Granule of BU-XIN RUAN-MAI Attenuates the Patients’ Angina Pectoris of Coronary Heart Disease via Regulating miR-542-3p/GABARAP Signaling

Dong Yan,1,2 Li-li Zhao,2 Bo-wen Yue,2 Hui Qian,2 Zi-han Zhang,2 Ning Wang,2 Shi-hai Yan,1,2 and Yu-liang Qian1

1Department of Pharmacology, Affiliated Hospital of Nanjing University of TCM, Nanjing, China
2Nanjing University of TCM, Nanjing, China

Correspondence should be addressed to Shi-hai Yan; sea-y@qq.com and Yu-liang Qian; drqyl1972@163.com

Received 2 September 2019; Revised 15 October 2019; Accepted 23 October 2019

Objective. Coronary heart disease (CHD) has been regarded as a serious and common disease in the modern society. This study aims to investigate the effect of Granule of BU-XIN RUAN-MAI (BXRM) on angina pectoris of coronary heart disease and to explore the molecular mechanisms underlying Granule of BU-XIN RUAN-MAI-mediated protective activity against this disease.

Methods. The effects of Granule of BU-XIN RUAN-MAI on clinical symptoms of patients’ angina were indicated by hemorheology indicators including high shear of blood viscosity, low shear of blood viscosity, plasma viscosity, erythrocyte rigidity index, D-D dimer, fibrinogen content, and lipid content. The effects of Granule of BU-XIN RUAN-MAI on isoprenaline-induced myocardial cell injury were determined by conducting H&E staining and by performing ELISA to examine the serum content of MDA, SOD, Na+/K+-ATPase, cAMP, and the content of inflammatory factors in isoprenaline-induced rats. Meanwhile, western blot and real-time PCR were used to determine the expression of genes involved in oxidation and energy metabolism, and real-time PCR was also used for determination of miR-542-3p expression. Luciferase reporter assay was conducted to test the binding sites of miR-542-3p on GABARAP 3’UTR. The chemical compositions of Granule of BU-XIN RUAN-MAI were determined by liquid LC-QTOF-MS.

Results. Granule of BU-XIN RUAN-MAI significantly attenuated the clinical symptoms of patients’ angina by improving the patients’ heart rate and by decreasing the level of hemorheology indicators and also by reducing the serum content of TC, TG, LDL, and elevated HDL content. H&E staining demonstrated that Granule of BU-XIN RUAN-MAI ameliorated the myocardial ischemia in a dose-dependent manner. Besides, Granule of BU-XIN RUAN-MAI downregulated serum MDA content and upregulated the content of SOD, Na+/K+-ATPase, and cAMP in isoprenaline-induced rats. Granule of BU-XIN RUAN-MAI significantly improved oxidation stress by increasing PPARα expression and it inhibited inflammation by downregulating expression and contents of IL-6, IL-1β, and TNF-α. Then, Granule of BU-XIN RUAN-MAI-containing serum increased the MDA content in angiotensin II-stimulated HUVEC cells. The granule of BU-XIN RUAN-MAI-containing serum obviously downregulated protein expressions of P40phox, P47phox, and P67phox in plasma membrane, and it significantly increased protein levels of P40phox, P47phox, and P67phox in the cytoplasm of HUVEC cells. Furthermore, GABARAP was reduced in heart tissues of ISO-induced rats and in angiotensin II-stimulated cell lines, and GABARAP was required for the inhibitory activity of Granule of BU-XIN RUAN-MAI on oxidation and inflammation in vivo and in vitro. GABARAP could be upregulated by Granule of BU-XIN RUAN-MAI by inhibiting the expression of miR-542-3p, which may significantly enhance oxidation and inflammation by targeting GABARAP in cardiomyocytes. Moreover, the silencing of GABARAP could obviously reverse the granule of BU-XIN RUAN-MAI-mediated protective activity against coronary heart disease, and interfering GABARAP expression also could partly block the anti-miR-542-3p-controlled oxidation and inflammation in cardiomyocytes. Besides, salidroside, loganin, and polydatin were the main compounds of granules of BU-XIN RUAN-MAI.

Conclusion. Granule of BU-XIN RUAN-MAI is an excellent prescription for treatment of coronary heart disease by suppressing inflammation and NAPDH-mediated oxidative stress. The miR-542-3p/GABARAP axis is required for Granule of BU-XIN RUAN-MAI, exhibiting its protective activity against the pectoris of coronary heart disease.
1. Introduction

Coronary heart disease (CHD) has been regarded as a serious and common disease in the modern society [1]. A variety of pathophysiological dysfunctions has been observed in patients with coronary heart disease, including lipid content [2], oxidative stress, autonomic system dysfunction [3], inflammation [4], genetic susceptibility, smooth muscle hypercontraction [5], and endothelial dysfunction [6]. In recent years, although the western medical technology is used to reduce the mortality rate of acute coronary heart disease, it fails to improve the high incidence and poor prognosis of this disease. Angina pectoris is a major cause of disability worldwide, and angina pectoris is mainly caused by coronary artery disease or atherosclerosis [7]. Previous findings showed that there are treatment options for coronary artery disease, including medical treatment (cholesterol-lowering medications [8, 9], aspirin [10], beta blockers [11], ranolazin [12], nitroglycerin [13], calcium channel blockers [14]), coronary interventions (angioplasty and coronary stent), and coronary artery grafting [15].

Ancient traditional Chinese medicine (TCM) has been practiced for over 2,000 years [16], and modern Western medicine was introduced in the 19th century. Both the Chinese medicine and Western medicine have their own understanding and common grounds about human heart diseases and its treatment [17, 18]. In our opinion, a comprehensive analysis of the pathological situation is needed to diagnose syndrome of the coronary heart disease. Syndrome research has been a difficult and hot topic in traditional Chinese medicine because it is conducive to the management of this heart failure [19], and then the specific treatment of Chinese medicine could be prepared for patients.

In this study, the Granule of BU-XIN RUAN-MAI (BXRM) has been developed by professor Shu-Hua Tang, who is one of the fourth and fifth batch of instructors on traditional Chinese medicine. This granule of BU-XIN RUAN-MAI has gained the independent intellectual property rights of Chinese government, and it has been produced for improving the angina pectoris of coronary heart disease. He has devoted himself to traditional Chinese medicine for 50 years shaping him as an excellent and experienced master on coronary heart disease following Yellow Emperor’s Classic of Internal Medicine and JIN BIAN YAO LUE. In his thoughts, the key causes of angina pectoris are deficiency of both Qi and Yin and syndrome of stagnant-heat invading collaterals. During the formation of angina pectoris, asthenic cardiac Qi and Yin deficiency of heart and kidney always induce blood stagnation and blood stasis, failure of Qi-transforming fluid leading to phlegm aggregation and dysfunction of spleen in transportation resulting in phlegm turbidity. Combination blood stasis with phlegm can block the vein flow of patients. Finally, both Yin deficiency and blood stasis are going to develop endogenous heat in vivo. In accordance with these above cognition of Chinese medicine, Western medicine has demonstrated that coronary heart disease is a chronic inflammatory process (termed Yin deficiency with internal heat in the Chinese medicine), lipid accumulation (termed sputum in the Chinese medicine), and migration and proliferation of smooth muscle cells (termed blood stasis in the Chinese medicine) in Western medicine [2–6].

According to the above theories of coronary heart disease (angina pectoris) Chinese medicine, the Granule of BU-XIN RUAN-MAI was prepared for treatment of angina pectoris. This Granule of BU-XIN RUAN-MAI may nourish Qi and Yin to promote blood circulation for clearing internal heat and removing obstruction in collaterals. The Granule of BU-XIN RUAN-MAI contains *Rhodiola rosea* L., *Ophiopogon japonicas* (Linn. f.) Ker-Gawl., *Cornus officinalis* Sieb. et Zucc., *Whitmania pigra* Whitman, *Ginkgo biloba* L., and *Polygonum cuspidatum* Sieb. et Zucc. Importantly, products of these herbs in Western medicine exhibit perfect activity of anti-inflammation, antioxidative stress, and anti-CHD [20–31]. Consequently, the granule of BU-XIN RUAN-MAI may be considered as an effective treatment of heart diseases.

Interestingly, overexpression of GABARAP may improve the development of coronary heart disease by promoting autophagy which can attenuate the anginal pectoris of heart disease [32–44]. In our study, we investigated the effect of Granule of BU-XIN RUAN-MAI on clinical angina pectoris of coronary heart disease. Furthermore, we determined the molecular mechanism underlying Granule of BU-XIN RUAN-MAI-regulated GABARAP expression in the treatment of coronary heart disease. Additionally, the compositions of Granule of BU-XIN RUAN-MAI were detected by time-of-flight mass spectrometry.

2. Materials and Methods

2.1. Preparation of Buxin Ruanmai Granule. First, the Buxin Ruanmai (granule of BU-XIN RUAN-MAI) granule was provided by Jiang Yin Tian Jiang Pharmaceutical Corporation (Nanjing, Jiangsu, China). The granule of BU-XIN RUAN-MAI contains *Rhodiola rosea* L. (12 g), *Ophiopogon japonicas* (Linn. f.) Ker-Gawl. (20 g), *Cornus officinalis* Sieb. et Zucc. (12 g), *Whitmania pigra* Whitman (6 g), *Ginkgo biloba* L. (20 g), and *Polygonum cuspidatum* Sieb.et Zucc. (15 g). All the plants were mixed together and grinded into powder. Then, they were shaped into Granule of BU-XIN RUAN-MAI.

2.2. Clinical Participants. In our study, the clinical serum samples were collected. The patients in this study were collected according to the Western medicine diagnosed by the works “Diagnostic Criteria for Coronary Atherosclerotic Heart Disease (2010, in Chinese)” and “Practice internal medicine (2017, in Chinese).” Meanwhile, we also collected the patients following “Guideline for Clinical Study of Novel Chinese Medicine (third edition, 2002, in Chinese).” The patients were excluded if they met the following criteria: (1) the patients did not meet the above principles of Western and Chinese medicine; (2) they were confirmed that they...
Evidence-Based Complementary and Alternative Medicine

subjected to acute myocardial infarction, aortic dissection, cardiac valvular disease, cardiomyopathy, and other chest pain not caused by heart disease within half a year; (3) the patients with severe hypertension, diabetes, shock, arrhythmia, severe hepatic and renal dysfunction, hematopoietic system disorder, and psychotic disease; (4) the pregnant or lactating women; and (5) allergic constitution. Then, the patients were terminated if they met these criteria: (a) the patients were collected mistakenly; (b) the patients are not conducive to the judgment of drug efficacy; (c) the patients with incomplete personal information; (d) the patients were found with severe complications or exacerbations during treatment; (e) the patients were diagnosed with severe adverse events to treatment, or they were allergic to the treatment; and (f) the patients belonged to voluntary withdrawal from the trial. Finally, 40 patients were collected from March to December in 2015 (Department of Cardiology, Jiangsu Province Hospital of Traditional Chinese Medicine). They were divided into the placebo group and Granule of BU-XIN RUAN-MAI group randomly, with 20 patients for each group.

The patients in placebo group were treated with basic Western medicine following the Guideline for the American College of Physicians (2004). Other patients in the Granule of BU-XIN RUAN-MAI group were administrated with Granule of BU-XIN RUAN-MAI (traditional Chinese medicine) as well as the same Western medicine above. The Granule of BU-XIN RUAN-MAI was administrated at the dose of one packet/patient every 12h (per os). This Chinese medicine would be given until the end of the experiment (8 weeks). Then, we judged the treatment effectiveness, clinical syndrome score, and electrocardiogram of Granule of BU-XIN RUAN-MAI on patients with angina based on the items in “Diagnostic Criteria for Angina and Electrocardiogram in Coronary Heart Disease (1974, in Chinese)” and “Guideline for Clinical Study of Novel Chinese Medicine (third edition, 2002, in Chinese).”

2.3. Acute Myocardial Ischemia in Rats. The male Sprague-Dawley rats (200 ± 20 g) were purchased from Nanjing University, China. With free access to food and water, they were kept in a clean cage with 12 h/12 h light/dark cycles and at a temperature of 23 ± 1°C. This subject was approved by Jiangsu Province Hospital of Traditional Chinese Medicine. All the experimental rats were housed under the indicated conditions for four days’ acclimation. Then, myocardial ischemia of rats was caused by subcutaneous injection of isoproterenol hydrochloride (ISO, 30 mg/kg body weight), dissolved in physiologic saline, for two consecutive days [45, 46]. The animals were sacrificed on the 6th day of experiment. 60 rats were put into 6 groups randomly: (1) normal control (Sham group, 5% stroke-physiological saline solution, i.g.); (2) the model group (ISO injection only, 5% stroke-physiological saline solution, i.g.); (3) the positive group (valsartan, 0.027 g/kg body weight, i.g. for 6 day) and this chemical was purchased from Sigma (Shanghai, China); (4) the model group of BU-XIN RUAN-MAI groups: each group administered with 29.2 g/kg, 14.6 g/kg and 7.2 g/kg Granule of BU-XIN RUAN-MAI i.g., for 6 days. The rats were anesthetized with diethyl ether, and the blood samples were collected from the femoral arteries. The serum was inactivated at 56°C for 30 min, and then it was filtrated with 0.22 μm membrane for sterilization (Sangon, Shanghai, China). The serum was preserved at −80°C following centrifugation at 4°C at 3500 rpm for 20 min.

2.4. Cell Culture and Angiotensin II-Stimulated HUVEC and H9C2 Cell Model. The cultured HUVEC or H9C2 cells (National Infrastructure of Cell Line Resource, Beijing, China) were incubated with 10−6 M angiotensin II (Sigma, Shanghai, China) for 4 h to establish the cell model of NADPH-mediated oxidative stress. Then, the cells were treated with the indicated concentration of the granule of BU-XIN RUAN-MAI-containing serum for 24 h. The cells and the corresponding medium were collected for further Western analysis, ELISA assay, and real time PCR.

2.5. Granule of BU-XIN RUAN-MAI-Containing Serum Preparation. The 30 rats were divided into 3 groups: (1) the sham group (normal control, 5% stroke-physiological saline solution, i.g.); (2) the positive group (valsartan, 0.027 g/kg body weight, i.g. for 6 day) and this chemical was purchased from Sigma (Shanghai, China); (3) the Granule of BU-XIN RUAN-MAI groups: each group administered with 29.2 g/kg, 14.6 g/kg and 7.2 g/kg Granule of BU-XIN RUAN-MAI i.g., for 6 days.

2.6. Histology. The myocardial tissues were fixed in 10% parafomaldehyde buffer. The specimens were embedded in paraffin blocks, and they were subjected to sectioning and hematoxylin-eosin (H&E) staining for histological analysis using standard protocols performed as previously described [47].

2.7. The Enzyme-Linked Immunosorbent Assay. The effects of Granule of BU-XIN RUAN-MAI on contents of MDA, SOD, Na+/K+-ATPase, cAMP, TNF-α, IL-1β and IL-6, and TC, TG, LDL, HDL, hs-CRP, and D-Dimer, fibrinogen in serum of patients or animals, or in cytoplasm of HUVEC cells were determined with enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s instructions (R&D Systems, Inc., 614 Mickinley Placene, MN, USA) [48].

2.8. MTT Assay. The cell viability or cell proliferation was determined by 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan Thiazolyl blue formazan (MTT) assay. Granule of BU-XIN RUAN-MAI has been used in China
for many years. Granule of BU-XIN RUAN-MAI-containing serum was prepared by our lab in Traditional Chinese Medicine of Nanjing University, and the details were showed in Section 2.5. The prepared Granule of BU-XIN RUAN-MAI-containing serum was diluted in DMEM at 1:1, 1:10, 1:100, and 1:1000. The HUVEC cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 0.1 mL DMEM and were exposed to increasing concentrations of Granule of BU-XIN RUAN-MAI-containing serum at the indicated concentrations in this study for 24 h. The control cells were incubated with Granule of BU-XIN RUAN-MAI-free serum. Cell viability was determined by the MTT assay (cat. no. 57360-69-7, Sigma-Aldrich, Merck KGaA). MTT was dissolved in DMSO at 5 mg/mL, and the cells were cultured in an incubator for 4 h. The absorbance of the samples was measured using a microtiter plate reader at OD490 nm and OD655 nm.

2.9. Dual-Luciferase Reporter Assays. At the beginning, 6×10^4 cells (HUVEC or H9C2) per well were seeded in 24-well plates. The cells were cotransfected with pmirGLO-GABARAP-3′-UTR WT (wide type) or pmirGLO-GABARAP-3′-untranslated region (UTR)-mut (mutant), reporter plasmids and Mock (negative control), miR-542-3p mimics, and anti-miR-542-3p. After 24 h transfection, we determined luciferase activity using the Dual-Luciferase Assay Kit on GloMax 20/20 Luminometer (Promega, Madison, USA).

2.10. Western Blotting. To prepare total protein, the myocardial tissues of model animals or HUVEC cells were lysed for 30 min with lysis buffer (20 mM sucrose, 1 mM EDTA, 20 μM Tris-Cl pH 7.2, 1 mM DTT, 1 mM KCl 1.5 mM MgCl2, 5 μg/mL peptatin A, 10 μg/mL leupeptin, and 2 μg/mL aprotinin). Additionally, we isolated P40phox, P47phox, and P67phox proteins in plasma membrane or cytoplasm of HUVEC cells using the kit from Sangon (Membrane, Nuclear and Cytoplasmic Protein Extraction kit, cat. no. C510002, Shanghai, China). Briefly, the cells were washed with PBS, and they were centrifuged to collect for further use. The cells were suspended with CER A buffer, and they were incubated on ice for 15 min, and then the suspension was added with CER B buffer for following incubation on ice. They were vortexed for 5 seconds, and then they were centrifuged at 15,000 g at 4°C for 15 min to prepare the cytoplasmic proteins in a precooling tube. After removing the supernatant, the precipitate was suspended with NER buffer incubated on the ice for 15 min to prepare the nuclear proteins (supernatant) into a new precooling tube. All these isolated protein samples were stored at −80°C.

Then, 40 μg protein for each sample was used for electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels. The protein bands were transferred to PVDF membrane, and these membranes were incubated overnight at 4°C with the primary antibodies and then incubated with the HRP-carried secondary antibodies following by enhanced chemiluminescence detection. Importantly, the optical density of western blot bands was analyzed by ImageJ software. The enhanced chemiluminescence reagents were bought from Pierce Biotechnology (Rockford, IL, USA). The primary antibodies against GAPDH, PPARα, GABARAP, Beclin-1, and LC3-II antibodies were got from Cell Signaling Technology (Danvers, MA, USA).

2.11. Real-Time PCR Analysis. Total RNA was extracted using TRIzol reagent, and cDNA was produced using the Superscript III RT kit, and real-time PCR was carried out using the SYBR Green PCR Master Mix in an ABI 7500 thermal cycler. All these agents were bought from Thermo Fisher Scientific, Inc. The primers (Genscript Co., Ltd., Nanjing, China) were as follows: GAPDH (human): forward, 5′-CACCATCTCCAAGCAGAGAG-3′ and reverse, 5′-GCAAAGTGGCTATCTG-3′; GAPDH (Rattus norvegicus): forward, 5′-AGTGCAGACCTGTCATGCA-3′ and reverse, 5′-GCAGGCCGATAAGAACCTG-3′; IL-1β (human): forward, 5′-CATGGTCTGTTGTTGTGTTG-3′ and reverse, 5′-CTTCCGAGAGGCTGTTGAC-3′; IL-1β (Rattus norvegicus): forward, 5′-AGGCTCACTGCCGCTCATT-3′ and reverse, 5′-TCACGGAGAGCTGTTGAC-3′; and PPARα 5′-AGCCTGGTGTGTGAGGC-3′ and reverse, 5′-GCAAAGTGGCTATCTG-3′. In this study, the OD490 nm and OD655 nm.

2.9. Dual-Luciferase Reporter Assays. At the beginning, 6×10^4 cells (HUVEC or H9C2) per well were seeded in 24-well plates. The cells were cotransfected with pmirGLO-GABARAP-3′-UTR WT (wide type) or pmirGLO-GABARAP-3′-untranslated region (UTR)-mut (mutant), reporter plasmids and Mock (negative control), miR-542-3p mimics, and anti-miR-542-3p. After 24 h transfection, we determined luciferase activity using the Dual-Luciferase Assay Kit on GloMax 20/20 Luminometer (Promega, Madison, USA).

2.10. Western Blotting. To prepare total protein, the myocardial tissues of model animals or HUVEC cells were lysed for 30 min with lysis buffer (20 mM sucrose, 1 mM EDTA, 20 μM Tris-Cl pH 7.2, 1 mM DTT, 10 mM KCl 1.5 mM MgCl2, 5 μg/mL peptatin A, 10 μg/mL leupeptin, and 2 μg/mL aprotinin). Additionally, we isolated P40phox, P47phox, and P67phox proteins in plasma membrane or cytoplasm of HUVEC cells using the kit from Sangon (Membrane, Nuclear and Cytoplasmic Protein Extraction kit, cat. no. C510002, Shanghai, China). Briefly, the cells were washed with PBS, and they were centrifuged to collect for further use. The cells were suspended with CER A buffer, and they were incubated on ice for 15 min, and then the suspension was added with CER B buffer for following incubation on ice. They were vortexed for 5 seconds, and then they were centrifuged at 15,000 g at 4°C for 15 min to prepare the cytoplasmic proteins in a precooling tube. After removing the supernatant, the precipitate was suspended with NER buffer incubated on the ice for 1 h. Finally, these samples were centrifuged at 15,000 g at 4°C for 15 min to prepare the nuclear proteins (supernatant) into a new precooling tube. All these isolated protein samples were stored at −80°C.

Then, 40 μg protein for each sample was used for electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels. The protein bands were transferred to PVDF membrane, and these membranes were incubated overnight at 4°C with the primary antibodies and then incubated with the HRP-carried secondary antibodies following by enhanced chemiluminescence detection. Importantly, the optical density of western blot bands was analyzed by ImageJ software. The enhanced chemiluminescence reagents were bought from Pierce Biotechnology (Rockford, IL, USA). The primary antibodies against GAPDH, PPARα, GABARAP, Beclin-1, and LC3-II antibodies were got from Cell Signaling Technology (Danvers, MA, USA).

2.11. Real-Time PCR Analysis. Total RNA was extracted using TRIzol reagent, and cDNA was produced using the Superscript III RT kit, and real-time PCR was carried out using the SYBR Green PCR Master Mix in an ABI 7500 thermal cycler. All these agents were bought from Thermo Fisher Scientific, Inc. The primers (Genscript Co., Ltd., Nanjing, China) were as follows: GAPDH (human): forward, 5′-CACCATCTCCAAGCAGAGAG-3′ and reverse, 5′-GCAAAGTGGCTATCTG-3′; GAPDH (Rattus norvegicus): forward, 5′-AGTGCAGACCTGTCATGCA-3′ and reverse, 5′-GCAGGCCGATAAGAACCTG-3′; IL-1β (human): forward, 5′-CATGGTCTGTTGTTGTGTTG-3′ and reverse, 5′-CTTCCGAGAGGCTGTTGAC-3′; IL-1β (Rattus norvegicus): forward, 5′-AGGCTCACTGCCGCTCATT-3′ and reverse, 5′-TCACGGAGAGCTGTTGAC-3′; and PPARα 5′-AGCCTGGTGTGTGAGGC-3′ and reverse, 5′-GCAAAGTGGCTATCTG-3′. In this study, the
2.12. Statistical Analysis. All the data are showed as the mean ± standard deviation. We compared two groups using Student’s t-test, and the comparisons among multiple groups were performed with one-way analysis of variance followed by Tukey’s honestly significant difference test. Finally, we used SPSS 17.0 software to conduct statistical analyses (SPSS Inc., Chicago, IL, USA), and $P < 0.05$ was significant.

3. Result

3.1. The Chemical Compounds in Granule of BU-XIN RUAN-MAI and Granule of BU-XIN RUAN-MAI Alleviate the Clinical Symptoms of Angina Pectoris Diagnosed by Expert of Chinese Medicine. In this study, we observed that salidroside (275 nm), loganin (240 nm), polydatin (306 nm), resveratrol (306 nm), and emodin (280 nm) were contained in Granule of BU-XIN RUAN-MAI by using high-performance liquid chromatography (HPLC). In this study, 40 patients were collected from Jiangsu Province Hospital of TCM (traditional Chinese medicine). There were 50.00% males (10) and 50.00% (10) females in the placebo group, and 40.00% (8) males and 60.00% (12) females in the Granule of BU-XIN RUAN-MAI group before treatment (Table 1). They were randomly divided into the placebo and Granule of BU-XIN RUAN-MAI group, with 20 patients for each group. Patients’ age and sex of the two groups showed no statistical difference (Table 1).

To determine the effect of Granule of BU-XIN RUAN-MAI on treatment of patients with angina pectoris, the patients in the placebo group (also named as the control group) were administrated with Western medicine, and the patients in the Granule of BU-XIN RUAN-MAI group were administrated with both Granule of BU-XIN RUAN-MAI and the same Western medicine. The total effective percentage was 90% when patients were administrated with both Western medicine and Granule of BU-XIN RUAN-MAI (Table 2). However, the total percentage was 65.00% when patients were treated with Western medicine alone (Table 2). Consequently, Granule of BU-XIN RUAN-MAI may obviously improve the angina pectoris of patients.

Then, no statistical difference existed in symptom scores of pretreatment between placebo and Granule of BU-XIN RUAN-MAI groups (Table 3). The symptom score of posttreatment patients was significantly reduced in the placebo group as well as in the Granule of BU-XIN RUAN-MAI group ($\Delta P < 0.05$ versus the pretreatment patients in the placebo or Granule of BU-XIN RUAN-MAI group) (Table 3). Meanwhile, significant difference was found in symptom scores of posttreatment between the placebo and Granule of BU-XIN RUAN-MAI groups ($\Delta P < 0.05$ versus the placebo group after treatment) (Table 3). Thus, Granule of BU-XIN RUAN-MAI may be a good choice for treatment of the patients.

3.2. Granule of BU-XIN RUAN-MAI Improves Patients’ Heart Rate. To evaluate the effect of Granule of BU-XIN RUAN-MAI on patients’ heart rate, the electrocardiogram was determined. The total effective percentage was 70.00% in the placebo group, and the total effective rate was 95% in the Granule of BU-XIN RUAN-MAI group (Table 4). The clinical curative effect of angina pectoris between the placebo and Granule of BU-XIN RUAN-MAI groups was statistically significant (Table 4). Therefore, Granule of BU-XIN RUAN-MAI may obviously better the heart rate of patients.

3.3. Effect of Granule of BU-XIN RUAN-MAI on Patients’ Serological Indicators. No difference was found in the blood lipid level between the placebo and Granule of BU-XIN RUAN-MAI group before treatment was administrated ($P > 0.05$) (Table 5). Compared with the pretreatment patients, TC, TG, LDL, and HDL contents were significantly lower in posttreatment patients of the placebo or Granule of BU-XIN RUAN-MAI groups (Table 5). Compared with the posttreatment patients in placebo group, the TC, TG, LDL, and HDL contents were significantly reduced in the Granule of BU-XIN RUAN-MAI-administrated group (Table 5).

Furthermore, no difference was found in hemorheology indicators between the placebo and Granule of BU-XIN RUAN-MAI group before treatment was performed ($P > 0.05$) (Table 6). Compared with the pretreatment patients in the placebo, high shear of blood viscosity, low shear of blood viscosity, plasma viscosity, and erythrocyte rigidity index were downregulated when treatment was administrated ($\Delta P < 0.05$) (Table 6). Compared with the posttreatment patients in the placebo group, all the indicators were significantly reduced in the Granule of BU-XIN RUAN-MAI group (Table 6).

Next, no difference was found about hs-CRP content between the placebo and Granule of BU-XIN RUAN-MAI group before treatment was administrated ($P > 0.05$) (Table 7). Compared with the pretreatment patients, the hs-CRP content was significantly downregulated after treatment in the placebo or Granule of BU-XIN RUAN-MAI group, respectively ($\Delta P < 0.05$) (Table 7). Compared with the posttreatment patients in the placebo group, the hs-CRP content was significantly decreased in the Granule of BU-XIN RUAN-MAI-administrated patients ($\Delta P < 0.05$) (Table 7).

In order to determine the patients’ coagulation, we found that compared with the pretreatment patients in the Granule of BU-XIN RUAN-MAI groups, the D-Dimer and fibrinogen contents were downregulated after treatment ($\Delta P < 0.05$), and compared with the pretreatment patients, the D-Dimer and fibrinogen contents were also significantly decreased in the placebo groups ($\Delta P < 0.05$) (Table 8). Moreover, in the Granule of BU-XIN RUAN-MAI-administrated group, the D-Dimer and fibrinogen contents were significantly downregulated compared with the placebo group ($\Delta P < 0.05$) (Table 8).

3.4. Granule of BU-XIN RUAN-MAI Attenuates the Oxidation and Inflammation in Isoprenaline-Caused Myocardial Ischemia of Rats. To test the effect of Granule of BU-XIN RUAN-MAI on myocardial cell injury caused by isoprenaline, H&E staining was used. The results showed that the myocardial cells were seriously injured in cardiac
muscles of isoprenaline-induced rats (Figure 1(a)). Importantly, valsartan may significantly inhibit the myocardial cell injury, and Granule of BU-XIN RUAN-MAI also ameliorated this dysfunction at a dose-dependent manner (Figure 1(a)). Moreover, Granule of BU-XIN RUAN-MAI as well as valsartan obviously downregulated the MDA content.

Table 1: Patients’ sex and age.

| Group               | Number of cases | Male  | Female | Average age  |
|---------------------|-----------------|-------|--------|--------------|
| Placebo             | 20              | 10    | 10     | 66.75 ± 8.10 |
| Granule of BU-XIN RUAN-MAI | 20         | 8     | 12     | 64.30 ± 9.69 |

The statistical difference between the placebo and Granule of BU-XIN RUAN-MAI group (BXRM) was tested by Student’s t test ($P > 0.05$ versus the placebo group).

Table 2: Clinical curative effect of Granule of BU-XIN RUAN-MAI on angina pectoris diagnosed by experts of Chinese medicine.

| Group               | Number of cases | Excellence | Improvement | Failure | Effective percentage (%) |
|---------------------|-----------------|------------|-------------|---------|--------------------------|
| Placebo             | 20              | 2          | 11          | 7       | 65.00                    |
| Granule of BU-XIN RUAN-MAI | 20         | 5          | 13          | 2       | 90.00                    |

The clinical curative effect of angina pectoris in the two groups was statistically significant ($\Delta P < 0.05$ versus the placebo group).

Table 3: Effect of Granule of BU-XIN RUAN-MAI on clinical syndrome score evaluated by experts of Chinese medicine.

| Group               | Number of cases | Symptom score of pretreatment | Symptom score of posttreatment |
|---------------------|-----------------|------------------------------|--------------------------------|
| Placebo             | 20              | 23.70 ± 5.13                 | 17.75 ± 3.06                   |
| Granule of BU-XIN RUAN-MAI | 20         | 23.35 ± 4.54                 | 10.15 ± 1.76                   |

In this table, there was no statistical difference in symptom scores of pretreatment between two groups (Student’s t test, $P > 0.05$). The $\# P < 0.05$ versus the pretreatment patients in the placebo or Granule of BU-XIN RUAN-MAI group; $\Delta P < 0.05$ versus posttreatment patients in the placebo group.

Table 4: Effect of Granule of BU-XIN RUAN-MAI on patients’ heart rate evidenced by electrocardiogram.

| Group               | Number of cases | Excellence | Improvement | Failure | Heart rate (%) |
|---------------------|-----------------|------------|-------------|---------|----------------|
| Placebo             | 20              | 4          | 10          | 6       | 70.00          |
| Granule of BU-XIN RUAN-MAI | 20         | 6          | 13          | 1       | 95.00\#        |

The clinical curative effect of angina pectoris in the two groups was statistically significant (Student’s t test); $\Delta P < 0.05$ versus the placebo group.

Table 5: Effect of Granule of BU-XIN RUAN-MAI on patients’ blood lipid content.

| Group               | Number of cases | Stage       | TC      | TG      | LDL    | HDL    |
|---------------------|-----------------|-------------|---------|---------|--------|--------|
| Placebo             | 20              | Pretreatment| 5.91 ± 0.73 | 2.11 ± 0.13 | 3.94 ± 0.68 | 0.87 ± 0.12 |
| Granule of BU-XIN RUAN-MAI | 20         | Posttreatment| 4.67 ± 0.75\# | 1.64 ± 0.15\# | 3.24 ± 0.65\# | 0.56 ± 0.13\# |
|                     | 20              | Pretreatment| 5.95 ± 0.76 | 2.12 ± 0.14 | 3.92 ± 0.67 | 0.73 ± 0.11 |
| Granule of BU-XIN RUAN-MAI | 20         | Posttreatment| 4.10 ± 0.45\# | 1.09 ± 0.13\# | 2.58 ± 0.44\# | 0.39 ± 0.08\# |

There was no significant difference in blood lipid level between the two groups before treatment was administrated (Student’s t test, $P > 0.05$). $\# P < 0.05$ versus the pretreatment patients in the placebo or Granule of BU-XIN RUAN-MAI group; $\Delta P < 0.05$ versus posttreatment patients in the placebo group.

Table 6: Effect of Granule of BU-XIN RUAN-MAI on hemorheology indicators in patents.

| Group               | Number of cases | Stage       | High shear of blood viscosity | Low shear of blood viscosity | Plasma viscosity | Erythrocyte rigidity index |
|---------------------|-----------------|-------------|-------------------------------|-------------------------------|-----------------|---------------------------|
| Placebo             | 20              | Pretreatment| 4.52 ± 0.24                   | 7.99 ± 0.95                   | 1.57 ± 0.17     | 7.84 ± 0.51               |
| Granule of BU-XIN RUAN-MAI | 20         | Posttreatment| 3.87 ± 0.27\#                | 7.14 ± 0.89\#                | 1.52 ± 0.16\#  | 7.02 ± 0.59\#            |
|                     | 20              | Pretreatment| 4.56 ± 0.26                   | 8.24 ± 0.98                   | 1.61 ± 0.16     | 7.73 ± 0.58               |
| Granule of BU-XIN RUAN-MAI | 20         | Posttreatment| 3.25 ± 0.17\#\#              | 6.53 ± 0.87\#\#              | 1.22 ± 0.09\#\# | 5.98 ± 0.43\#\#          |

There was no significant difference in hemorheology indicators between the placebo and Granule of BU-XIN RUAN-MAI groups before treatment was administrated (Student’s t test, $P > 0.05$). $\# P < 0.05$ versus the pretreatment patients in the placebo or Granule of BU-XIN RUAN-MAI group; $\Delta P < 0.05$ versus the posttreatment patients in the placebo group.
Table 7: Effect of Granule of BU-XIN RUAN-MAI on hs-CRP level in patents.

| Group                     | Number of cases | Stage      | hs-CRP content |
|---------------------------|-----------------|------------|---------------|
| Placebo                   | 20              | Pretreatment | 5.49 ± 0.86   |
|                           | 20              | Posttreatment | 3.76 ± 0.34   |
| Granule of BU-XIN RUAN-MAI| 20              | Pretreatment | 5.35 ± 1.08   |
|                           | 20              | Posttreatment | 2.82 ± 0.39   |

There was no significant difference in hs-CRP content between the two groups before treatment was administrated (Student’s t test, \( P > 0.05 \)). \(^a P < 0.05\) versus the pretreatment patients in the placebo or Granule of BU-XIN RUAN-MAI group; \(^b P < 0.05\) versus the posttreatment patients in the placebo group.

Table 8: Effect of Granule of BU-XIN RUAN-MAI on indicators of patients’ coagulation.

| Group                     | Number of cases | Stage      | D-Dimer | Fibrinogen |
|---------------------------|-----------------|------------|---------|------------|
| Placebo                   | 20              | Pretreatment | 2.03 ± 1.12 | 3.97 ± 1.13 |
|                           | 20              | Posttreatment | 1.23 ± 0.86 \(^a\) | 2.57 ± 1.27 \(^a\) |
| Granule of BU-XIN RUAN-MAI| 20              | Pretreatment | 2.09 ± 1.08 | 4.01 ± 1.22 |
|                           | 20              | Posttreatment | 0.66 ± 0.42 \(^a\) | 1.83 ± 0.68 \(^a\) |

There was no significant difference in patients’ coagulation indicator (D-Dimer and fibrinogen) contents between the placebo and Granule of BU-XIN RUAN-MAI group before treatment was administrated (\( P > 0.05 \)). \(^a P < 0.05\) versus the pretreatment patients in the placebo or Granule of BU-XIN RUAN-MAI group; \(^b P < 0.05\) versus the posttreatment patients in the placebo group.

Figure 1: Continued.
Figure 1: Continued.
in serum (Figure 1(b)). We also found that Granule of BU-XIN RUAN-MAI can increase the SOD, Na⁺/K⁺-ATPase, and cAMP contents in serum of isoprenaline-induced rats (Figure 1(b)).

On the other hand, Granule of BU-XIN RUAN-MAI significantly upregulated the PPArα protein expression and mRNA expression in myocardial ischemia of rats caused by isoprenaline (Figures 1(c)–1(e)). Granule of BU-XIN RUAN-MAI showed anti-inflammation activity in vivo. Granule of BU-XIN RUAN-MAI remarkably elevated the IL-6, IL-1β, and TNF-α contents evidenced by ELISA (Figures 1(f)–1(h)). Additionally, Granule of BU-XIN RUAN-MAI increased the IL-6, IL-1β, and TNF-α mRNA expression (Figures 1(i)–1(k)).

3.5. *Granule of BU-XIN RUAN-MAI Improves the Angiotensin II-Stimulated Oxidative Stress in HUVEC Cells.* To determine the effect of Granule of BU-XIN RUAN-MAI-containing serum on cell oxidation and cell energy metabolism, the HUVEC cells were stimulated by angiotensin II. Firstly, the indicated concentration of Granule of BU-XIN RUAN-MAI-containing serum as well as valsartan exhibited no toxicity on HUVEC cells (Figure 2(a)). Granule of BU-XIN RUAN-MAI-containing serum and valsartan may significantly increase the SOD content and reduce the MDA content in angiotensin II-stimulated HUVEC cells (Figure 2(b)). Proteins including P40phox, P47phox, and P67phox were isolated from plasma membrane or cytoplasm of HUVEC cells using the protein extraction kit from Sangon (Membrane, Nuclear and Cytoplasmic Protein Extraction kit, cat. no. CS10002, Shanghai, China). Secondly, Granule of BU-XIN RUAN-MAI-containing serum obviously downregulated P40phox, P47phox, and P67phox protein levels in the plasma membrane of HUVEC cells (Figure 2(c)). Expectedly, Granule of BU-XIN RUAN-MAI-containing serum significantly restored protein expressions of P40phox, P47phox, and P67phox in the cytoplasm of HUVEC cells (Figure 2(d)).

3.6. *GABARAP Is Required for the Inhibitory Activity of Granule of BU-XIN RUAN-MAI on Oxidation and Inflammation In Vivo and In Vivo.* To investigate the role of GABARAP in Granule of BU-XIN RUAN-MAI-mediated suppression of oxidation and inflammation, we knocked down the GABARAP expression in rat and in HUVEC cells, and then the cells were incubated with Granule of BU-XIN RUAN-MAI. Firstly, GABARAP was decreased in the heart of ISO-induced rats, whereas it was upregulated by Granule of BU-XIN RUAN-MAI (Figures 3(a)–3(c)). Similarly, GABARAP was reduced in angiotensin II-induced HUVEC cells; however, it was upregulated by Granule of BU-XIN RUAN-MAI (Figure 3(d)). Moreover, knockdown of GABARAP could significantly block the Granule of BU-XIN RUAN-MAI-controlled upregulation of PPArα in ISO-induced rats and in angiotensin II-incubated HUVEC cells (Figures 3(e)–3(g)). Meanwhile, suppression of GABARAP could obviously block the Granule of BU-XIN RUAN-MAI-regulated gene expression of inflammatory factors including IL-6, IL-1β, and TNF-α.
Figure 2: Effect of Granule of BU-XIN RUAN-MAI-containing serum on antioxidation and energy metabolism in angiotensin II-stimulated HUVEC cells. (a) The effect of Granule of BU-XIN RUAN-MAI on HUVEC cell viability. Data shown are mean ± SD; \#P < 0.001 versus the placebo group. (b) Effect of Granule of BU-XIN RUAN-MAI-containing serum on the SOD content and MDA content in angiotensin II-stimulated HUVEC cells; data shown are mean ± SD; \#P < 0.001 versus the sham group and \#P < 0.05 versus the model group. (c) Effect of Granule of BU-XIN RUAN-MAI-containing serum on energy metabolism indicators (P40phox, P47phox, and P67phox) at the cell membrane detected by western blot in angiotensin II-incubated HUVEC cells and the corresponding semi-quantitative analysis was based on optical density with ImageJ software; data shown are mean ± SD; \#P < 0.001 versus the sham group and \#P < 0.05 versus the model group. (d) Effect of Granule of BU-XIN RUAN-MAI-containing serum on energy metabolism indicators (P40phox, P47phox, and P67phox) in the cytoplasm detected by western blot in angiotensin II-incubated HUVEC cells, and the corresponding semi-quantitative analysis was based on optical density with ImageJ software; data shown are mean ± SD; \#P < 0.001 versus the sham group and \#P < 0.05 versus the model group. The data are representative of three independent experiments.
**Figure 3: Continued.**

- (a) GABARAP mRNA level (fold change) in Rat.
  - (b) GABARAP mRNA level (fold change) in HUVEC.
  - (c) GABARAP mRNA level (fold change) in Rat (AAV).
  - (d) GABARAP mRNA level (fold change) in HUVEC.
  - (e) PPARα mRNA level (fold change) in Rat.
  - (f) PPARα mRNA level (fold change) in HUVEC.
  - (g) IL-1β mRNA level (fold change) in Rat.
  - (h) IL-6 mRNA level (fold change) in HUVEC.
  - (i) TNFα mRNA level (fold change) in HUVEC.

Legend:
- Black: Vector
- Red: sh-GABARAP

Groups:
- Sham
- Placebo
- Valsartan
- BXRM(H)
- BXRM(L)
- AAV-vector
- sh-GABARAP

**Evidence-Based Complementary and Alternative Medicine**
in rat and HUVEC cells (Figures 3(h)–3(m)). Finally, down-regulation of GABARAP could significantly block the Granule of BU-XIN RUAN-MAI-controlled expression of oxidative stress-associated gene SOD1 in vivo and in vitro (Figures 3(n) and 3(o)).

3.7. miR-542-3p Is Involved in Granule of BU-XIN RUAN-MAI-Induced Upregulation of GABARAP Expression. The above data showed that Granule of BU-XIN RUAN-MAI could significantly attenuate oxidation and inflammation in coronary heart disease by upregulating GABARAP expression. However, the regulatory molecular mechanism underlying the Granule of BU-XIN RUAN-MAI-controlled GABARAP expression remains unknown. In this study, we found that Granule of BU-XIN RUAN-MAI significantly upregulated GABARAP expression by stabilizing its 3′UTR (Figure 4(a)). In this study, GABARAP might be targeted by miR-542-3p (Figure 4(b)). Further data revealed that miR-542-3p was increased in the heart of ISO rats and in angiotensin-incubated HUVEC cells, while its level was suppressed in Granule of BU-XIN RUAN-MAI (Figures 4(c) and 4(d)). More importantly, GABARAP was upregulated by Granule of BU-XIN RUAN-MAI partly depending on miR-542-3p (Figure 4(e)). Next, miR-542-3p significantly inhibited luciferase activity of GABARAP 3′UTR, whereas suppression of miR-542-3p could enhance the luciferase activity of GABARAP 3′UTR (Figures 4(f) and 4(g)). Consistently, overexpression of miR-542-3p markedly suppressed GABARAP expression both at the mRNA and protein levels (Figures 4(h) and 4(i)).

3.8. miR-542-3p Promotes Oxidation and Inflammation by Targeting GABARAP in Cell Lines. To explore the role of miR-542-3p/GABARAP axis in coronary heart disease, we
Binding site of miR-542-3p on GABARAP 3'UTR

Mutant of GABARAP 3'UTR

Position 252-268 of GABARAP 3' UTR 5' ...UUGAUUGUCAGUCUGU... 3'

hsa-miR-542-3p

Mutant of GABARAP 3'UTR

Position 247-254 of GABARAP 3' UTR 5' ...CUGGAUGUGUGUGUGUCUCAGU... 3'

mo-miR-542-3p

Figure 4: Continued.
examined the effects of miR-542-3p/GABARAP axis on oxidation and inflammation (Figure 5). Firstly, knockdown of miR-542-3p could significantly reverse angiotensin II-induced downregulation of oxidation indicator PPARα, whereas silencing of GABARAP obviously blocked anti-miR-542-3p-controlled PPARα expression in H9C2 and HUVEC cells (Figures 5(a)–5(c)). Then, interfering miR-542-3p suppressed angiotensin II-mediated upregulation of inflammatory factors including IL-6, IL-1β, and TNFα, while knockdown of GABARAP markedly reversed these miR-542-3p-induced gene expressions (Figures 5(d)–5(i)). Similarly, silencing of GABARAP could significantly block the miR-542-3p-controlled expression of oxidative stress-associated genes including SOD1 in H9C2 and HUVEC cells (Figures 5(j) and 5(k)). Besides, according to our previous studies (written in Chinese) [49, 50], we analyzed the compositions of Granule of BU-XIN RUAN-MAI. LC-QTOF-MS analysis showed that salidroside, loganin, and polydatin were the main compounds of Granule of BU-XIN RUAN-MAI (Supporting Figure 1).

4. Discussion
Coronary heart disease (CHD) is a common disease nowadays [1]. The western medical technology fails to improve poor prognosis of this disease. In this study, we investigated...
Figure 5: miR-542-3p regulates oxidation and inflammation by targeting GABARAP in cell lines. (a) Real-time PCR analysis of miR-542-3p or GABARAP in cell lines after transfection for 48 h; \( P < 0.001 \) versus Mock and \( \Delta P < 0.05 \) versus vector. (b, c) Real-time PCR analysis of PPARα expression in H9C2 and HUVEC cells after 48 h transfection followed by 4 h angiotensin II-stimulation; \( P < 0.001 \) versus the sham + Mock + vector, \( \Phi P < 0.05 \) versus the angiotensin II + Mock + vector, and \( \Delta P < 0.05 \) versus the angiotensin II + anti-miR-542-3p + vector. (d, e) Real-time PCR analysis of IL-6 expression in H9C2 and HUVEC cells after 48 h transfection followed by 4 h angiotensin II-stimulation; \( P < 0.001 \) versus the sham + Mock + vector, \( \Phi P < 0.05 \) versus the angiotensin II + Mock + vector, and \( \Delta P < 0.05 \) versus the angiotensin II + anti-miR-542-3p + vector. (f, g) Real-time PCR analysis of IL-1β expression in H9C2 and HUVEC cells after 48 h transfection followed by 4 h angiotensin II-stimulation; \( P < 0.001 \) versus the sham + Mock + vector, \( \Phi P < 0.05 \) versus the angiotensin II + Mock + vector, and \( \Delta P < 0.05 \) versus the angiotensin II + anti-miR-542-3p + vector. (h, i) Real-time PCR analysis of TNFα expression in H9C2 and HUVEC cells after 48 h transfection followed by 4 h angiotensin II-stimulation; \( P < 0.001 \) versus the sham + Mock + vector, \( \Phi P < 0.05 \) versus the angiotensin II + Mock + vector, and \( \Delta P < 0.05 \) versus the angiotensin II + anti-miR-542-3p + vector. (j, k) Real-time PCR analysis of SOD1 expression in H9C2 and HUVEC cells after 48 h transfection followed by 4 h angiotensin II-stimulation; \( P < 0.001 \) versus the sham + Mock + vector, \( \Phi P < 0.05 \) versus the angiotensin II + Mock + vector, and \( \Delta P < 0.05 \) versus the angiotensin II + anti-miR-542-3p + vector.
Evidence-Based Complementary and Alternative Medicine

the effect of Granule of BU-XIN RUAN-MAI, a clinical Chinese medicine, on CHD patients with angina pectoris. The data showed that Granule of BU-XIN RUAN-MAI can ameliorate the clinical coronary heart disease and inhibit isoprenaline-induced myocardial cell injury. In the end, Granule of BU-XIN RUAN-MAI may significantly trigger autophagy in myocardial cells.

Ancient traditional Chinese medicine (TCM) has been practiced for a long history [16]. Granule of BU-XIN RUAN-MAI has been prepared by professor Shu-Hua Tang, an excellent expert of traditional Chinese medicine. The Granule of BU-XIN RUAN-MAI contains Rhodiola rosea L., Ophiopogon japonicas (Linn. f.) Ker-Gawl., Cornus officinalis Sieb. et Zucc., Whitmania pigra Whitman, Ginkgo biloba L., Polygonum cuspidatum Sieb. et Zucc., and so on [20–31]. Professor Shu-Hua Tang considered that asthenic cardiac Qi and Yin deficiency of heart and kidney often occurred in the shape of coronary heart disease. Western medicine has stated that coronary heart disease is a chronic inflammatory process (Yin deficiency with internal heat), lipid accumulation (sputum) and migration and proliferation of smooth muscle cells (blood stasis). Luckily, Granule of BU-XIN RUAN-MAI is just the compound prescription that it may nourish Qi and Yin to enhance blood circulation for clearing internal heat and removing obstruction in collaterals, and products of these herbs exhibit perfect activity of anti-inflammation, anti-oxidative stress, anti-thrombosis, and cardiovascular protective activity [20–31, 51–56]. Consequently, Granule of BU-XIN RUAN-MAI is a perfect prescription for treatment of ischemic heart disease (angina pectoris).

In the present study, to determine the effect of Granule of BU-XIN RUAN-MAI on treatment of patients with angina pectoris, the patients in the placebo group were administrated with Western medicine, and the patients in the Granule of BU-XIN RUAN-MAI group were administrated with Western medicine and Granule of BU-XIN RUAN-MAI (traditional Chinese medicine). The data showed that patients with angina pectoris were improved more effectively by administration of Granule of BU-XIN RUAN-MAI. Furthermore, the results demonstrated that Granule of BU-XIN RUAN-MAI can keep patients’ heart rate at the proper level. To investigate the effect of Granule of BU-XIN RUAN-MAI on patients’ serological indicators, the data proved that Granule of BU-XIN RUAN-MAI could significantly decrease the TC, TG, LDL, and HDL contents, and Granule of BU-XIN RUAN-MAI may downregulate the patients’ high shear of blood viscosity, low shear of blood viscosity, plasma viscosity, erythrocyte rigidity index, hs-CRP content, and D-Dimer and fibrinogen contents. These results suggested that Granule of BU-XIN RUAN-MAI was able to improve patients’ heart rate, promote blood circulation, reduce lipid content in serum, and inhibit the formation of thrombus. Importantly, Granule of BU-XIN RUAN-MAI contained salidroside, loganin, and polydatin determined by LC-QTOF-MS analysis. Salidroside [57], loganin [58], and polydatin [59] can protect against oxidative stress, which is the main cause of coronary heart disease. Thus, Granule of BU-XIN RUAN-MAI may be considered as a good prescription for treatment of ischemic heart disease.

Similarly, we determined the effect of Granule of BU-XIN RUAN-MAI in the myocardial injury model. In isoprenaline-induced rats, Granule of BU-XIN RUAN-MAI sustained the shape of myocardial cells evidenced by H&E staining, and Granule of BU-XIN RUAN-MAI significantly inhibited the myocardial cell injury by reducing the MDA content in serum and elevating the SOD1, Na+/K+-ATPase, and cAMP content in serum. Additionally, in isoprenaline-induced rats, Granule of BU-XIN RUAN-MAI ameliorated myocardial ischemia by downregulating the inflammatory factor levels of IL-6, IL-1β, and TNF-α. These data implied that Granule of BU-XIN RUAN-MAI could improve heart functions during the myocardial ischemia.

The production of oxidative stress may be enhanced by activating the angiotensin II-associated NADPH oxidase (Nox) in endothelial cells [60]. Oxidative stress is an important factor to endothelial dysfunction [61]. The NADPH oxidase family members are multicomponent protein complexes composed of catalytic subunits including Nox1-5, organizer subunits such as p47phox or Noxol1, activator subunits such as p67phox or Noxa1, and other regulatory subunits such as p22phox and p40phox and the binding partner Rac [62]. In our study, Granule of BU-XIN RUAN-MAI-containing serum obviously downregulated protein levels of P40phox, P47phox, and P67phox in the plasma membrane of HUVEC cells, and it significantly increased protein expressions of P40phox, P47phox, and P67phox in the cytoplasm of HUVEC cells. These findings suggest that Granule of BU-XIN RUAN-MAI-containing serum can downregulate the NADPH-mediated oxidative stress level in angiotensin II-stimulated HUVEC cells. Subsequently, Granule of BU-XIN RUAN-MAI can inhibit coronary heart disease by regulating NADPH-mediated oxidative stress.

GABARAP has been considered as a novel and essential regulator that it could improve coronary heart disease including angina pectoris and arteriosclerosis by increasing autophagy, which is a beneficial biological process for treatment of coronary heart disease [38–44]. Thus, we investigated whether GABARAP was involved in the Granule of BU-XIN RUAN-MAI-induced inhibition of angina pectoris. Firstly, GABARAP was decreased in the heart of ISO-induced rats and in angiotensin II-incubated HUVEC cells, whereas it was upregulated by Granule of BU-XIN RUAN-MAI, implying that GABARAP-mediated autophagy might be involved in Granule of BU-XIN RUAN-MAI alleviating heart disease. Furthermore, knockdown of GABARAP could significantly reverse the Granule of BU-XIN RUAN-MAI-controlled expression of genes including PPARα, IL-6, IL-1β, and TNFα, SOD1, and MDA in vivo and in vitro, suggesting that Granule of BU-XIN RUAN-MAI shows its inhibitory activity against oxidation and inflammation in angina pectoris of heart disease partly depending on the GABARAP expression. Thus, GABARAP is required for Granule of BU-XIN RUAN-MAI exhibiting its protective effect against heart disease, and it is meaningful for us to investigate the role of GABARAP in Granule of BU-XIN RUAN-MAI against heart disease. Based on these studies, we investigated the molecular mechanisms of Granule of BU-XIN RUAN-MAI in modulating the
GABARAP expression. The data demonstrated that Granule of BU-XIN RUAN-MAI upregulated GABARAP by keeping stability of GABARAP mRNA 3'UTR, suggesting that Granule of BU-XIN RUAN-MAI could increase the GABARAP expression through modulating miRNAs level.

Previous studies have pointed out that miRNAs play an important role in coronary heart disease. For example, downregulated miRNA-26a-5p enhances the apoptosis of endothelial cells in coronary heart disease by suppressing PI3K/AKT signaling [63]. MiR-590 increases endothelial cell apoptosis by inactivating TLR4/NF-κB signaling in atherosclerosis [64]. Inhibition of microRNA-429 alleviates myocardial injury of rats with coronary heart disease [65]. MicroRNA-7b ameliorates ischemia/reperfusion-induced H9C2 cardiomyocyte apoptosis through the hypoxia-inducible factor-1/p-p38 pathway [66]. miR-542-3p often serves as a tumor suppressor in various cancers including epithelial ovarian cancer [67], hepatocellular carcinoma [68], osteosarcoma [69], and meanwhile, miR-542-3p promotes hepatic stellate cell activation and fibrosis by regulating BMP-7 [70]. In our study, we observed that miR-542-3p was increased in ISO-induced rats and in angiotensin II-induced HUVEC cells, and it could be inhibited by Granule of BU-XIN RUAN-MAI. Then, miR-542-3p can significantly promote oxidation and inflammation in cardiomyocytes of coronary heart disease by targeting GABARAP. Additionally, overexpression of miR-542-3p markedly inhibited GABARAP expression while interfering miR-542-3p increased the GABARAP level in cardiomyocytes. Finally, Granule of BU-XIN RUAN-MAI upregulated the GABARAP expression partly by depending on miR-542-3p. Together, these findings indicate that miR-542-3p may aggravate oxidation and inflammation in coronary heart disease by targeting GABARAP, and miR-542-3p/GABARAP axis is required for Granule of BU-XIN RUAN-MAI showing its protective activity against angina pectoris of coronary heart disease. Further data demonstrated that knockdown of miR-542-3p-mediated inhibition of oxidation and inflammation could be partly blocked by silencing of GABARAP in cardiomyocytes.

5. Conclusions

The data from clinic and animal model demonstrated that Granule of BU-XIN RUAN-MAI is an excellent prescription for treatment of coronary heart disease by suppressing the inflammation and NAPDH-mediated oxidative stress. MiR-542-3p/GABARAP axis is required for Granule of BU-XIN RUAN-MAI exhibiting its protective activity against pectoris of coronary heart disease.

Abbreviations

BXRM: Granule of BU-XIN RUAN-MAI
CHD: Coronary heart disease
TCM: Traditional Chinese medicine
MDA: Malondialdehyde
SOD: Superoxide dismutase
cAMP: Cyclic adenosine monophosphate

TNF-α: Tumor necrosis factor alpha
IL-1β: Interleukin-1 beta
IL-6: Interleukin-6
TC: Total cholesterol
TG: Triglyceride
LDL: Low-density lipoprotein cholesterol
HDL: High-density lipoprotein cholesterol
hs-CRP: High-sensitivity C-reactive protein
H&E: Hematoxylin and eosin staining
SDS: Sodium dodecyl sulfate
ECL: Enhanced chemiluminescence
ISO: Isoproterenol hydrochloride
ELISA: Enzyme-linked immunosorbent assay
PVDF: Polyvinylidene fluoride
PCR: Polymerase chain reaction.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Dong Yan and Li-li Zhao contributed equally to this study. Y.S.H., Q.Y.L., Y.D., and Z.L.L. conceived and designed the experiments. Y.D., Q.H., Y.B.W., Z.L.L., Z.Z.H., and W.N. carried out the experiments. Z.L.L. and Y.D. analyzed the data. Y.S.H., Q.Y.L., and Y.D. contributed the reagents and materials. Y.D. drafted the manuscript. All authors reviewed the final manuscript.

Supplementary Materials

Supporting Figure 1: the compositions of Granule of BU-XIN RUAN-MAI. In this study, the compositions of Granule of BU-XIN RUAN-MAI were determined by LC-QTOF-MS analysis. The results showed that salidroside (a), loganin (b), and polydatin (c) were the main compounds of Granule of BU-XIN RUAN-MAI granule. (Supplementary Materials)

References

[1] P. Ong, A. Athanasiadis, S. Hill, H. Vogelsberg, M. Voehringer, and U. Sechtem, “Coronary artery spasm as a frequent cause of acute coronary syndrome—the CASPAR (coronary artery spasm in patients with acute coronary syndrome) study,” Journal of the American College of Cardiology, vol. 52, no. 7, pp. 523–527, 2008.
[2] T. V. Varga, A. Kurbić, M. Aine et al., “Novel genetic loci associated with long-term deterioration in blood lipid concentrations and coronary artery disease in European adults,” International Journal of Epidemiology, vol. 46, no. 4, pp. 1211–1222, 2016.
[3] J. F. Keaney, M. G. Larson, R. S. Vasan et al., “Obesity as a source of systemic oxidative stress: clinical correlates of oxidative stress in the Framingham study,” Circulation, vol. 106, no. 19, p. 467, 2002.
[4] R. O. Escárciga, M. J. Lipinski, M. García-Carrasco, C. Mendoza-Pinto, J. L. Galvez-Romero, and R. Cervera, "Inflammation and atherosclerosis: cardiovascular evaluation in patients with autoimmune diseases," *Autoimmunity Reviews*, vol. 17, no. 7, pp. 703–708, 2018.

[5] T. Itoh, Y. Mizuno, E. Harada, M. Yoshimura, H. Ogawa, and H. Yasue, "Coronary spasm is associated with chronic low-grade inflammation," *Circulation Journal*, vol. 71, no. 7, pp. 1074–1078, 2007.

[6] M. V. Paul and S. Hiroaki, "Endothelium-derived relaxing factor and coronary vasospasm," *Circulation*, vol. 80, no. 1, pp. 1–9, 1989.

[7] C. L. Grines, M. W. Watkins, G. Helmer et al., "Angiogenic Gene Therapy (AGENT) trial in patients with stable angina pectoris," *Circulation*, vol. 105, no. 11, pp. 1291–1297, 2002.

[8] N. C. Tan, C. C. Goh, S. C. P. Goh, Y. L. E. Koh, and K. H. Koh, "The effect of the intensity of lipid-lowering medications on the LDL cholesterol treatment goals of Asian patients with dyslipidemia in primary care," *Journal of Clinical Pharmacy and Therapeutics*, vol. 41, no. 6, pp. 677–683, 2016.

[9] C. Melloni, B. R. Shah, F.-S. Ou et al., "Lipid-lowering intensification and low-density lipoprotein cholesterol achievement from hospital admission to 1-year follow-up after an acute coronary syndrome event: results from the Medications Applied and SusTAINed over Time (MAIN-TAIN) registry," *American Heart Journal*, vol. 160, no. 6, pp. 1121–1129, 2010.

[10] E. M. Ohman, M. T. Roe, P. G. Steg et al., "Clinically significant bleeding with low-dose rivaroxaban versus aspirin, in addition to P2Y12 inhibition, in acute coronary syndromes (GEMINI-ACS-1): a double-blind, multicentre, randomised trial," *The Lancet*, vol. 389, no. 10081, pp. 1799–1808, 2017.

[11] T. Tsujimoto, T. Sugiyama, and H. Kajio, "Effects of β-blockers on all-cause mortality in patients with type 2 diabetes and coronary heart disease," *Diabetes, Obesity and Metabolism*, vol. 19, no. 6, pp. 800–808, 2017.

[12] A. C. Fanaroff, S. K. James, G. Weisz et al., "Ranolazine after incomplete percutaneous coronary revascularization in patients with versus without diabetes mellitus: RIVER-PICI trial," *Journal of the American College of Cardiology*, vol. 69, no. 18, pp. 2304–2313, 2017.

[13] P. G. Lund-Larsen, "Nitroglycerin and beta blockers: the only current drugs in angina pectoris: report of the information booklet from the Swedish social governmental agency "Drugs in angina pectoris" 1971 part 2:1 and 2:II," *Tidskrift for Den Norske Lægeforening*, vol. 93, no. 18, p. 1405, 1973.

[14] D. Sueta, N. Tabata, and S. Hokimoto, "Clinical roles of calcium channel blockers in ischemic heart diseases," *Hypertension Research*, vol. 40, no. 5, pp. 423–428, 2017.

[15] D. B. Hogan, "Did Osler suffer from "paranoia antitherapeutic" baltimoremoria? A comparative content analysis of the principles and practice of medicine and harrison’s principles of internal medicine, 11th edition," *CMAJ: Canadian Medical Association Journal*, vol. 161, no. 7, pp. 842–845, 1999.

[16] X. Yang, N. Liu, X. Li et al., "A review on the effect of traditional Chinese medicine against anthracline-induced cardiac toxicity," *Frontiers in Pharmacology*, vol. 9, p. 444, 2018.

[17] E. S. Spatz, Y. Wang, A. L. Beckman et al., "Traditional Chinese medicine for acute myocardial infarction in western medicine hospitals in China," *Circulation: Cardiovascular Quality and Outcomes*, vol. 11, no. 3, Article ID e004190, 2018.

[18] X. Wang and H. M. Li, "The agreement and differences between Chinese medicine and western medicine from the evolution process of heart failure," *Zhongguo Zhong Xi Yi Jie He Za Zhi Zhongguo Zhongxiyi Jiehe Zazhi Chinese Journal of Integrated Traditional and Western Medicine*, vol. 34, no. 2, pp. 138–140, 2014.

[19] Q. Shi, H. Zhao, J. Chen et al., "Study on TCM syndrome identification modes of coronary heart disease based on data mining," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 697028, 11 pages, 2012.

[20] T.-H. Lee, C.-C. Hsu, G. Hsiao, J.-Y. Fang, W.-M. Liu, and C.-K. Lee, "Anti-MMP-2 activity and skin-penetrating capability of the chemical constituents from Rhodiola rosea," *Planta Medica*, vol. 82, no. 8, pp. 698–704, 2016.

[21] K. Kobayashi, K. Yamada, T. Murata et al., "Constituents of Rhodiola rosea showing inhibitory effect on lipase activity in mouse plasma and alimentary canal," *Planta Medica*, vol. 74, no. 14, pp. 1716–1719, 2008.

[22] J. W. Zhao, D. S. Chen, C. S. Deng et al., "Evaluation of anti-inflammatory activity of compounds isolated from the rhizome of Ophiopogon japonicas," *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, p. 7, 2017.

[23] G. Y. Li, Y. X. Yao, and X. Ding, "Studies on chemistry component and the biological activity of petroleum ether extraction from pre-and post-processed of Cornus officinalis," *Zhong Yao Cai Journal of Chinese Medicinal Materials*, vol. 33, no. 2, pp. 192–195, 2010.

[24] M. J. Wu, Q. S. Guo, H. Z. Shi et al., "Effect of ingestion on antithrombin activity in different tissues of Whithmania pigra," *Zhongguo Zhong Yao Za Zhi Zhongguo Zhongyao Zazhi China Journal of Chinese Materixa Medica*, vol. 43, no. 5, pp. 934–937, 2018.

[25] Y. Shan, J. M. Zhang, Y. Z. Ding et al., "In vitro anticoagulant activity of different processed products of Whithanma pigra by water extraction and bionic extraction," *Zhongguo Zhong Yao Za Zhi Zhongguo Zhongyao Zazhi China Journal of Chinese Materixa Medica*, vol. 41, no. 10, pp. 1843–1848, 2016.

[26] H. Z. Shi, H. Liu, Q. S. Guo et al., "Studies on digestive enzyme activity of Whithnma pigra in different months old," *Zhongguo Zhong Yao Za Zhi Zhongguo Zhongyao Zazhi China Journal of Chinese Materixa Medica*, vol. 40, no. 14, pp. 2796–2799, 2015.

[27] B. Yin, Y. Xu, R. Wei, and B. Luo, "Ginkgo biloba on focal cerebral ischemia: a systematic review and meta-analysis," *The Journal of American Journal of Chinese Medicine*, vol. 42, no. 4, pp. 769–783, 2014.

[28] X. J. Xiong, W. Liu, X. C. Yang et al., "Ginkgo biloba extract for essential hypertension: a systemic review," *Phytomedicine*, vol. 21, no. 10, pp. 1131–1136, 2014.

[29] C. Ude, M. Schubert-Zsilavecz, and M. Wurglics, "Ginkgo biloba extracts: a review of the pharmacokinetics of the active ingredients," *Clinical Pharmacokinetics*, vol. 52, no. 9, pp. 727–749, 2013.

[30] G. B. Mahady, "Ginkgo biloba for the prevention and treatment of cardiovascular disease: a review of the literature," *The Journal of Cardiovascular Nursing*, vol. 16, no. 4, pp. 21–32, 2002.

[31] W. Peng, R. Qin, X. Li, and H. Zhou, "Botany, phytochemistry, pharmacology, and potential application of Polygonum cuspidatum Sieb.et Zucc.: a review," *Journal of Ethnovetpharmacology*, vol. 148, no. 3, pp. 729–745, 2013.

[32] J. Joachim and S. A. Tooze, "Control of GABARAP-mediated autophagy by the Golgi complex, centrosome and centriolar satellites," *Biology of the Cell*, vol. 110, no. 1, pp. 1–5, 2018.
[33] J. Joachim, M. Razi, D. Judith et al., “Centriolar satellites control GABARAP ubiquitination and GABARAP-mediated autophagy,” Current Biology, vol. 27, no. 14, pp. 2123–2136, 2017.

[34] J. Dancourt and T. J. Melia, “Lipidation of the autophagy proteins LC3 and GABARAP is a membrane-curve-dependent process,” Autophagy, vol. 10, no. 8, pp. 1470-1471, 2014.

[35] S. Nath, J. Dancourt, V. Shteyn et al., “Lipidation of the LC3/ GABARAP family of autophagy proteins relies on a membrane-curve-sensing domain in Atg3,” Nature Cell Biology, vol. 16, no. 5, pp. 415–424, 2014.

[36] D. Colecchia, A. Strambi, S. Sanzone et al., “MAPK15/ERK8 stimulates autophagy by interacting with LC3 and GABARAP proteins,” Autophagy, vol. 8, no. 12, pp. 1724–1740, 2012.

[37] M. Schwarten, J. Mohrländer, P. Ma et al., “Nix directly binds to GABARAP: a possible crosstalk between apoptosis and autophagy,” Autophagy, vol. 5, no. 5, pp. 690–698, 2009.

[38] S. Yamamoto, M. Yoshimura, M.-C. Shin, M. Wakita, K. Nonaka, and N. Akaike, “GABAA receptor-mediated presynaptic inhibition on glutamatergic transmission,” Brain Research Bulletin, vol. 84, no. 1, pp. 22–30, 2011.

[39] H. I. D. Mack and K. Munger, “Modulation of autophagy-like processes by tumor viruses,” Cells, vol. 1, no. 3, pp. 204–247, 2012.

[40] B.-K. Ban, M.-H. Jun, H.-H. Ryu, D.-J. Jang, S. T. Ahmad, and J.-A. Lee, “Autophagy negatively regulates early axon growth in cortical neurons,” Molecular and Cellular Biology, vol. 33, no. 19, pp. 3907–3919, 2013.

[41] Y.-M. Wei, X. Li, M. Xue et al., “Enhancement of autophagy by simvastatin through inhibition of Rac1-mTOR signaling pathway in coronary arterial myocytes,” Cellular Physiology and Biochemistry, vol. 31, no. 6, pp. 925–937, 2013.

[42] F. Wang, J. Jia, and B. Rodrigues, “Autophagy, metabolic disease, and pathogenesis of heart dysfunction,” Canadian Journal of Cardiology, vol. 33, no. 7, pp. 850–859, 2017.

[43] S. Y. Ren and X. Xu, “Role of autophagy in metabolic syndrome-associated heart disease,” Biochimica et Biophysica Acta (BBA)—Booenergetics, vol. 1852, no. 2, pp. 225–231, 2013.

[44] L. A. Kirshenbaum, “Regulation of autophagy in the heart in health and disease,” Journal of Cardiovascular Pharmacology, vol. 60, no. 2, pp. 109, 2012.

[45] G. Rona, C. I. Chappel, T. Balazs et al., “An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat,” AMA Archives of Pathology, vol. 64, no. 4, pp. 443–455, 1959.

[46] S. D. Seth, M. Maulik, C. K. Katiyar et al., “Role of Lipistat in protection against isoproterenol induced myocardial necrosis in rats: a biochemical and histopathological study,” Indian Journal of Physiology and Pharmacology, vol. 42, no. 1, pp. 101–106, 1998.

[47] L. Zhou, L. Xu, J. Ye et al., “Cidea promotes hepatic steatosis by sensing dietary fatty acids,” Hepatology, vol. 56, no. 1, pp. 95–107, 2012.

[48] F. Aktan, S. Henness, V. Tran, C. Duke, B. Roufogalis, and A. Ammit, “Gingerol metabolite and a synthetic analogue capsarol inhibit macrophage NF-κB-Mediated iNOS gene expression and enzyme activity,” Planta Medica, vol. 72, no. 8, pp. 727–734, 2006.

[49] X. Wang, L. Fan, C. Lu et al., “Determination of salidroside in Buxin Ruinai granules by a multi-variation method, the content of saponin and polydatin,” China Journal of Traditional Chinese Medicine and Information, vol. 21, no. 9, pp. 93–97, 2014.

[50] L. Fan, S. Li, X. Ma et al., “Optimization of extraction and purification process of Buxin soft vein granules,” Chinese Journal of Experimental Forensiology, vol. 18, no. 22, pp. 32–35, 2012.

[51] L. V. Maslova, B. Konrad'ev, L. N. Maslov et al., “The cardioprotective and antiadrenergic activity of an extract of Rhodiola rosea in stress,” Ekspertimental'naia I Klinicheskaiia Farmakologii, vol. 57, no. 6, pp. 61–63, 1994.

[52] M. Déciga-Campos, M. E. González-Trujano, R. Ventura-Martínez, R. M. Montiel-Ruiz, G. E. Angeles-López, and F. Brindis, “Antihyperalgesic activity of Rhodiolarosea in a diabetic rat model,” Drug Development Research, vol. 77, no. 1, pp. 29–36, 2016.

[53] Z.-Q. Qu, Y. Zhou, Y.-S. Zeng, Y. Li, and P. Chung, “Pre-treatment with Rhodiola rosea extract reduces cognitive impairment induced by intracerebroventricular streptozotocin in rats: implication of anti-oxidative and neuroprotective effects,” Biomedical and Environmental Sciences, vol. 22, no. 4, pp. 318–326, 2009.

[54] J. Zhang, S. Fan, Y. Yao et al., “Cardiovascular protective effect of polysaccharide from Ophiopogon japonicus in diabetic rats,” International Journal of Biological Macromolecules, vol. 82, pp. 505–513, 2016.

[55] Y.-L. Zhang, M.-Z. Xi, Y.-B. Choi, and B.-H. Lee, “Antithrombotic effect of fermented Ophiopogon japonicus in thrombosis-induced rat models,” Journal of Medicinal Food, vol. 20, no. 7, pp. 637–645, 2017.

[56] J. Kou, Y. Tian, Y. Tang, J. Yan, and B. Yu, “Antithrombotic activities of aqueous extract from Radix Ophiopogon japonicus and its two constituents,” Biological & Pharmaceutical Bulletin, vol. 29, no. 6, pp. 1267–1270, 2006.

[57] D.-M. Wu, X.-R. Han, X. Wen et al., “Salidroside protection against oxidative stress injury through the wnt/β-catenin signaling pathway in rats with Parkinson’s disease,” Cellular Physiology and Biochemistry, vol. 46, no. 5, pp. 1793–1806, 2018.

[58] H. Xu, J. Shen, H. Liu, Y. Shi, L. Li, and M. Wei, “Morroniside and loganin extracted from Cornus officinalis have protective effects on rat mesangial cell proliferation exposed to advanced glycation end products by preventing oxidative stress,” Canadian Journal of Physiology and Pharmacology, vol. 84, no. 12, pp. 1267–1273, 2006.

[59] R. Lv, L. Du, L. Zhang et al., “Polydatin attenuates spinal cord injury in rats by inhibiting oxidative stress and microglia apoptosis via Nrf2/NO-1 pathway,” Life Sciences, vol. 217, pp. 119–127, 2019.

[60] R. Harfouche, N. A. Malak, R. P. Brandes, A. Karsan, K. Irani, and S. N. A. Hussain, “Roles of reactive oxygen species in angiopoietin-1/tie-2 receptor signaling,” The FASEB Journal, vol. 19, no. 12, pp. 1728–1730, 2005.

[61] J. Siegrist and H. Sies, “Disturbed redox homeostasis in oxidant stress to coronary heart disease?,” Free Radical Biology and Medicine, vol. 57, no. 6, pp. 61–63, 1994.

[62] M. Déciga-Campos, M. E. González-Trujano, R. Ventura-Martínez, R. M. Montiel-Ruiz, G. E. Angeles-López, and F. Brindis, “Antihyperalgesic activity of Rhodiolarosea in a diabetic rat model,” Drug Development Research, vol. 77, no. 1, pp. 29–36, 2016.
[64] L. Yang and C. Gao, “MiR-590 inhibits endothelial cell apoptosis by inactivating the TLR4/NF-κB pathway in atherosclerosis,” *Yonsei Medical Journal*, vol. 60, no. 3, pp. 298–307, 2019.

[65] Q. Yang, J. Li, H. Zhang, H. Zuo, Q. Zhang, and J. Cheng, “Down-regulation of microRNA-429 alleviates myocardial injury of rats with coronary heart disease,” *Cell Cycle*, vol. 18, no. 19, pp. 2550–2565, 2019.

[66] Z. Sheng, W. Lu, Z. Zuo et al., “MicroRNA-7b attenuates ischemia/reperfusion-induced H9C2 cardiomyocyte apoptosis via the hypoxia inducible factor-1/p-p38 pathway,” *Journal of Cellular Biochemistry*, vol. 120, no. 6, pp. 9947–9955, 2019.

[67] J. Li, W. Shao, and H. Feng, “MiR-542-3p, a microRNA targeting CDK14, suppresses cell proliferation, invasiveness, and tumorigenesis of epithelial ovarian cancer,” *Biomedicine & Pharmacotherapy*, vol. 110, pp. 850–856, 2019.

[68] X.-P. Wang, J. Yao, J. Guan, Z.-Q. Zhou, Z.-Y. Zhang, and J. Yang, “MicroRNA-542-3p functions as a tumor suppressor via directly targeting survivin in hepatocellular carcinoma,” *Biomedicine & Pharmacotherapy*, vol. 99, pp. 817–824, 2018.

[69] Q. Li, S. Song, G. Ni, Y. Li, and X. Wang, “Serum miR-542-3p as a prognostic biomarker in osteosarcoma,” *Cancer Biomarkers*, vol. 21, no. 3, pp. 521–526, 2018.

[70] F. Ji, K. Wang, Y. Zhang et al., “MiR-542-3p controls hepatic stellate cell activation and fibrosis via targeting BMP-7,” *Journal of Cellular Biochemistry*, vol. 120, no. 3, pp. 4573–4581, 2019.