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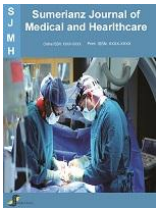

Original Article Open Access

Effects of Different Proportions of Salvianolic Acid and Hydroxysafflor Yellow A on the Myocardial Ischemia Model Induced by Pituitrin in Rats

Miao-Miao Lin
College of Chinese Medicine, Hebei University, Baoding, 071002, Hebei province, China

Mei-Yu Cui
College of Chinese Medicine, Hebei University, Baoding, 071002, Hebei province, China

Li-Na Zhang
College of Chinese Medicine, Hebei University, Baoding, 071002, Hebei province, China

Bo Zhao
College of Chinese Medicine, Hebei University, Baoding, 071002, Hebei province, China

Guo-Wei Zhang*
College of Chinese Medicine, Hebei University, Baoding, 071002, Hebei province, China

Abstract

Aim: In this experiment, SD rats were injected intravenously with different proportions of test samples to observe the protective effect of intravenous injection of pituitrin (PIT)-induced myocardial ischemia in rats, and to determine the lipid peroxidation product of Malondialdehyde (MDA) in rat brain and the content and activity of Superoxide Dismutase (SOD), Creatine Kinase (CK) and Lactate Dehydrogenase (LDH). The role of the anti-myocardial ischemia model in the test and its optimal ratio were studied. **Materials and methods:** Experimental SD rats sensitive to pituitrin were randomly divided into normal saline group, model group, positive control group, and administration group (7 groups). Each group of test samples was injected through the tail vein and the lead ECG was traced. After administration for 30 min, the rats were sublingually injected with 2U/kg pituitrin, and Ⅱ lead ECG was recorded for 5 min. After 3 hours of observation, the rats were sacrificed from the cervical spine, and the hearts were removed and placed in a refrigerator at -20°C to measure various biochemical indicators. **Results:** The contents of MDA, LDH, and CK in myocardial ischemia model group were significantly higher than those in saline control group, and SOD activity was significantly decreased. Intravenous injection of each group of tested products has the effect of reducing the content of MDA, LDH, and CK in myocardial tissue, which can enhance the activity of SOD in myocardial tissue, and has statistical significance compared with the model group ($P \leq 0.05$ or $P \leq 0.01$). In a comprehensive comparison, the A:B=1:10 dose group had the best results. **Conclusion:** Under the experimental conditions, each dose group of the test article can significantly reduce the levels of MDA, LDH, and CK in the myocardial tissue of ischemic rats, and increase the activity of SOD. The effect of this dose has an increasing trend with the increase of B components, and has a certain degree of Dose-effect relationship.

Keywords: Salvianolic acid; Hydroxysafflor yellow A; Myocardial ischemia; Rats.

Abbreviation: PIT, pituitrin; MDA, Malondialdehyde; SOD, Superoxide Dismutase; CK, Creatine Kinase ; LDH, Lactate Dehydrogenase.

1. Introduction

Myocardial ischemia refers to a decrease in blood perfusion of the heart, resulting in decreased oxygen supply to the heart, abnormal myocardial energy metabolism, and a pathological condition that cannot support the normal work of the heart [1]. Ischemic cardiomyopathy is a public health concern with a rising incidence that results in high morbidity and mortality worldwide [2]. Despite optimal treatment, ischemic heart disease - is the leading cause of death worldwide [3], and the second leading cause of cardiovascular death in China [4]. Treatment for myocardial ischemia includes cholesterol-lowering medications, beta-blockers, nitroglycerin, and calcium antagonists [5-7]. Recently, constituents from natural herbs have attracted attention with regard to pharmaceutical development.

Comprehensive investigations have been focusing on combination drugs in order to optimize or amplify the therapeutic effects [8, 9]. In traditional Chinese medicine (TCM), a number of herbs are paired together in order to attenuate toxicity, as well as to enhance the therapeutic effects [10]. Radix Salvia miltiorrhiza (S. miltiorrhiza) and Carthamus tinctorius L. (C. tinctorius; also known as Flos Carthami) are usually used as a combination herbal formulation, known as Danhong injection, which can relieve the symptoms of angina pectoris, attenuate myocardial ischemia and promotes atherosclerotic plaque regression [11].

The Traditional Chinese Medicine Salvia miltiorrhiza is widely used in the treatment of coronary artery disease and cerebrovascular diseases and as a remedy to improve microcirculation [12, 13]. Sal B is a major water-soluble polyphenolic acid extracted from Lamiaceae radix Salvia miltiorrhiza, which is a common herbal medicine that has

*Corresponding Author

been clinically used in China for thousands of years as a blood circulation-accelerating agent and antioxidant [14]. Salvianolic acid is an important active ingredient of Salvia miltiorrhiza Bunge. There are nearly 20 kinds of monomer components including Danshensu and Salvianolic acid B. Pharmacological experiments show that salvianolic acid compounds have strong anti-oxidation effect and can eliminate superoxide anion and hydroxyl group. Free radicals inhibit lipid peroxidation and have a protective effect on myocardial and brain cell damage induced by ischemia-reperfusion [15].

Safflower is a traditional Chinese medicine that promotes blood circulation. The main pharmacological components of safflower are glycosides and safflower yellow pigments.Safflor yellow clinically has the effect of dispelling stasis and relieving pain, lowering cholesterol, lowering blood pressure, and is easily soluble in water. It belongs to chalcone compounds and has a wide range of clinical applications [16]. In 1993, Meselhy, *et al.* [17] isolated hydroxysafflor yellow A from safflower, which is the main active ingredient of safflower yellow to activate blood circulation and it is the most effective water-soluble ingredient in the pharmacological efficacy of medicinal safflower. According to the Chinese Pharmacopoeia 2010 edition, hydroxysafflor yellow A is determined as the most representative active ingredient in safflower. Hydroxysafflor yellow A can exert its anti-ischemic effect by improving hemorheology and coagulation function.

In this experiment, SD rats were injected intravenously with different proportions of salvianolic acid and hydroxysafflor yellow A to observe the protective effect of intravenous injection of pituitrin (PIT) on myocardial ischemia in animal models, and to study the optimal ratio of salvianolic acid and hydroxysafflor yellow A in the treatment of myocardial ischemic diseases. It is applied to myocardial ischemic diseases to maximize their benefits.

2. Materials and Methods

2.1. Test Samples

2.1.1. Name

Salvianolic acid (A: 60.23%; light brown powder), hydroxysafflor yellow A (B: 94.85%; yellow loose powder). Storage conditions: 4℃ refrigerator cold storage. Providers: Shanxi Yuanbang Biotechnology Co., Ltd.

2.1.2. Positive Control

Danhong injection (light brown liquid; refrigerator at 4℃; provided by Shanxi Buchang Pharmaceutical Group).

2.1.3. Solvent

Normal saline injection

2.1.4. Preparation of the Test Sample

Accurately weigh a certain amount of the test articles A and B, and add the normal saline to the required concentration in different proportions.

2.2. Experimental Animals

2.2.1. Strains

SD rats used in this experiment were SPF-grade animals. A total of 80 SD rats were used in the experiment. They were male and female, weighing between 180 and 200g. Animals were sourced from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (License No.: SCXK (Beijing) 2014-0001).All animals were handled accordance with the Principles for Care and Use of Experimental Animals from Hebei University and approved by the institutional committee on animal care. All animals were maintained under standard environmental conditions (23±2℃, 55±5% humidity and 12h/12h light/dark cycle). All animals were allowed free access to tap water and standard laboratory rat food. Euthanasia method: the experimental animals were injected with 3 times dose of sodium pentobarbiturate for 30mg, and the subsequent experiment was carried out after the death was confirmed.

2.2.2. Animal Identification and Grouping Methods

Animals are marked with picric acid. Animals were weighed after the quarantine period and grouped according to the animal's body weight by stratified randomized grouping. And animals were divided into normal saline group, model group, positive control group, A:B (10:10) group, A:B (8:10) group, A:B (6:10) group, A:B (4:10) group, A: B (2:10) group, A: B (1:10) group, A: B (1:20) group, a total of 10 groups, six in each group,half male and female.

2.3 Experimental Design

2.3.1. Experimental Design Basis

2.3.1.1. Adoption of Standards

The experimental design was conducted in accordance with the Guidelines for the Study of New Drugs of Traditional Chinese Medicines - Pharmaceutical Pharmacology Toxicology issued by the State Food and Drug Administration.

2.3.1.2. Pre-experimental Research Data

The effective amount of myocardium ischemia induced by pituitary ligone in this batch was 2U/kg (Concentration: 2U/mL, Providers:Nanjing Xinbai Pharmaceutical Co., Ltd., batch number: 120204, specification: 1mL: 6U).

2.3.2. Dosage and Grouping

Table-1. Dose design of pharmacodynamics test

Group	AB(mg/kg)
Saline group	0
Model group	0
Positive control group	100
A:B 10:10	50mgA+50mgB
A:B 8:10	44.45mgA+55.56mgB
A:B 6:10	37.5mgA+62.5mgB
A:B 4:10	28.57mgA+71.43mgB
A:B 2:10	16.67mgA+83.33mgB
A:B 1:10	9mgA+91mgB
A:B 1:20	4.76mgA+95.24mgB

2.3.3. Experimental Methods

2.3.3.1. SD rats were injected sublingually with 2U/kg pituitrin (concentration:1U/mL). The BIOPAC MP150 multi-channel physiological recording instrument (BIOPAC Corporation) was used to observe the Ⅱ lead electrocardiogram changes, and pituitrin sensitive rats were selected for experiment. (The T-wave is high, exceeding the R-wave 1/2 of the lead; the ST elevation of the lead> 0.1 mv; the ST shift of the lead> 0.1 mv; the T-wave is accompanied by ST segment shift; the R-wave amplitude is reduced; T wave inversion).

2.3.3.2. The experimental rats, half male and female, were randomly divided into normal saline group, model group, positive control group and administration group (7 groups).

2.3.3.3. Each group was intravenously administered 30 minutes in advance and administered at a dose of 1.0 mL/100 g.bw once. Anesthetized with urethane (1g/kg) in a 10% mass fraction and fixed in a supine position on a rat stern, and connected to a multi-channel physiological recorder. The Ⅱ lead electrocardiogram was traced and given 30 minutes later. Pituitary chlorophyll 2U/kg was injected into the sublingual vein of the rat and injection was completed within 5 s. The Ⅱ lead electrocardiogram was traced for 5 min.

2.3.3.4. After 3 hours of observation, the rats were sacrificed from the cervical spine, the heart was removed by thoracotomy, and frozen at -20℃.

2.3.3.5. The homogenate was used to make the heart homogenate with 10% myocardial tissue saline. The content of MDA and the activity of SOD and CK and LDH in the myocardial tissue were measured.

2.3.4. Test items

MDA, SOD, LDH, CK, reagents provided by Nanjing Jiancheng Biological Engineering Institute, the batch numbers were 20111216, 20111217, 20120423, and 20120423 respectively, and were measured by using a KC-junior type microplate reader from Bio TEK, USA.

2.4. Statistical Analysis

Data were presented as mean±standard deviation (S.D.). One-way ANOVA was used to compare means in SPSS 19.0. $p < 0.05$ was considered significantly.

3. Results

3.1. Effect of on MDA Content in Rats with Myocardial Ischemia

The MDA content in the myocardial ischemia model group was higher than that in the normal saline control group. The intravenous injection of the tested products in each group had the effect of reducing the MDA content in the myocardial tissue and had a good dose-effect relationship. The effect of inhibiting MDA production increased with the increase of B content, and there was a significant difference between each dose group and the model group($P \leq 0.01$).

3.2. Effect of SOD Activity on Myocardial Ischemia Rats

The activity of SOD in myocardial ischemia model group was significantly lower than that in saline control group. Intravenous injection of the test article could enhance SOD activity in myocardium, except that the A/B=1/10 dose group had larger sample standard deviation, the remaining groups were statistically significant compared with the model group ($P \leq 0.05$ or $P \leq 0.01$), but the dose-effect relationship was not significant.

3.3. Effect of on LDH Levels in Rats with Myocardial Ischemia

LDH levels in the myocardial ischemia model group were significantly higher than those in the normal saline control group. Intravenous injection of the test article significantly decreased the LDH level in the myocardium. There was a statistically significant difference between the model group and the model group ($P \leq 0.05$ or $P \leq 0.01$). And it has a certain dose-effect relationship.

3.4. Effect of CK Activity on Myocardial Ischemia Rats

The CK level in the myocardial ischemia model group was significantly higher than that in the normal saline control group. Intravenous injection of the test article could reduce the myocardial CK content, and there was a statistically significant difference compared with the model group (P≤0.05 or P≤0.01).

3.5. Effect of ST and ST on the Rate of Change of Cardiac Electrocardiogram in Rats

Compared with the normal control group, the change of ST segment value and change rate at 1-3 min after injection of Pit in the model group rats was significant (P≤0.05 or P≤0.01), indicating that the Pit replication myocardial ischemia model was successful. The test sample can significantly reduce the ST segment of myocardial ischemia induced by Pit. Compared with the model group, the difference is significant (P≤0.05 or P≤0.01).

Table-2. Effect of PIT on myocardial ischemia, MDA, SOD, LDH, and CPK in rats ($\bar{x}$±s, n=6)

Group	MDA (nmol/mgprot)	SOD (U/ mgprot)	LDH (U/ gprot)	CK (U/ mgprot)
Normal saline	20.921±5.089	48.595±6.415	1930.88±417.55	1.827±0.303
Models	26.321±1.491^{a}	30.834±1.722^{aa}	2646.12±208.73^{aa}	2.428±0.134^{aa}
Positive	19.812±3.209^{bb}	37.098±4.834^{aab}	1890.89±91.96^{bb}	2.014±0.203^{bb}
A:B 10:10	20.130±4.531^{bb}	34.873±3.557^{aab}	2055.20±134.34^{bb}	2.018±0.398^{b}
A:B 8:10	20.175±3.989^{bb}	38.100±6.013^{ab}	2037.06±300.16^{bb}	2.009±0.228^{bb}
A:B 6:10	19.455±2.967^{bb}	38.864±5.760^{abb}	2168.95±314.96^{b}	2.000±0.124^{bb}
A:B 4:10	19.024±4.528^{bb}	41.305±9.565^{b}	2098.01±377.37^{b}	1.960±0.259^{bb}
A:B 2:10	19.073±4.560^{bb}	38.604±7.208^{b}	1871.11±308.92^{bb}	2.001±0.416^{b}
A:B 1:10	17.922±1.201^{bb}	39.841±10.202	1854.17±210.92^{bb}	1.867±0.165^{bb}
A:B 1:20	17.673±2.977^{bb}	42.442±9.888^{b}	1981.11±369.79^{bb}	1.966±0.244^{bb}

Note: Compared with the saline group: ^{a}P≤0.05, ^{aa}P≤0.01;
Compared with the model group: ^{b}P≤0.05, ^{bb}P≤0.01.

Table-3. Effect of ST-variation on myocardial ischemia in rats （ $\bar{x}$±s，n=6）

Group	Dose mg/kg	ST Change（MV）						
		Normal	10s	1min	2min	3min	4min	5min
Normal saline	---	0	0.025	0.043	0.026	0.025	0.038	0.047
			±0.024	±0.026	±0.026	±0.020	±0.030	±0.023
Models	100	0	0.056	0.086	0.118	0.093	0.071	0.044
			±0.034	±0.022^{a}	±0.014^{aa}	±0.038^{aa}	±0.029	±0.022
Positive	100	0	0.037	0.080	0.054	0.024	0.055	0.074
			±0.036	±0.044	±0.034^{bb}	±0.023^{b}	±0.036	±0.053
A/B 10/10	100	0	0.038	0.050	0.079	0.047	0.052	0.067
			±0.020	±0.033	±0.061	±0.042	±0.050	±0.045
A/B 8/10	100	0	0.060	0.059	0.073	0.077	0.056	0.056
			±0.046	±0.040	±0.057	±0.031^{a}	±0.034	±0.029
A/B 6/10	100	0	0.035	0.035	0.050	0.030	0.009	0.019
			±0.026	±0.025^{bb}	±0.035^{bb}	±0.026^{b}	±0.006^{bb}	±0.007^{b}
A/B 4/10	100	0	0.062	0.067	0.059	0.068	0.074	0.070
			±0.048	±0.029	±0.029^{bb}	±0.013^{a}	±0.013	±0.014
A/B 2/10	100	0	0.051	0.038	0.048	0.037	0.048	0.032
			±0.059	±0.053	±0.037^{bb}	±0.042	±0.021^{b}	±0.036
A/B 1/10	100	0	0.047	0.038	0.015	0.056	0.022	0.100
			±0.034	±0.028^{bb}	±0.021^{bbb}	±0.026	±0.005^{b}	±0.112
A/B 1/20	100	0	0.041	0.026	0.068	0.068	0.102	0.113
			±0.047	±0.013^{bb}	±0.020^{abb}	±0.058	±0.129	±0.107

Note: Compared with the saline group: ^{a}P≤0.05, ^{aa}P≤0.01;
Compared with the model group: ^{b}P≤0.05, ^{bb}P≤0.01.

Table-4. Effect of ST-rate change on myocardial ischemia in rats （ $\bar{x}\pm s$，n＝6）

Group	Dose mg/kg	ST-rate of change						
		Normal	10s	1min	2min	3min	4min	5min
Normal saline	---	0	11.39	19.47	12.85	11.48	18.65	21.68
			±9.69	±9.43	±13.39	±7.91	±13.85	±9.95
Models	100	0	21.98	35.10	48.04	37.64	27.69	17.31
			±12.45	±11.97^{a}	±8.85^{aa}	±13.72^{aa}	±10.14	±8.20
Positive	100	0	17.47	31.33	23.73	9.43	23.95	32.44
			±21.06	±12.03	±12.98^{bb}	±7.67^{bb}	±14.72	±24.12
A/B 10/10	100	0	16.18	21.11	28.73	17.76	18.78	25.55
			±9.32	±12.16	±18.03	±13.04^{b}	±16.00	±13.68
A/B 8/10	100	0	39.10	40.60	50.09	44.58	34.95	31.59
			±32.59	±32.90	±45.84	±20.20^{aa}	±27.70	±18.87
A/B 6/10	100	0	18.44	18.44	28.00	16.66	5.32	9.70
			±15.08	±14.31	±22.48	±15.32	±3.82^{bb}	±4.01^{a}
A/B 4/10	100	0	25.94	28.95	28.67	31.55	33.26	32.09
			±17.30	±10.10	±13.21^{b}	±3.98^{aa}	±2.68	±8.28^{b}
A/B 2/10	100	0	28.64	15.34	24.02	15.34	19.96	12.02
			±41.16	±16.66	±20.75^{b}	±12.65^{b}	±8.07	±12.17
A/B 1/10	100	0	20.10	15.26	6.45	25.49	10.00	40.64
			±15.38	±9.51^{b}	±9.03^{bb}	±11.28^{a}	±2.92^{b}	±40.68
A/B 1/20	100	0	13.70	9.55	24.73	21.87	31.01	34.09
			±15.90	±3.64^{b}	±9.67^{bb}	±15.62	±35.93	±28.90

Note: Compared with the saline group: $^{a}P\leq0.05$, $^{aa}P\leq0.01$;
Compared with the model group: $^{b}P\leq0.05$, $^{bb}P\leq0.01$.

4. Discussion

In this study, we selected rats that were sensitive to pituitrin and successfully built a rat model of myocardial ischemia. Based on this model, we studied the different ratios of salvianolic acid and safflower yellow A in myocardial ischemia. In this experiment, the MDA, LDH, and CK levels in the myocardial ischemia model group were significantly higher than those in the saline control group, and the SOD activity was significantly reduced. Intravenous injection of each group of tested products has the effect of reducing the content of MDA, LDH, and CK in myocardial tissue, which can enhance the activity of SOD in myocardial tissue, and has statistical significance compared with the model group ($P\leq0.05$ or $P\leq0.01$). In a comprehensive comparison, the A:B=1:10 dose group had the best results.

Traditional Chinese medicinal herbs and their ingredients have been widely used as important therapeutic agents in China since ancient times. In clinical practice, they are commonly prescribed in combination to solve the complexity of a disease [18]. For example, the treatment of myocardial ischemia. In traditional Chinese medicine theory, salvia miltiorrhiza and safflower are commonly used to promote blood circulation and improve myocardial ischemic symptoms.

Salvianolic acids are the most abundant water-soluble compounds extracted from Radix Salvia miltiorrhiza (Danshen). In China, Danshen has been wildly used to treat cardiovascular diseases for hundreds of years. Salvianolic acids, especially salvianolic acid A (Sal A) and salvianolic acid B (Sal B), have been found to have potent anti-oxidative capabilities due to their polyphenolic structure. Recently, intracellular signaling pathways regulated by salvianolic acids in vascular endothelial cells, aortic smooth muscle cells, as well as cardiomyocytes, have been investigated both in vitro and in vivo upon various cardiovascular insults. It is discovered that the cardiovascular protection of salvianolic acids is not only because salvianolic acids act as reactive oxygen species scavengers, but also due to the reduction of leukocyte-endothelial adherence, inhibition of inflammation and metalloproteinases expression from aortic smooth muscle cells, and indirect regulation of immune function. Competitive binding of salvianolic acids to target proteins to interrupt protein-protein interactions has also been found to be a mechanism of cardiovascular protection by salvianolic acids [19]. The modern pharmacological effects of salvianolic acid mainly include the ability to significantly dilate coronary arteries, increase coronary blood flow, inhibit myocardial contractility, reduce myocardial consumption of oxygen, effectively improve brain microcirculation and anti-cerebral ischemia. It is also proved that salvianolic acid is the main active ingredient of salvia miltiorrhiza for removing blood stasis and invigorate the circulation of blood in modern pharmacological research [20, 21]. In experimental myocardial infarction in rat, Jiang et al reported that administration of salvianolic acids significantly decreased infarct size, improved left ventricular function and decreased myocardial malondialdehyde levels compared with the control group. The cardioprotection of salvianolic acids against infarct-induced left ventricle remodeling was significantly contributed by the down-regulation of MMP-9 mRNA expression level and its activity at the infarct area [22].

The cardioprotective effects of Hydroxysafflor Yellow A in myocardial ischemia operate partially through reducing oxidative stress induced damage and apoptosis. The protection is achieved by scavenging of ROS and

mediating the PI3K signaling pathway [23]. Hydroxysafflor yellow A has protective effects on myocardial ischemia and reperfusion injury in rats, and its protective mechanism is similar to that of myocardial ischemic preconditioning.Hydroxysafflor yellow A can significantly inhibit the expression of creatine kinase isoenzymes in serum (CK-MB) and can increase endogenous active substances CGRP, FIA (6-Keto-PGF1A) 6-keto-prostate F1α content, this mechanism mimics the protective effect of IPC on ischemia-reperfusion myocardium [24]. Dong Wenbin, *et al.* [25] used rat myocardial ischemia and blood stasis models to conclude that hydroxysafflor yellow A can significantly reduce the myocardial infarct size in ischemic rats and significantly reduce lactate dehydrogenase (LDH) and produce serum creatine kinase MB type (CK-MB). Hydroxysafflor yellow can reduce the occurrence of blood viscosity and platelet aggregation in rats that have developed myocardial ischemia.

Salvianolic acid and hydroxysafflor yellow A are one of the few important Chinese medicine injections. They have a wide range of pharmacological effects and have good medicinal potential. Currently, they are emerging drug with promising prospects. And they have been widely recognized and valued by the medical community at home and abroad. Therefore, the best ratio for treating myocardial ischemic diseases is studied and applied to myocardial ischemic diseases to better serve humans. It provides a theoretical basis for clinical safety and reasonable use of traditional Chinese medicine.

5. Conclusions

Under the experimental conditions, the tested products can significantly reduce the levels of MDA, LDH, and CK in the myocardial tissue of ischemic rats, and increase the activity of SOD. The effect of the test products has the tendency of increasing with the increase of B components, and has a certain dose-effect relationship. In a comprehensive comparison, the A:B=1:10 dose group had the best results.

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Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] Xu Bohua and Li., X., 2011. "Research progress of Chinese traditional medicine against myocardial ischemia." *China Journal of Experimental Traditional Medical Formulae,* vol. 17, pp. 265-269.

[2] Yellon, D. M. and Hausenloy, D. J., 2007. "Myocardial reperfusion injury." *New England Journal of Medicine,* vol. 357, pp. 1074–1135.

[3] Forouzanfar, M. H., Moran, A. E., Flaxman, A. D., Roth, G., Mensah, G. A., and Ezzati, M., 2012. "Assessing the global burden of ischemic heart disease, part 2: analytic methods and estimates of the global epidemiology of ischemic heart disease in 2010." *Global Heart,* vol. 7, pp. 331–342.

[4] Zhang, X. H., Lu, Z. L., and Liu, L., 2008. "Coronary heart disease in China." *Heart,* vol. 94, pp. 1126–1131.

[5] Rader, D. J. and Hovingh, G. K., 2014. "HDL and cardiovascular disease." *Lancet,* vol. 384, pp. 618–625.

[6] Sadamatsu, K., Koide, S., K., N., and Yoshida, K. J., 2015. "Heart rate control with single administration of a long-acting beta-blocker at bedtime before coronary computed tomography angiography." *J. Cardiogr,* vol. 65, pp. 293-297.

[7] Sharma, K. K., Mathur, M., Gupta, R., Guptha, S., Roy, S., and Khedar, R. S., 2013. "Epidemiology of cardioprotective pharmacological agent use in stable coronary heart disease." *Indian Heart J.,* vol. 65, pp. 250-255.

[8] Wagner, H., 2011. "Synergy research: approaching a new generation of phytopharmaceuticals." *Fitoterapia,* vol. 82, pp. 34–37.

[9] Combination Pharmacotherapy and Public Health Research Working Group Combination Pharmacotherapy For Cardiovascular Disease, 2005. *Ann. Intern. Med.,* vol. 143, pp. 593–599.

[10] Wang, L., Zhou, G. B., Liu, P., Song, J. H., Liang, Y., Yan, X. J., Xu, F., Wang, B. S., Mao, J. H., *et al.*, 2008. "Dissection of mechanisms of Chinese medicinal formula Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia." *Proc. Natl. Acad. Sci. USA,* vol. 105, pp. 4826–4831.

[11] Li, S. J., Tang, Y. P., Shen, J., Li, J. P., Guo, J. M., and Duan, J. A., 2013. "Research of Chinese medicine pairs (VIII) - Salviae Miltiorrhizae Radix et Rhizoma-Carthami flos." *Zhongguo Zhong Yao Za Zhi,* vol. 38, pp. 4227–4231.

[12] Cheng, T. O., 2007. "Cardiovascular effects of Danshen." *Int. J. Cardiol.,* vol. 121, pp. 9-22.

[13] Han, J. Y., Fan, J. Y., Horie, Y., Miura, S., Cui, D. H., Ishii, H., Hibi, T., Tsuneki, H., and Kimura, I., 2008. "Ameliorating effects of compounds derived from Salvia miltiorrhiza root extract on microcirculatory disturbance and target organ injury by ischemia and reperfusion." *Pharmacol Ther,* vol. 117, pp. 280–295.

[14] He, Q., Zhang, J., Chen, F., Guo, L., Zhu, Z., and Shi, J., 2010. "An anti-ROS/hepatic fibrosis drug delivery system based on salvianolic acid B loaded mesoporous silica nanoparticles." *Biomaterials,* vol. 31, pp. 7785–7796.

[15] Lai Yujuan, Liu Hanqing, Li Junsong, and Cai Baochang, 2011. "Research progress on the pharmacokinetics and component interaction of salvianolic acid." *China Journal of New Pharmacy,* vol. 20, pp. 1287-1291.

[16] Cai, G., 2003. "Progress in Clinical application of safflor yellow." *South China Journal of Defence Medical Science,* pp. 13-15.

[17] Meselhy, M., Kadota, S., and Momose, Y., 1993. "Two new quinoch-alcone yellow pigments from Carthamus tinctorius and Ca2+ an-tagonistic activity of tinctormine." *Chen Pharm Bull,* vol. 41, pp. 1796-1802.

[18] Ma, X., Zheng, C., Han, L., Xie, B., Jia, J., Cao, Z., Li, Y., and Chen, Y., 2009. "Synergistic therapeutic actions of herbal ingredients and their mechanisms from molecular interaction and network perspectives." *Drug Discov Today,* vol. 14, pp. 579–588.

[19] Ho, J. H. and Hong, C. Y., 2011. "Salvianolic acids: small compounds with multiple mechanisms for cardiovascular protection." *J. Biomed Sci.,* pp. 18-30.

[20] Fu Xu and Li Jun, 2013. "Research progress of water-soluble components of Salvia miltiorrhiza in chronic kidney disease." *Global Chinese Medicine,* vol. 6, pp. 697-700.

[21] Feng Fan, Zhang Yongping, Song Xinli, and Xu Jian, 2013. "Process for purification of Salvia miltiorrhiza total phenolic acids by macroporous adsorption resin." *Central South Pharmacy,* vol. 11, pp. 27-30.

[22] Jiang, B., Wu, W., Li, M., Xu, L., Sun, K., Yang, M., Guan, S., Liu, X., and Guo, D. A., 2009. "Cardioprotection and matrix metalloproteinase-9 regulation of salvianolic acids on myocardial infarction in rats." *Planta Med.,* vol. 75, pp. 1286–1292.

[23] Han, S. Y., Li, H. X., Ma, X., Zhang, K., Ma, Z. Z., and Tu, P. F., 2009. "Protective effects of purified safflower extract on myocardial ischemia in vivo and in vitro." *Phytomedicine,* vol. 16, pp. 694–702.

[24] Xu Aibin, Liu Jianguo, Zhang Jian, and Li Junxia, 2014. "Hydroxysafflor yellow A improves myocardial ischemia/reperfusion injury in SD rats." *Chinese Journal of Evidence-Based Cardiovascular Medicine,* vol. 6, pp. 733- 736.

[25] Dong Wenbin, Ye Xiaodi, Cheng Min, Wang Junqing, and Zheng Gaoli, 2014. "Antioxidation effect of hydroxysafflor yellow A on myocardial ischemia." *Chinese Journal of Clinical Pharmacology and Therapeutics,* vol. 19, pp. 1001- 1005.