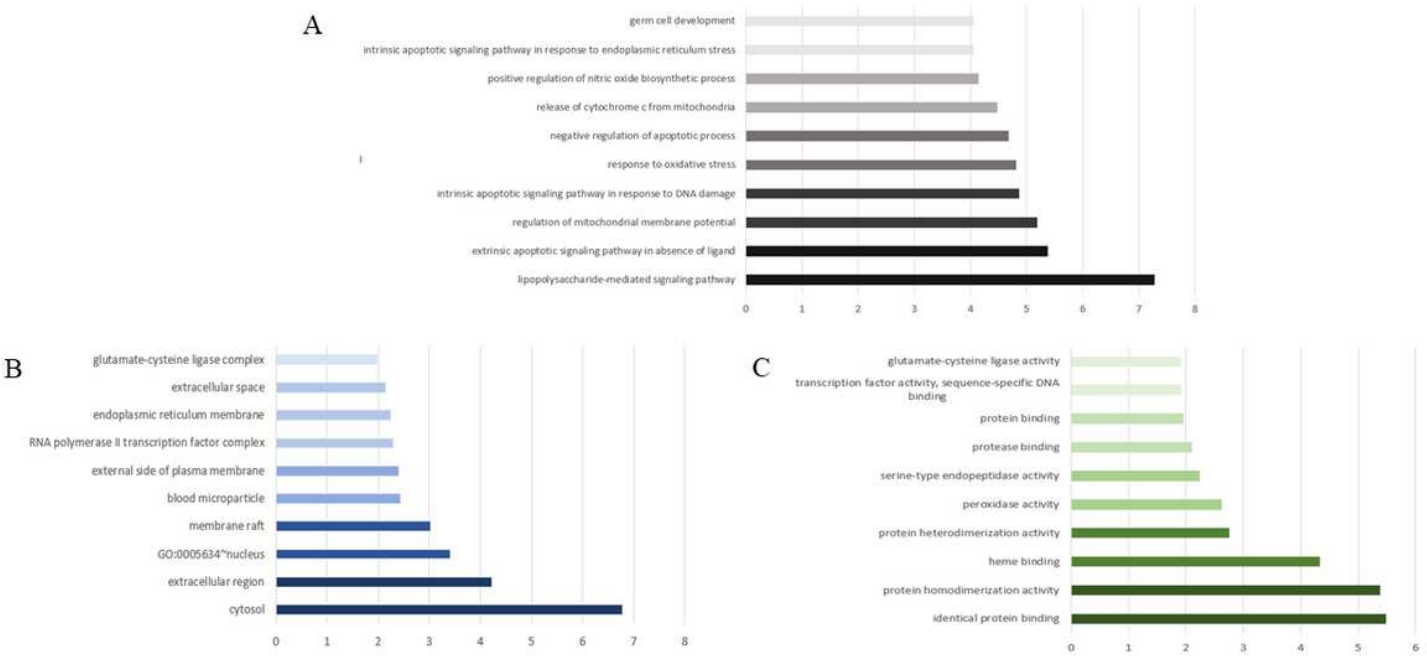

Figure 3

The gene ontology (GO) enrichment analysis for ganjiang fuzi decoction (GFD) anti - MI/RI targets. (A) Biological processes of GFD against MI/RI targets. (B) Cellular component of GFDG against MI/RI targets. (C) Molecular function of GFD anti-MI /RI target.

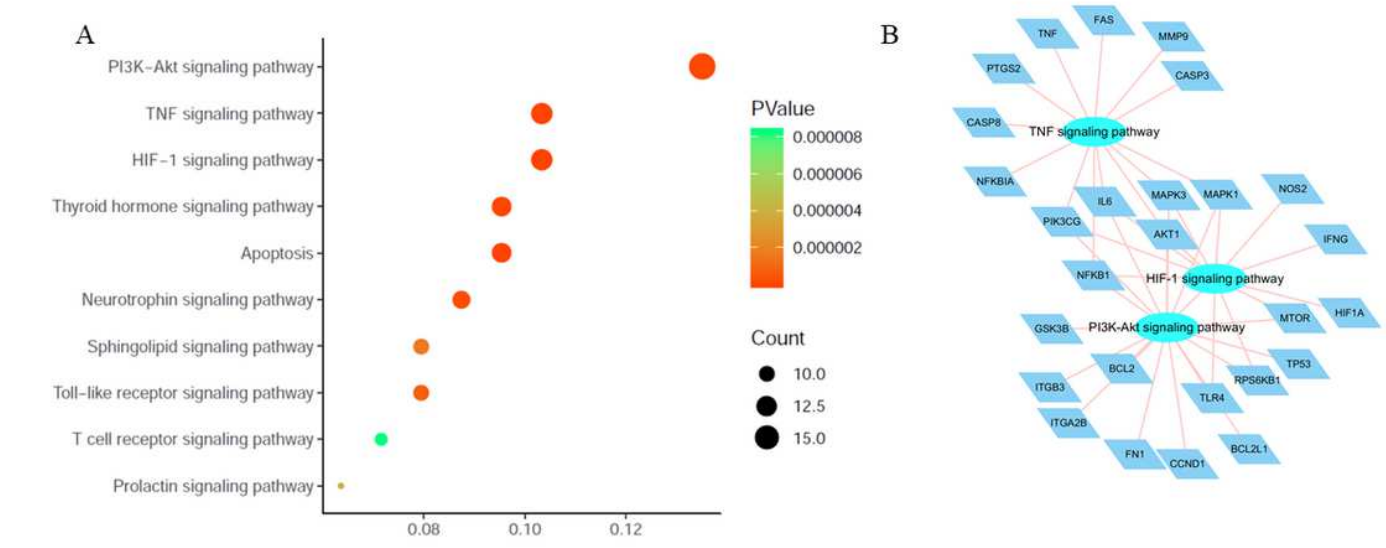

Figure 4

KEGG pathway enrichment analysis of ganjiang fuzi decoction (GFD) anti - MI/RI targets. (A) Top 10 Signal Pathway Bubble Chart. The size and color of the circle are proportional to the importance of the signaling pathway. The bigger the dot and the redder the color, the more important the signaling pathway. (B) Target-Pathway network. The ellipse represents the signaling pathway and the diamond represents the target.

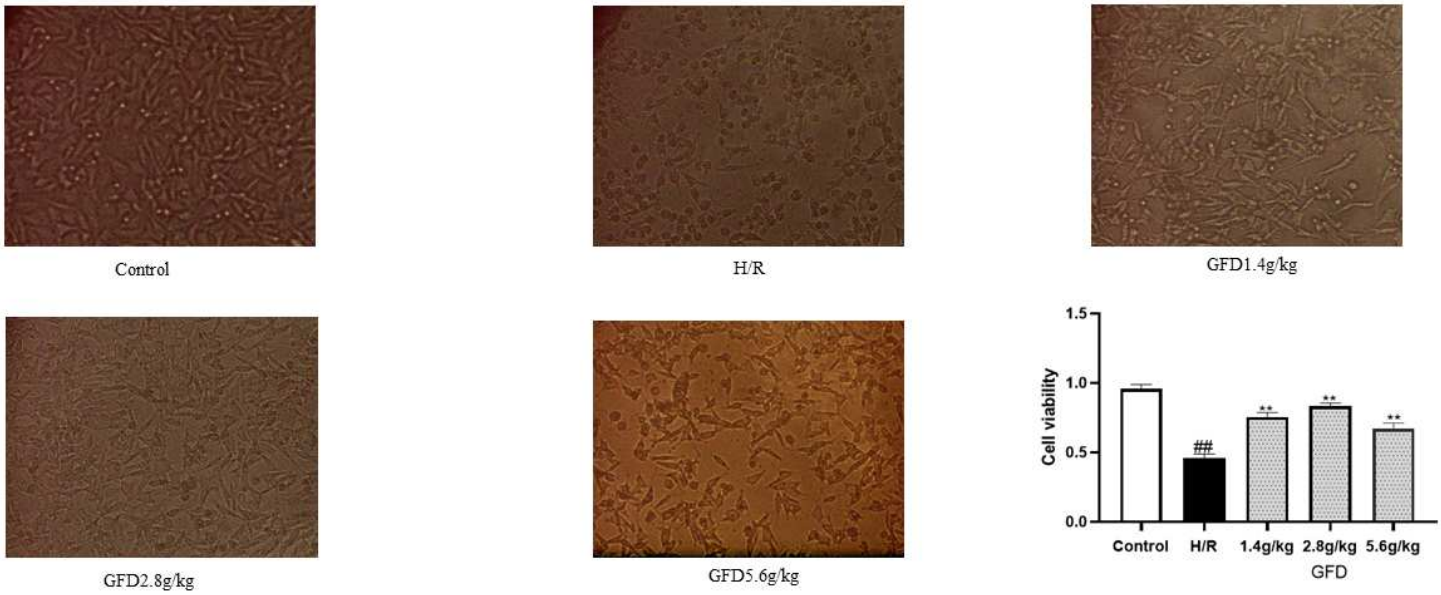

Figure 5

Effects of ganjiang fuzi decoction (GFD) on morphology, number and survival rate of vascular endothelial cells in H/R injured rats(100x). The morphology of vascular endothelial cells in normal rats is like rice grains. After H / R injury, cell morphology became atrophied. Data were expressed as mean ± SD. N=5. #p< 0.05, ##p < 0.01 vs. control group. *p < 0.05, **p < 0.01 vs. H/R group.

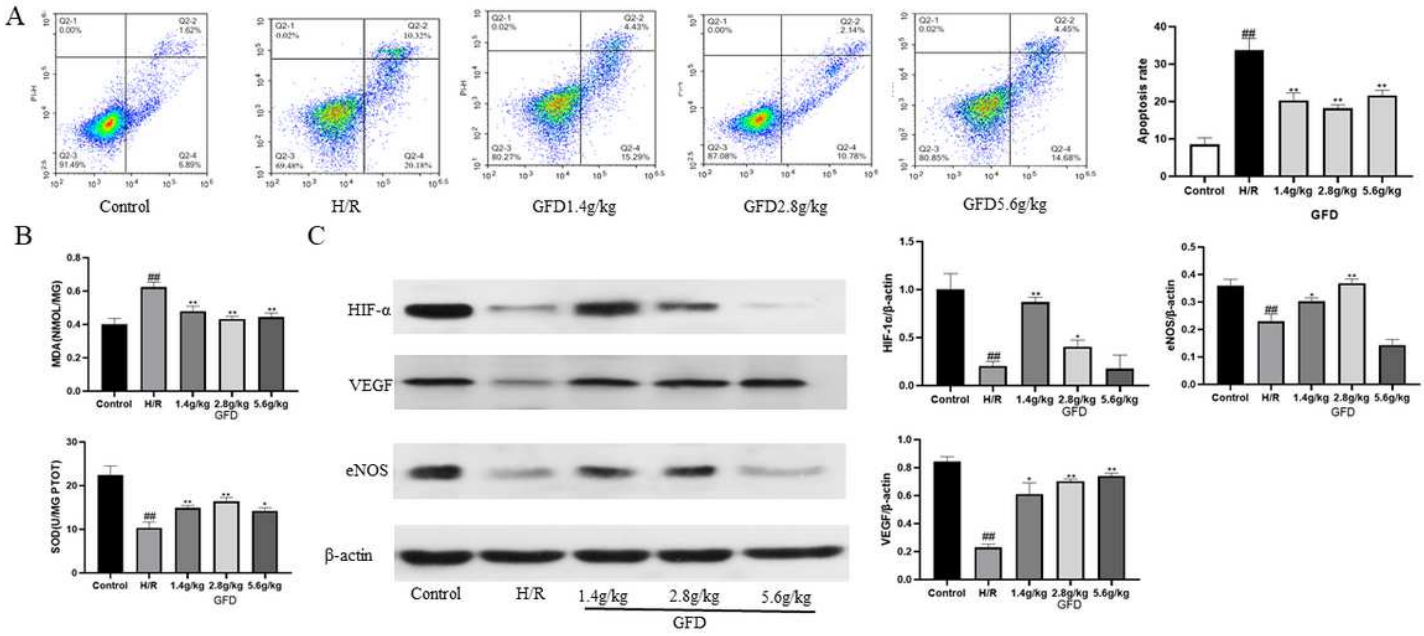

Figure 6

Effects of ganjiang fuzi decoction (GFD) on apoptosis, oxidative damage and expression of HIF-α signaling pathway in H/R injured rat vascular endothelial cells. (A) Effect of GFD on apoptosis of H/R injured rat vascular endothelial cells.(B) Effects of GFD on SOD and MDA expression in H/R injured rat vascular endothelial cells. (c) Effects of GFD on the expression of HIF-α, VEGF and eNOS in H/R injured rat vascular endothelial cells. Data were expressed as mean ± SD. N=5. #p< 0.05, ##p < 0.01 vs. control group. *p < 0.05, **p < 0.01 vs. H/R group.

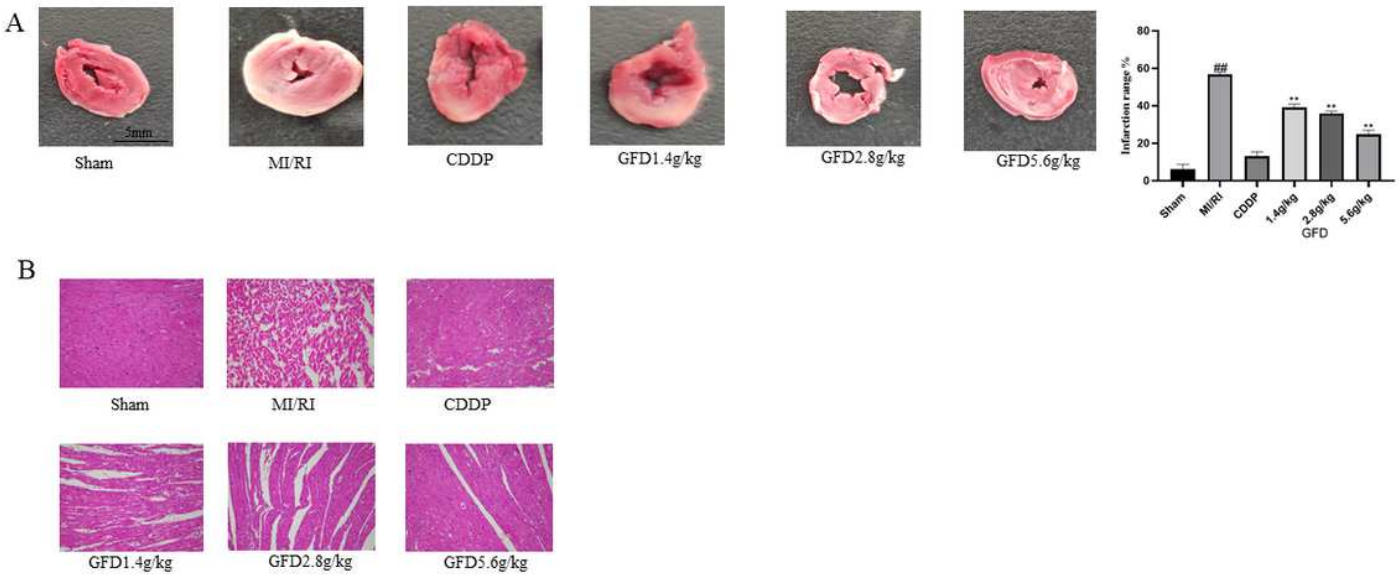

Figure 7

Protective effect of ganjiang fuzi decoction (GFD) on MI/RI myocardial tissue. (A) Effect of GFD on myocardial infarction size in MI/RI rats. White areas represent infarct areas, while red areas represent non-infarct areas (B)Representative myocardial tissue histopathological sections on the effects of GFD on myocardial infarction size in MI/RI rats (200×) , scale bar indicated 100 μm. Data were expressed as mean ± SD. N=3 #p< 0.05, ##p < 0.01 vs. Sham group. *p < 0.05, **p < 0.01 vs. MI/RI group.

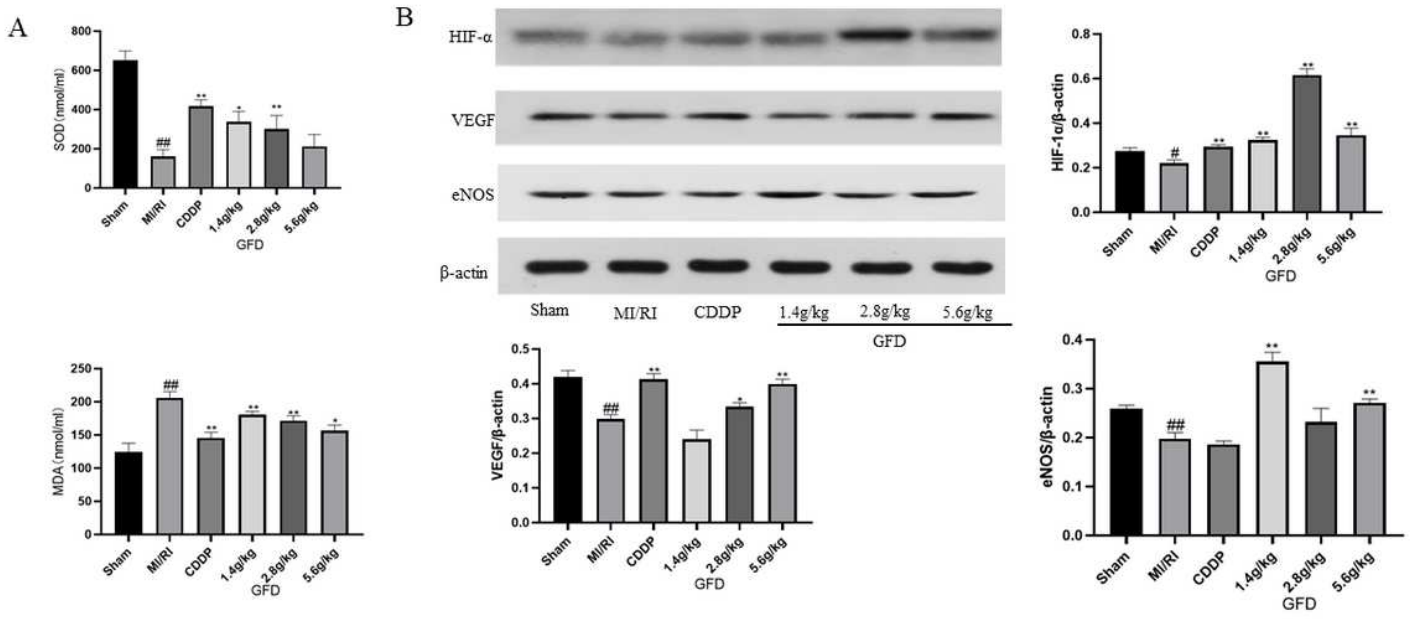

Figure 8

Effects of ganjiang fuzi decoction (GFD) on oxidative damage and expression of HIF-α signaling pathway in MI/RI rats. (A) Effects of GFD on the expression of SOD and MDA in serum of MI/RI rats. (B) Effects of GFD on the expression of HIF-α, VEGF and eNOS in vascular tissue of MI/RI rats. Data were expressed as mean ± SD. N=3. #p< 0.05, ##p < 0.01 vs. Sham group. *p < 0.05, **p < 0.01 vs. MI/RI group.

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