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Kaempferide-7-*O*-(4″-*O*-acetylrhamnosyl)-3-*O*-rutinoside reduces myocardial infarction Size after coronary artery ligation in rats

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Kaempferide-7-*O*-(4″-*O*-acetylrhamnosyl)-3-*O*-rutinoside (A-F-B) is a novel flavonoid extracted from the leaves of *Actinidia kolomikta*. We recently reported that A-F-B administration could improve lipid profiles. A-F-B actions are associated with regulating the activities of PAP and HMG-CoA reductase in hepatic tissue. This study evaluated the effects of A-F-B on acute myocardial infarction (AMI) in rats. An AMI model was established by ligating the left anterior descending coronary artery. The myocardial infarct size (MIS), creatine kinase (CK-MB) activity, troponin T level, endothelial nitric oxide synthase (eNOS) activity, superoxide dismutase (SOD) activity, catalase activity, malondialdehyde (MDA) content, nitric oxide (NO) content were measured. The results showed that the groups treated with A-F-B showed a dose-dependent reduction in MIS. A-F-B markedly inhibited the elevation of the activity of CK-MB, troponin T level, and the content of MDA induced by AMI. A-F-B also showed a capacity to increase the activities of SOD, catalase, and eNOS. The NO content in A-F-B-treated groups also augmented. The findings suggest that A-F-B exerted cardioprotective effects against acute myocardial ischemic injury by regulating antioxidative enzymes activity and endothelial nitric oxide synthase activity.

1. Introduction

Ischemic heart diseases, especially acute myocardial ischemia, remain the leading cause of death in both developed and developing countries as over the past quarter century. Reduction of mortality rate and prevention of myocardial infarction are of utmost importance. Reactive oxygen species, which possess highly reactive and toxic properties, can be generated as a result of ischemia and exacerbate the degree of myocardial damage sustained by the ischemic myocardium (Wattanapitayakul and Bauer 2001). As a consequence, animals have developed an effective defense system to cope with unwanted and toxic oxygen species. In the heart, defense mechanisms include enzymes such as SOD, catalase, and glutathione peroxidase, plus other endogenous antioxidants. In pathological or disease conditions, such as myocardial infarction, stroke and others, the production of free radicals may override the scavenging effects of antioxidants leading to oxidative stress (Zhu et al. 2004).

In recent years, Chinese medicinal herbs and their extracts have been given more attention for prevention and treatment of ischemic heart diseases. The kiwifruit (*Actinidia deliciosa*), originally known as the Chinese gooseberry, is native to northern China, Korea, Siberia and Japan. It is commercially available in New Zealand as well as several other fruit-producing countries. The fruit and flowers of kiwifruit contain a number of nutritional compounds, including vitamin C, vitamin E, folate, potassium, and magnesium (Rush et al. 2002). Kiwifruit also contain plant secondary metabolites such as ursolic acid, carotenoids, and a range of polyphenols. It is reported that kiwifruit has antioxidant activity *in vitro* (Fiorentino et al. 2009). Kiwifruit can also protect against oxidative DNA damage or oxidative stress *in vivo* (Collins et al. 2003). *Actinidia kolomikta* belongs to the family of kiwifruit. Recently, seven new compounds have been isolated and identified from the leaves of *Actinidia kolomikta*. Kaempferide-7-*O*- (4″-*O*- acetylrhamnosyl)-3-*O*-rutinoside (A-F-B), a kind of flavonoid, is one of the seven new compounds. We recently reported that A-F-B administration could improve lipid profiles associated with regulation of the activities of PAP and HMG-CoA reductase in hepatic tissue (Park et al. 2009). Here, we hypothesize that A-F-B might exert cardioprotective effects on acute myocardial ischemia through reducing oxidant stress. In this study, we investigated the experimental therapeutic effects and mechanisms of A-F-B on acute myocardial ischemia damage by a left anterior descending coronary artery ligation model in rats.

2. Investigations and results

2.1. Effect of A-F-B on myocardial infarct size (MIS)

The MIS calculated by a ration of weight of ischemic zone over total weight of the left ventricle was $32.5 \pm 4.8\%$ in AMI group. However, treatment with A-F-B at doses of 12.5, 25, and 50 mg/kg resulted in a significant reduction in

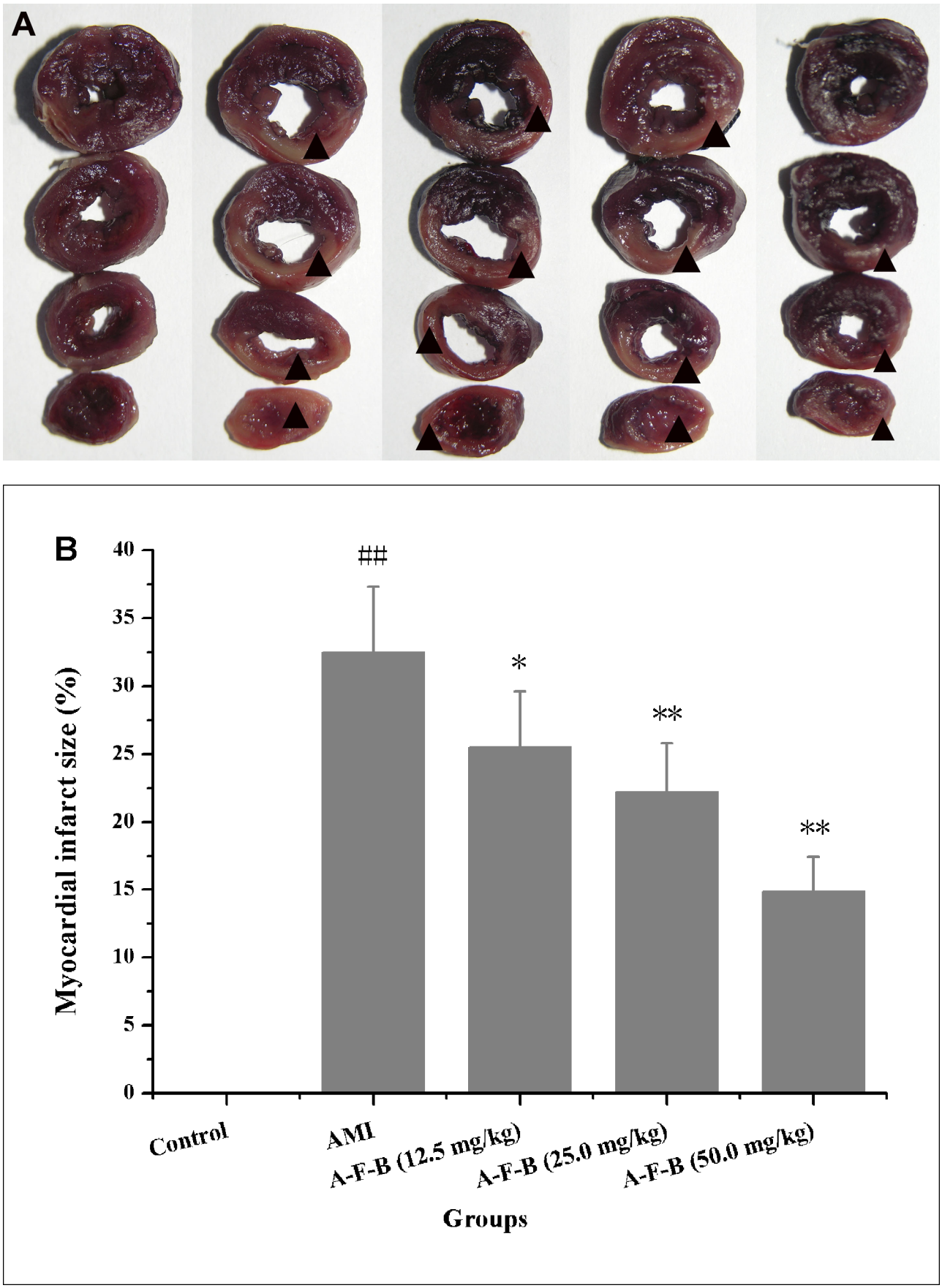

Fig. 1: Effect of A-F-B on myocardial infarct size in acute myocardial ischemic rats. A: Representative photographs showing the appearance of myocardial infarct size (Δ) after triphenyltetrazolium chloride staining in rats (From left to right: control group, AIM group, A-F-B (12.5 mg/kg) group, A-F-B (25.0 mg/kg) group, A-F-B (50.0 mg/kg) group). B: Calculation of the overall myocardial infarct size, each column represented the means ± standard deviation (n = 8). Differences among groups were analyzed by one-way ANOVA, then Student-Newman-Keuls test. ## $P < 0.01$ versus control group; * $P < 0.05$ or ** $P < 0.01$ versus AIM group

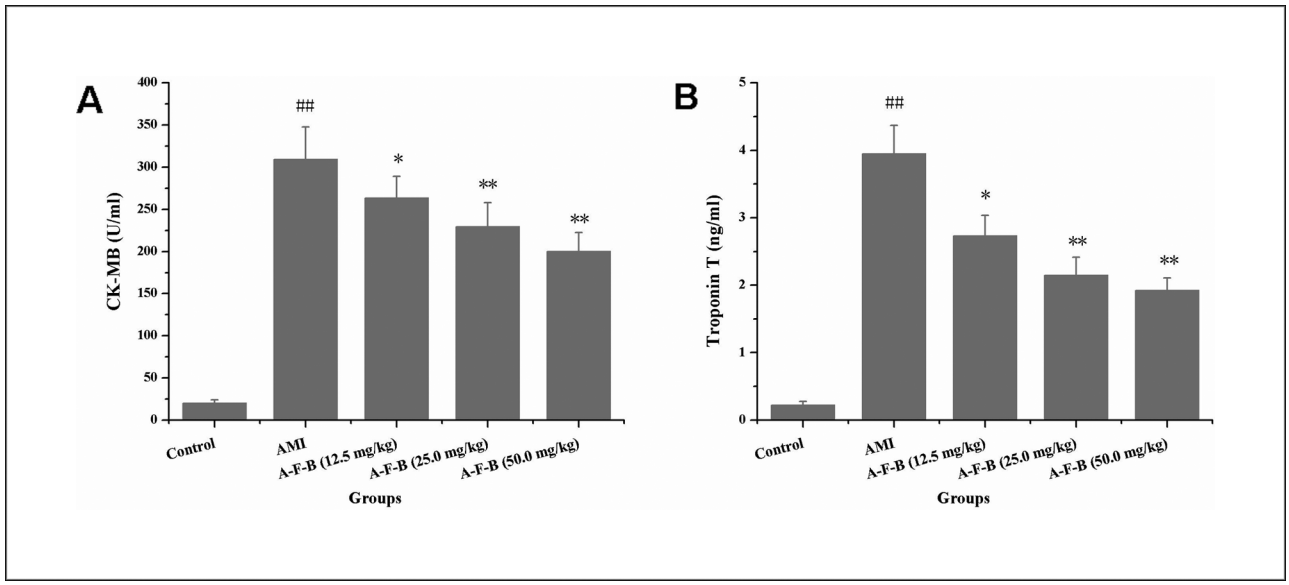

Fig. 2: Effect of A-F-B on CK-MB (A) activity and troponin T level (B) in the serum of acute myocardial ischemic rats. All data are expressed as the means ± standard deviation (n = 8). Differences among groups were analyzed by one-way ANOVA, then Student-Newman-Keuls test. ## $P < 0.01$ versus control group; * $P < 0.05$ or ** $P < 0.01$ versus AIM group

MIS (25.5 ± 4.1%, 22.2 ± 4.6%, and 18.9 ± 2.5%, respectively) ($P < 0.05$ or $P < 0.01$), (Fig. 1).

2.2. Effects of A-F-B on CK-MB activity and troponin T level

Left anterior descending coronary artery (LAD) ligation resulted in a significant increase of serum CK-MB activity and troponin T level. Compared with the AIM group, administration with A-F-B at doses of 12.5, 25, and 50 mg/kg led to reductions in the increased CK-MB activity and troponin T level ($P < 0.05$ or $P < 0.01$), (Fig. 2).

2.3. Effects of A-F-B on the content of MDA

Compared with the control group, the content of MDA in AIM group augmented ($P < 0.01$). Pretreatment with A-F-B at doses of 12.5, 25, and 50 mg/kg significantly reduced the content of MDA in left ventricle ($P < 0.05$ or $P < 0.01$), (Fig. 3).

2.4. Effects of A-F-B on the activities of SOD and catalase

SOD activity and catalase activity in the left ventricles showed a significant decrease in the AIM group ($P < 0.01$). The decreases of SOD activity and catalase activity in the group treated with A-F-B at doses of 12.5, 25, and 50 mg/kg were inhibited when compared with that in the AIM group ($P < 0.05$ or $P < 0.01$), (Fig. 4).

2.5. Effect of A-F-B on eNOS activity and NO content

As shown in Fig. 5, eNOS activity and NO content showed a significant decrease in AIM group ($P < 0.01$). eNOS activity and NO content increased in the group treated with A-F-B at dose of 12.5, 25, and 50 mg/kg when compared with that in AIM group ($P < 0.05$ or $P < 0.01$), (Fig. 5).

3. Discussion

It is well known that coronary artery occlusion plays a key role in acute myocardial infarction. To investigate the cardioprotective action of A-F-B, we employed a typical acute myocardial infarction model induced by LAD ligation. Our results showed that A-F-B had obvious cardioprotective effects and could significantly reduce myocardial infarction size induced by LAD ligation.

Myocardium contains high concentrations of CK-MB. CK-MB, which serves as the diagnostic marker of myocardial tissue damage, will leak out from the damaged tissues to the blood stream when the myocardium membrane becomes permeable or ruptured (Gurgun et al. 2008). Hence, CK-MB activity in serum reflects the alterations of membrane integrity and the degree of myocardial injury. Cardiac troponins are regulatory proteins that control the calcium-mediated interaction of actin and myosin, which results in contraction and relaxation of striated muscle. An elevated troponin level predicts the risk of both cardiac death and subsequent infarction. Recent clinical studies have proposed that serum troponin T concentration increases and can be used to identify asymptomatic myocardial damage and as a predictor of the risk of myocardial ischemia in patients (Afsar et al. 2009; Sabatine et al. 2009). The release of troponin T after myocardial infarction has been correlated with the severity of infarction in experimental animal models and can be used for the early detection of cardiotoxic effects in animals (Bertinchant et al. 2000; Remppis et al. 2000).

Oxidative stress is one of the major concerns in the treatment of ischemic heart diseases. Some studies have reported that acute myocardial ischemia generated numerous free radicals causing damage to lipids and DNA (Bolli et al. 1988). When the production of ROS in the tissues exceeds the ability of the antioxidant system to eliminate them, oxidative stress occurs (Halliwell 2007). ROS can attack the polyunsaturated fatty acid in the biomembrane and induce free radicals chain reactions, leading to the enhancement of MDA (a major lipid peroxidant end product). Free radical scavenging enzymes such as SOD and catalase (Sawyer et al. 2002) are the first line cellular defense against oxidative stress, eliminating reactive oxygen radicals such as superoxide and hydrogen peroxide, and preventing the formation of a more reactive radical of the hydroxyl radical. Main functions of the total antioxidant system are to balance

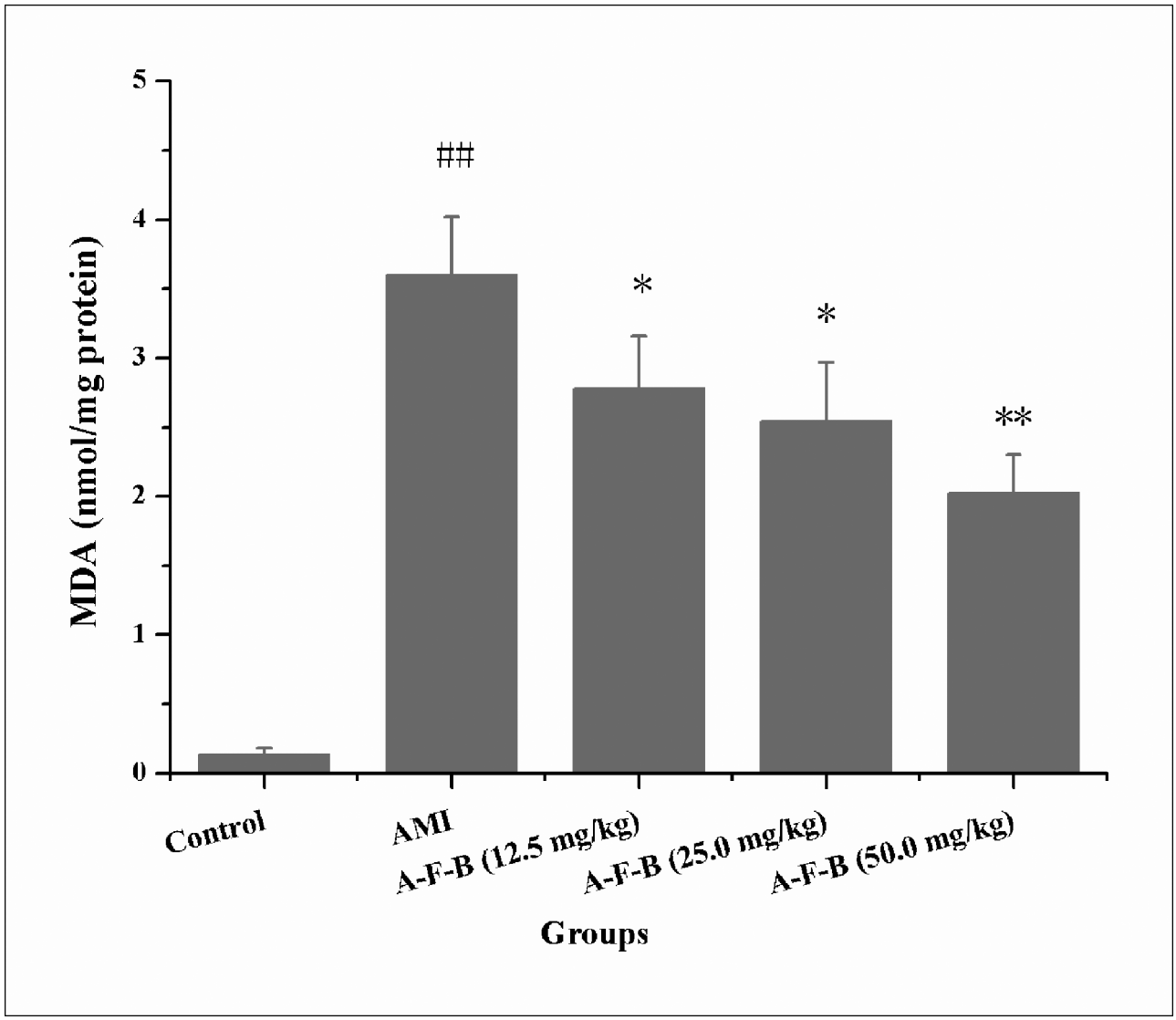

Fig. 3: Effect of A-F-B on MDA content of left ventricle in acute myocardial ischemic rats. All data are expressed as the means ± standard deviation (n = 8). Differences among groups were analyzed by one-way ANOVA, then Student-Newman-Keuls test. ## $P < 0.01$ versus control group; * $P < 0.05$ or ** $P < 0.01$ versus AIM group

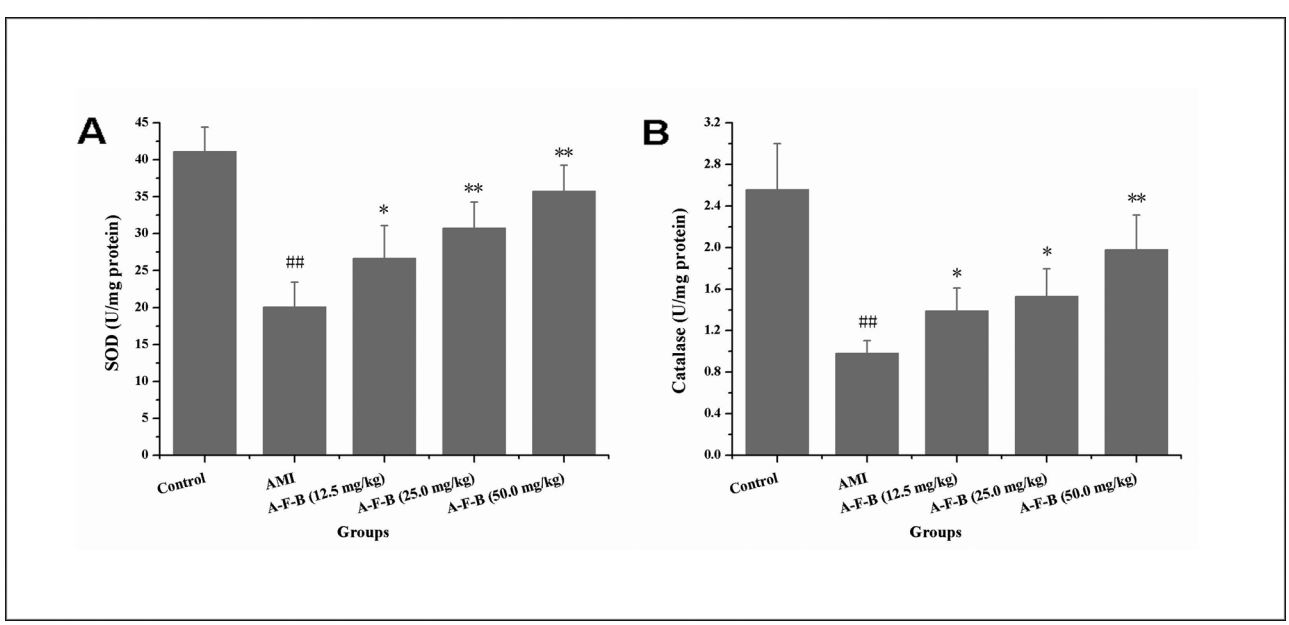

Fig. 4: Effect of A-F-B on SOD (A) and catalase (B) activities of left ventricle in acute myocardial ischemic rats. All data are expressed as the means ± standard deviation (n = 8). Differences among groups were analyzed by one-way ANOVA, then Student-Newman-Keuls test. ## $P < 0.01$ versus control group; * $P < 0.05$ or ** $P < 0.01$ versus AIM group

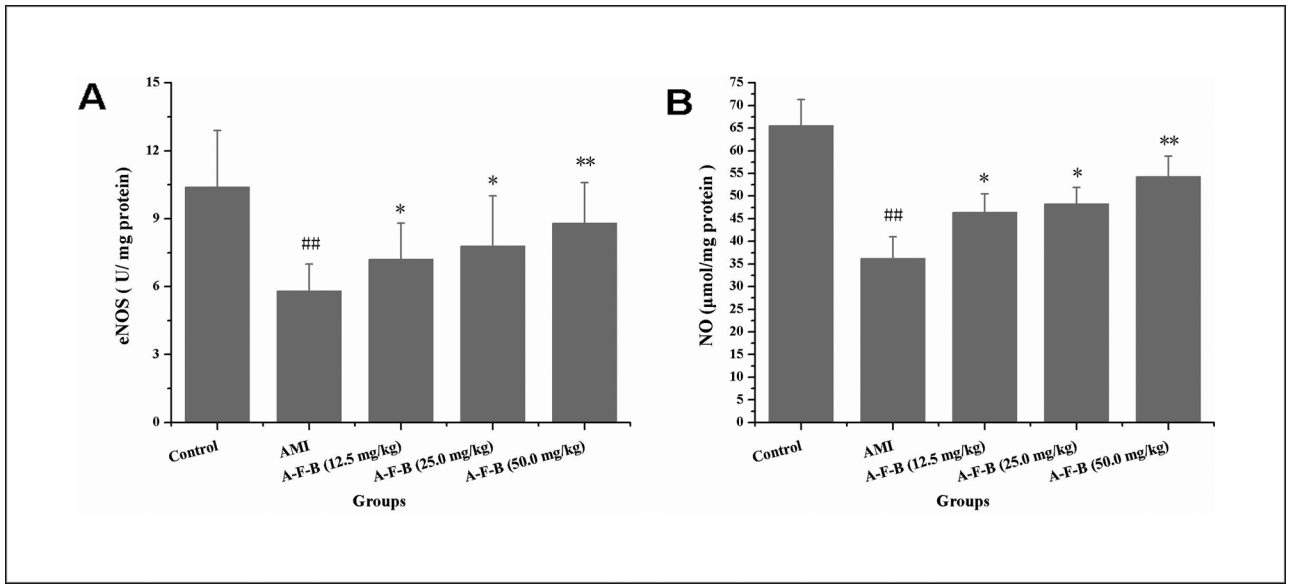

Fig. 5: Effect of A-F-B on eNOS (A) activity and NO content (B) of left ventricle in acute myocardial ischemic rats. All data are expressed as the means ± standard deviation (n = 8). Differences among groups were analyzed by one-way ANOVA, then Student-Newman-Keuls test. ## $P < 0.01$ versus control group; * $P < 0.05$ or ** $P < 0.01$ versus AIM group

the internal ROS environment, scavenge exorbitant ROS, and maintain redox balance. In our study, A-F-B appears to work by preserving SOD activity as well as inhibiting lipid peroxidation in the ischemic myocardium, as shown by the depressed formation of MDA content. This implies that the cardioprotective effects of A-F-B contribute to saving endogenous SOD activity under oxidative stress, at least, in part. However, we cannot be certain whether A-F-B exerts such effects by stimulating the expression of endogenous SOD at the mRNA or protein levels and then enhances SOD function subsequently.

NO formed by endothelial NO synthase (eNOS) causes vasodilatation, blood flow increase, decreased vascular resistance, hypotension, inhibitions of platelet aggregation and adhesion, and also leukocyte adhesion and migration, leading to prevention of thrombosis and atherosclerosis (Moncada et al. 1991). Furthermore, NO exerts other beneficial actions as an antioxidant (Forstermann 2008) and plays a role in protecting endothelial cells from apoptosis (Ho et al. 1999). Attenuated NO production and action may result in serious circulatory failure especially in the heart. In our experiment, A-F-B attenuated the reduction of eNOS activity induced by acute myocardial ischemia and thus enhanced NO content. Considering that NO can limit infarct size, maintain endothelial function, and improve myocardial contractility (Pernow et al. 1994), it is possible that the cardioprotective effects of A-F-B will contribute to saving eNOS activity under oxidative stress condition.

In conclusion, the present experiment showed that A-F-B exerted cardioprotective effects against acute myocardial ischemia injury in rats, and indicated A-F-B as an effective and promising medicine for the prevention of ischemic heart disease.

4. Experimental

4.1. Preparation of A-F-B

The leaves of *Actinidia kolomikta* were collected in Jingyu country in Changbai Mountain of China in June 2008 and authenticated by Prof. Minglu Deng at Changchun University. The dried, powdered leaves (2.0 kg) were mixed and added by 6 fold of 90% ethanol. They were refluxed 4 times for 2 h each time at room temperature. After filtration, the ethanol extracts were added to nonpolar macroporous resin (AB-8 Anhui, China) column and washed by water and 90% ethanol. Then, the eluent was treated by decompressed drying method to obtain extract of *Actinidia kolomikta*. The extract was dissolved with 75% ethanol again and added acetone to it. The suspension was filtered, and the precipitate was subjected to a silica gel column chromatography (2 cm × 20 cm) and eluted with a stepwise gradient of ethyl acetate:ethanol:H_2O (10:1:0.3) and then underwent further chromatography on silica gel columns, employing the same eluent systems, to give a A-F-B. The yield of the extract as a dried material was 1.4 wt.% of the original material. Further analysis by RP-HPLC showed that the content of A-F-B in the resultant extract was 99.4%.

4.2. Animals

Male Wistar rats weighing 180 - 220 g were from the Experimental Animal Center of Hebei University. They were housed individually in a well-ventilated animal unit (22 ± 2 °C, humidity 60 ± 5%, 12 h light/dark cycle) and had access to rodent chow and water *ad libitum*. All animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85 - 23, revised 1996) and Committee of Hebei University for the Care and Use of Laboratory Animals.

4.3. Chemicals and reagents

Thiobarbituric acid, nitroblue tetrazolium, H_2O_2, and triphenyltetrazolium chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The reagents for measuring serum creatine kinase activity and eNOS activity were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). NO detection kit was from Eastern Asia Radioimmunity Research Institute (Beijing, China). The ELISA kit of troponin T was purchased from Westang Biomedical Technology Company (Shanghai, China).

4.4. Induction of acute myocardial ischemia in rats

Male Wistar rats were randomly divided into five groups as follows: control group, AIM group, A-F-B groups. The rats in A-F-B groups were orally administered with A-F-B at the dose of 12.5, 25.0, or 50.0 mg/kg once a day for 7 days continually. While the rats in control group an AIM group were treated with the same volume of distill water once daily for 7 days. Acute myocardial infarction model was produced by ligation of the left coronary artery as described previously (Stanton et al. 2000) with minor modifications. Briefly, 30 min after the last dose, rats were anesthetized with chloralose (300 mg/kg, intraperitoneally). Endotracheal intubation was performed under direct visualization. The rats were ventilated with air (tidal volume, 3 mL/100 g; respiratory rate, 60 times/min) by a ventilator (DW-3000 Anhui, China). A thoracotomy was performed in the fourth intercostals space under sterile conditions. A 1.0 silk thread was passed around the LAD near its origin using a tapered needle. Both ends of the thread were passed through a 1 cm long polyethylene tube (outer diameter, 2.0 mm), which was used to ligate the coronary artery by pulling the thread. Then the thoracic cavity was sutured. Body temperature was measured by an electric thermometer placed in the rectum and maintained at 37 °C by a heating pad placed under the rats. The rats in control group were subjected to the same procedures without LAD ligation.

4.5. Measurement of MIS

At 6 h in post-occlusion, the heart was harvested and the ventricle of heart was cut from the apex to the base into four transverse slices and incubated in 1% (w/v) triphenyltetrazolium chloride for 10 min. By this staining, normal myocardium appeared brick red and the infarct area was unstained. The slices were immediately fixed with 10% formalin for 24 h. The ischemic zone was dissected from the nonischemic zone by the same lab assistant who was blind to the treatment. MIS was calculated as: weight of ischemic zone/total weight of the ventricle × 100% (Haiyun et al. 2004).

4.6. Assay of CK-MB and troponin T

To measure CK-MB activity and troponin T content, blood samples were collected in dry test tubes without anticoagulant at the end of each experiment. The samples were centrifuged (2500 *g*) at 4 °C for 15 min. Serum CK-MB activity was measured using a commercial kit according to the instructions provided by the manufacturer. The level of troponin T was assayed using an ELISA kit.

4.7. Measurement of eNOS, NO, SOD, Catalase, and MDA

Six heart tissues in control and eight heart tissues in other group were excised, rinsed in ice-cold isotonic saline, and then homogenized in 0.1 mol/L ice-cold phosphate buffer (pH 7.4; 1:10 w/v) for biochemical assays. The total protein was estimated by the method of Lowry et al. (1951). eNOS activity was measured with a commercial kit according to the instruction provided by the manufacturer. Because most of the NO is rapidly converted to nitrite and further to nitrate, the levels of nitrite/nitrate were measured with the NO detection kit according to the manufacturer's instruction. Briefly, nitrate was converted to nitrite with aspergillus nitrite reductase, and the total nitrite was measured with the Griess reagent. The absorbance was determined at 540 nm with a microplate reader (Bio-TEK,Windoski, VT, USA). SOD activity was determined by the NBT reduction method (McCord and Fridovich 1969). Catalase activity was assayed in cytosolic fraction, based on H_2O_2 decomposition followed at 240 nm for 30 s (Aebi 1984).The determination of MDA content in heart tissues was performed according to the method of Ohkawa et al. (1979).

4.8. Statistical analysis

All data are expressed as the means ± standard deviation (SD). Differences among groups were analyzed by one-way ANOVA, then Student-Newman-Keuls test. Values of $P < 0.05$ were considered statistically significant.

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