The pharmacologically active components of *Oxytropis falcata* bunge reduce ischemic-reperfusion injury in the rat heart

Dejun Zhang¹²*, Ruofei Jiang¹, Eun-Kyung Hong³, Guosheng Tan¹, Dianxiang Lu¹, Yongping Li¹ and Wangtao Yuan²

State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining 810016, P.R.China.

Received: 05-04-2016

Accepted: 29-11-2016

DOI: 10.18805/lr.v0i0.7592

**ABSTRACT**

*Oxytropis falcata* is used as the main material to prepare various Tibetan traditional medicine, which its activity was reported that anti-inflammation and analgesia. Flavonoids is the chemical basis for the anti-inflammatory and anti-oxidant efficacy. The influence of total flavonoids of Tibetan herb *O. falcata* on myocardial ischemic-reperfusion injury was investigated using ethanol, chloroform, and ethyl acetate extracts. Rats were pretreated with different concentrations of the extract for 7 days. The model of myocardial ischemic-reperfusion injury was established by ligating the left anterior descending (LAD) artery in the heart. Following reperfusion for 40 minutes, the enzyme activity of creatine kinase (CK), lactic acid dehydrogenase (LDH), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), and the concentration of malondialdehyde (MDA) in the rat serum were measured. The ultrastructural pathological change was observed under transmission electron microscopy (TEM). The serum levels of CK, LDH, and MDA were reduced, and the activity of SOD and GSH-Px were increased significantly, by chloroform and ethyl acetate extracts (*P*<0.01, *P*<0.05). The ultrastructural pathological change in myocardial ischemic-reperfusion injury was alleviated by both extracts. The major components in chloroform extract were 7-hydroxy-flavonone and 2',4'-dihydroxy chalcone. 7-Hydroxy-flavanon naringenine naringetol and 2',4'-dihydroxy chalcone were the major components in ethyl acetate extract. Total flavonoids of Tibetan herb *O. falcata* might alleviate myocardial ischemic-reperfusion injury.

**Key words:** Active ingredients, Myocardial ischemic-reperfusion injury, *O. falcata* Bunge.

**Abbreviations:** CK: creatine kinase, LDH: lactic acid dehydrogenase, SOD: superoxide dismutase, GSH-Px: glutathione peroxidase, MDA: malondialdehyde

**INTRODUCTION**

*O. falcata* Bunge belongs to *Oxytropis* DC (Leguminosae)(Peter et al. 2010). It is mainly produced in the Tibetan Plateau. The parts of the plant that have traditionally been used in folk medicine are the rhizome and whole plant. It is traditionally used for detoxification, anti-inflammation, pain relief, astringent pulse, removal of heatÿproduce muscle fibers, and cure sores. It is also used for the treatment of Malaria, haemorrhage, constipation, anthrax, topical treatment of tonsillitis, eliminating blood stasis, and remove osteoma. It controlled bleeding with astringents, and improved tissue regeneration including blood vessel. *O. falcata* Bunge, called the “King of Herbs”(« Chinese Materia Medica» editorial committee, 1999; Liu, 1997), is the main material used to prepare a variety of Tibetan medicinal compounds and traditional Tibetan medicine, as well as more mainstream clinical medicine. To date, although many scholars have paid close attention to the research on *O. falcata* Bunge (Zhao et al., 2009; Wang et al., 2010; Cheng et al., 2009; Huo et al., 2008), the knowledge regarding its pharmacological activity is still in its infancy. Reports relating to its pharmacological activity are almost entirely focused on its properties of astringent pulse, anti-inflammation, and analgesia. Recent research has shown that the total amount of flavonoids in *O. falcata* Bunge is the chemical basis for the anti-inflammatory and antioxidant efficacy (Yang, 2010). The question that needs to be addressed is, do the total flavonoids of *O. falcata* Bunge reduce myocardial ischemic-reperfusion injury? In order to answer this, we selected the ethanol, chloroform, and ethyl acetate extract as the main plant components, and the effect of these components on myocardial ischemic-reperfusion injury was investigated.

**MATERIALS AND METHODS**

**Preparation of extract:** *O. falcata* was collected from Gonghe County in Qinghai province, and was identified by
the Northwest Plateau Institute of Biology of the Chinese Academy of Science. A voucher specimen was deposited in the College of Eco-Environmental Engineering, Qinghai University, Xining, P. R. China.

Ethanol extract was prepared from the powder of *O. falcata*, which were soaked in 75% solution of ethanol for seven days. The supernatant was condensed by rotary evaporator (Seestar rotary evaporator, Wuxi City Seestar Five Biochemical Equipment Co., Ltd), and ethanol extract was obtained. The ethanol extract was mixed with water to form a solution and successively partitioned using chloroform and ethyl acetate. Finally, the refined liquid was condensed using a rotary evaporator and lyophilized.

**Analysis of *O. falcata* Bunge extract:** The chloroform extract of *O. falcata* was analyzed by HPLC-MS. HPLC was performed on a 4.6×250mm Diamonsil C18 column packed with 5um particles (Dikma, Beijing, China) at a flow of 1mL/min, of which 250uL/min was shunting for mass spectrometer. The gradient was from 5% mobile phase B containing CAN and 0.1% for micacid (both HPLC-grade, Sigma–Aldrich) to 35% Bin 25 min, followed by a washandre-equilibration step. Molecular weight analyses were conducted using an LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA) equipped with an electrospray ionization source. The mass spectrometer was operated in the positive ion mode with the spray voltage set at 4.0Kv and the heated capillary at 120°C.

**Animals and administration of extracts:** Sprague-Dawley rats (180±20 g) were purchased from the Experimental Animal Center at the Medical College of Lanzhou, China (Certificate of quality: SCXK (Gan) 2011-0001). The animals were housed in a 12-hour light-dark cycle at 25±2°C, in a relative humidity of 30-60%. The animals were fed ad libitum on a diet of standard pellets and water.

The dosage administration of the ethanol extract at a high, middle, and low dose, the chloroform extract at a high and low dose of total flavonoids, and the ethyl acetate extract at a high and low dose of total flavonoids was based on the LD_{50} of the acute toxicity of *O. falcata* Bunge, which was determined during a previous experiment. The ethanol extract at a high, middle, and low dose was given at 1.5 g·kg^{-1}, 1.0 g·kg^{-1}, and 0.5 g·kg^{-1} per day; the chloroform extract at a high and low dose was given at 1.0 g·kg^{-1} and 0.5 g·kg^{-1} per day, and the ethyl acetate extract at a high and low dose was given at 0.5 g·kg^{-1} and 0.25 g·kg^{-1} per day, respectively. The positive control group was given propranolol dinitrate at 0.02 g·kg^{-1} per day. The sham and model groups were given the same volume of normal saline (NS). The test extracts were administered by mouth once a day, seven days, at precisely 9am.

**Animal grouping:** The groups of experimental animals were randomly divided into 10 separate groups after having been together for seven days; (1) ischemic-reperfusion model group (abbreviation: IR model), (2) sham group, heart was exposed by pericardiomy but without ischemia-reperfusion (the normal serum control group), (3) ethanol extract high-dose group, (4) ethanol extract middle-dose group, (5) ethanol extract low-dose group, (6) chloroform high dose of total flavonoids group, (7) chloroform low dose of total flavonoids group, (8) ethyl acetate high dose of total flavonoids group, (9) ethyl acetate low dose of total flavonoids group, (10) propranolol dinitrate control group (control group).

The experiment was approved by the Institutional Committee on Ethics of Animal Experimentation at Qinghai University.

**Myocardial ischemic-reperfusion injury (MIRI) models and specimen collection:** The rat was anesthetized by injection with 20% urethane (0.5 ml·100 g^{-1}, i.p.), and then fixed in the supine position. Electrode needles were inserted under the skin of the rat’s limb (sub-dermally, not intramuscularly). The other side of the needles was linked to the ECG instrument. Standard II ECG of the experimental animals was detected. Animals with an abnormal ECG should be rejected. Following tracheal intubation, the rat continued to be ventilated by an artificial animal respirator under positive pressure breathing (frequency: 65 times min^{-1}; respiration ratio 1:3; tidal volume: 10 ml·200 g^{-1}) (Yan et al., 2007). According to reported methods, a thoracotomy should be carried out on the left sternum of the rats, roughly at the third or fourth intercostals, and the pericardium should be peeled back to completely expose the heart into the field of vision. At the same time, the left anterior descending coronary artery (LADC) needs to be located between the pulmonary conus and the left atrial appendage. Circular atrumatic suture needles and 5-0 sutures were used, which should be placed under 2 mm from the start of the LADC. With the exception of the sham group that crossed lines without ligating, the other groups were ligated instantly using 5-0 sutures. When the artery was ligated, a tiny plastic pipe, approximately 1.0 mm in diameter, was placed between the blood vessels and the sutures. The ECG must be observed when the LADC is being ligated. A successful sign of myocardial ischemia is when the ST segment of the II ECG is raised by 0.1 mV or the T-wave towers, the QRS-wave widens and the amplitude increases, and the myocardial color changes to light red (Wang et al., 2003). Ligation should be removed after 30 minutes of ischemia, and 40 minutes of reperfusion should be conducted. Successful reperfusion was seen as the decline of the elevated ST and the gradual recovery of the T-wave (Tan et al., 2009; Zhang et al., 2008).

Blood was collected from the celiac artery and placed at room temperature for approximately 15 minutes. The serum was obtained by use of a centrifuge and spun at a speed of 2000 revolutions per minutes for 15 minutes, and
kept at -20°C. The heart was put on ice after the rats had been sacrificed. Myocardial tissues from the ischemic area were cut and immediately placed into 4% glutaraldehyde.

**Observations of myocardial ultrastructure and serum enzymes:** Following the last administration, the rats were anesthetized by injection of 20% urethane. An electrode needle of a multichannel physiological recorder was inserted under the skin of the rat’s limb. ECG of standard limbs and basic heart rate was recorded, and the change in ST was observed at the time of ischemia and reperfusion. The principal indicators observed regarding the ischemic research were the offset degrees.

After reperfusion, the rat’s heart was immediately removed by dissection and washed with a standard saline solution. The blood was removed and the whole heart was used to determine the content of SOD, MDA, and NO, and the activity of GSH-Px, NOS, and iNOS. When blood was collected from the celiac artery, serum was separated by centrifugation. The content of LDH and CK was determined.

For each group, 3 samples were fixed in 3% glutaraldehyde solution for 2 hours (1/15 M PBS), rinsed three times, once every 10 minutes, and fixed in 1% osmium tetroxide for 1.5 hours. The samples were then rinsed again three times, once every 10 minutes, and dehydrated in ethanol at a gradient of 50%, 70%, 80%, 90%, and 100%, once every 10 minutes, and then twice using pure acetone, once every 10 minutes. Following this, the samples were soaked for 2 hours and then embedded in 1:1 EPON-812 epoxy, followed by polymerization at 35°C for 24 hours, 45°C for 24 hours, and 65°C for 24 hours. The samples were sliced into semi-thin sections, dyed with toluidine blue, observed under a light microscope, further sliced into ultra-thin slices and observed and recorded using a Japanese transmission electron microscope (JEOL-1230 TEM). Each sample was amplified 1,000 times, and 7-10 shots were randomly collected using a quantitative CCD camera. For each group qualitative photographic film of normal and abnormal myocardial ultrastructure was taken.

**Statistical analysis:** Statistical calculations were carried out using SPSS 13.0. Data are presented as x±s. Statistical differences between groups were tested by one-way ANOVA. The differences were considered significant at \( P<0.05 \).

**RESULTS AND DISCUSSION**

**Identified compounds in chloroform extract of \textit{O. falcata}:** Many compounds in chloroform extract of \textit{O. falcata} were identified from the data of HPLC-MS as following. Oxytriphine, N-benzyol-phenylethylamine, 7-hydroxy-flavonone, 2',4'-Dihydroxy chalcone, N-benzyol-hydroxyphenylethylamine, 2',4'-Dihydroxy dihydro- chalcone, anagyrine, luponine, N-cinnamoyl-2-phenylethylamine, 7-methoxyflavonone, 2'-methoxy-4'-hydroxychalcone, 2'-hydroxy-4'-methoxychalcone, 2',4',4'-trihydroxychalcone, Pinocembrin, glycyrrhizin, fermononetin, Pinostrobin, 5-hydroxy-7-methoxyflavanone, -baptigenin, Oxytropine C, rhamnetin, m-Methoxyaniline, 2-monolinoleoyin, Kaempferol(3-O-(6'-acetyl)-β-D-glucoside, β-daucosterol, and anagyrine were identified from the chloroform extract of \textit{O. falcata}. The major components are 7-hydroxy-flavonone, 2',4'-Dihydroxy chalcone, which are shown in Figure 1.

We have identified compounds in ethyl acetate extract of \textit{O. falcata} as following. Phenethylamine, 7-Hydroxyflavone, 7-hydroxy-flavanon Naringenine Naringetol, Apigenin,2',4'-Dihydroxychalcone, Phenyl ethyl, Chrysin, isoliquiritigenin, Salicin, Naringenin, luteolin, Quercetin, 2,4-Dihydroxy-4'-dimethoxychalcone, Kaempferol, 5,7-Dihydroxy-4'-methoxy-hydroxy-2-phenyl,3,7-Dihydroxy-2',4'-Dimethoxyisoflavan,
Isorhamnetin, beta-sitosterol, Myricetin, kaempferide-7-O-
beta-D-glucopyranoside, beta-Daucosterin, 5,7-Dihydroxy-4'-
methoxy-2-phenyl-4-benzo pyrone-3-O-beta-
galactopyranoside, 7-oxoisosterol, 7a-hydroxy-sitosterol, 
5,6-Dihydroxy-2,7,3',42 -Tetramethoxyflavone, 5,6-
Dihydroxy-2,7,42-Tri-methoxyflavone, 3',4',5,7-
Tetrahydroxy Matteucino, Diisobutyl phthalate, 2-hydroxy-
ethylbenzene-Cinnamamide, The major components are 7-
hydroxy-flavonon Naringenine Naringetol, and 2',4'-
Dihydroxy chalcone, which are shown in Figure 2.

Our research shows that the main active components 
of the chloroform and ethyl acetate extracts of O. falcata 
Bunge are flavonoids (Yang, 2010; Wang et al., 2012; Zhang 
et al., 2014).

Observations of ECG: MIRI is one of the pathological 
processes that is characterized by a series of complex 
physiological and pathological changes.

The electrocardiogram observations showed that 
the ethanol extract at high, middle, and low doses, 
the chloroform extract at high and low doses, and the ethyl 
acetate extract at high and low doses, significantly reduced 
the elevated level of the ST segment in ECG that was caused 
by myocardial ischemic-reperfusion injury in rats. Compared 
with the model group, the results were found to be 
significantly different (P<0.01). The reduction in the level 
of the ST segment was found to have a proportional 
relationship with the administered dose of ethanol extract. 
In addition, compared with the high dose of ethanol extract, 
the high and low dose of chloroform and ethyl extracts acetate showed statistical differences in the reduction of the ST 
segment level (P<0.05). The results are shown in Table 1.

The mechanism of MIRI is related to excessive 
radical production during the reperfusion period, calcium 
overload in cells, activated vascular endothelial cells and 
leukocytes, and disordered energy metabolism 
in cardiomyocytes (Jiang, 2007). Changes in myocardial 
ultrastructure and myofilament damage could be observed 
under the electron microscope.

The change of serum enzymes: LDH and CK, important 
cardiomyocytes, appear to leak from necrotic 
cardiomyocytes in some diseases. Myocardial ischemia and 
damage could cause increased release of LDH and CK. The 
content of LDH and CK in serum correlates with the severity 
of myocardial injury. These enzymes were chosen as indicators 
to evaluate the effect of different extracts of O. falcata against 
myocardial ischemic injury (Min et al., 2011; Xu, 2002).

Compared with the model group, the ethanol extract 
at high, middle, and low doses, the chloroform extract at 
high and low doses, and the ethyl acetate extract at high and low 
doses, reduced the level of LDH and CK in the serum 
(P<0.05). The reduction in enzyme levels by the ethanol, 
chloroform, and ethyl acetate extract was proportional to 
the administered doses (P<0.05, P<0.01). Compared with 
the high-dose ethanol extract, the chloroform extract at high 
and low doses were shown to significantly reduce the level 
of LDH (P<0.05). The ethyl acetate extract at high and low 
doses significantly reduced the levels of LDH (P<0.05) and 
CK (P<0.01). The results are shown in Table 2. Compared 
with a high dose of ethanol extract, the chloroform and ethyl 
acetate extract reduced the level of LDH and CK much more 
effectively. Additionally, it was proven that the pharmacological effect of the chloroform and ethyl acetate 
extracts was much better than that of the ethanol extract at a 
high dose.

The activity of myocardial enzymes: SOD is an important 
oxygen free radical scavenger used as the main indicator of 
the degree of injury from free radical damage. The activity 
of SOD indirectly reflects the body’s capability of removing 
free radicals. The activity of MDA shows the degree of lipid 
peroxidation and indirectly reflects the severity of cellular 
injury from free radicals. MDA has become an index to 
evaluate the degree of injury in myocardial ischemia (Li et 
et al., 2012; Guan, 2006). GSH-Px is one of the most important

Table 1: Comparison of the change in ST segment of the ECG among 10 groups (x±s), n=10

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Myocardial Ischemia</th>
<th>Myocardial Ischemia (U/mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10min</td>
<td>30min</td>
</tr>
<tr>
<td>IR Model</td>
<td>1.47±0.28</td>
<td>3.41±1.15</td>
</tr>
<tr>
<td>Sham Operation</td>
<td>1.45±0.29</td>
<td>2.32±0.30</td>
</tr>
<tr>
<td>Ethanol Extract High Dose</td>
<td>1.42±0.36</td>
<td>2.10±0.33**</td>
</tr>
<tr>
<td>Ethanol Extract Middle Dose</td>
<td>1.43±0.22</td>
<td>2.40±0.32**</td>
</tr>
<tr>
<td>Ethanol Extract Low Dose</td>
<td>1.44±0.28</td>
<td>2.07±0.38**</td>
</tr>
<tr>
<td>Chloroform High Dose</td>
<td>1.40±0.34</td>
<td>2.11±0.25**</td>
</tr>
<tr>
<td>Chloroform Low Dose</td>
<td>1.42±0.30</td>
<td>1.66±0.22**</td>
</tr>
<tr>
<td>Ethyl acetate High Dose</td>
<td>1.41±0.31</td>
<td>1.90±0.35**</td>
</tr>
<tr>
<td>Ethyl acetate Low Dose</td>
<td>1.42±0.33</td>
<td>1.75±0.33**</td>
</tr>
<tr>
<td>Control Group</td>
<td>1.42±0.32</td>
<td>2.51±0.49**</td>
</tr>
</tbody>
</table>

Note: Compared with the IR model: * P<0.05; compared with the ethanol extract of high dose: ▲ ▲ P<0.01, ▲ P<0.05.
enzymes in vivo, which catalyzes the decomposition of peroxide. It plays a fundamental role in the elimination of harmful peroxidation metabolites, the blockade of the lipid peroxidation chain reaction, and the protection of membrane integrity and cellular functionality (Qin et al., 2006).

As shown in Table 3, compared with the model group, the ethanol extract at high, middle, and low doses, the chloroform extract at high and low doses, and the ethyl acetate extract at high and low doses, increased the activity of SOD and GSH-Px in myocardial tissue and decreased the activity of MDA (P<0.05, P<0.01) dose dependently. Furthermore, the increase in SOD and GSH-Px activity, and the decrease in MDA activity, were proportional to the administered doses. Compared with the ethanol extract at a high dose, the chloroform and ethyl acetate extract at high doses increased the activity of SOD and GSH-Px, and decreased the activity of MDA significantly (P<0.05). It was proved that a high dose of chloroform and ethyl acetate extract had a greater effect than that of the ethanol extract.

Observations of myocardial ultrastructure by TEM: The

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH-Px (U.mg⁻¹pro)</th>
<th>SOD (n U.mg⁻¹pro)</th>
<th>MDA (n mol mg⁻¹pro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR model</td>
<td>39.29±3.77</td>
<td>118.22±16.13</td>
<td>14.59±2.31</td>
</tr>
<tr>
<td>Sham Operation</td>
<td>67.82±3.56</td>
<td>187.65±43.21</td>
<td>7.54±0.61</td>
</tr>
<tr>
<td>Ethanol Extract High Dose</td>
<td>50.67±6.85</td>
<td>142.54±12.64</td>
<td>8.98±1.90</td>
</tr>
<tr>
<td>Ethanol Extract Middle Dose</td>
<td>45.05±5.86</td>
<td>140.25±18.15</td>
<td>9.03±0.81</td>
</tr>
<tr>
<td>Ethanol Extract Low Dose</td>
<td>43.66±5.50</td>
<td>131.49±21.07</td>
<td>9.23±1.73</td>
</tr>
<tr>
<td>Chloroform High Dose</td>
<td>52.17±2.19</td>
<td>173.18±28.90</td>
<td>8.68±1.52</td>
</tr>
<tr>
<td>Chloroform Low Dose</td>
<td>47.51±2.20</td>
<td>168.75±39.79</td>
<td>9.01±2.75</td>
</tr>
<tr>
<td>Ethyl acetate High Dose</td>
<td>51.01±2.15</td>
<td>175.15±41.75</td>
<td>8.66±1.86</td>
</tr>
<tr>
<td>Ethyl acetate Low Dose</td>
<td>45.67±3.27</td>
<td>169.31±39.77</td>
<td>10.97±1.55</td>
</tr>
<tr>
<td>Control Group</td>
<td>54.30±4.20</td>
<td>165.83±16.28</td>
<td>7.95±0.96</td>
</tr>
</tbody>
</table>

Note: Compared with the IR model: "*P<0.01," *P<0.05; compared with the ethanol extract of high dose: ▲ ▲ P<0.01, ▲ P<0.05.
observations of myocardial histopathological change is very important method for evaluation of myocardial injury. In particular, mitochondria are an essential organelle for cardiomyocyte energy generation and a cell’s life. Ischemic-reperfusion can cause tissue injury when mitochondria are damaged (Yoshiki, 1997).

The results of the myocardial ultrastructure by TEM are shown in Figure 3. 1. In model group, numerous contraction bands were seen in the myofibrillar. The myoneme was sparsely arranged and the sarcoplasmic reticulum was expanded. The myocardial intercalated disc was dissociated and the mitochondria appeared to be swollen. The cristae had loosened and come apart, and some parts of the cristae seemed to have disappeared. 2. In the sham group, the muscle fibers were arranged sparsely in the sarcolemma, and parts of the myonem had dissolved. The size and shape of the mitochondria were abnormal. 3. In the ethanol extract at a high dose group, the structures of the cardiac fiber were completely normal. 4. In the ethanol extract at a middle dose group, the structures of the muscle fiber showed mild contraction. The density of the myonem in the sarcomere was slightly sparse, and the mitochondria were not significantly damaged. 5. In the ethanol extract at a low dose group, the myofascial was mildly swollen. The myoneme with partial myofibrillar were missing and arranged sparely. The density of the myonem was dissociated and the mitochondria were partially swollen. 6. In the chloroform extract at a high dose group, the structure of the cardiac muscle fiber was normal. The sarcomere, the myocardial nucleus, the sarcolemma, and the interstitial microvascular area appeared almost normal. 7. In the chloroform extract at a low dose group, the myocardial contraction bands were seen, but their degree was not obvious. 8. In the ethyl acetate extract at a high dose group, the muscle fibers were normal. 9. In the ethyl acetate extract at a low dose group, the muscle fibers were contracted slightly. 10. In the control group, the structure of the muscle fibers appeared to be normal. Normal myocardial fiber structures were also observed in the high-dose group of the ethanol and chloroform extract. In all three extract groups, the level of free radicals generated during reperfusion was inhibited, and the activity of endogenous antioxidant systems was improved.

In the present study, ultrastructure examination showed myocardial tissue injury after ischemia, which was aggravated after reperfusion. In comparison with the model group, the cardiac sarcomere appeared almost normal, had a myocardial nucleus, perinuclear muscle fibers, and cardiac sarcolemma, and the interstitial microvascular structure was observed in the ethanol, chloroform, and ethyl acetate extracts at a high dose. In addition, the muscle fiber arrangement was normal. On the other hand, myolysis, rare muscle fiber degeneration and necrosis, and contraction bands, were observed in the ethanol, chloroform, and ethyl acetate extract low-dose groups. Compared with the ethanol extract at a high dose, normal cardiac muscle fiber structure was also observed in the chloroform and ethyl acetate high-dose groups. It was demonstrated that chloroform and ethyl acetate had the same effect as ethanol extract at a high dose. It was also proven that a high dose of chloroform and ethyl acetate had a much greater effect than that of the ethanol extract in reducing myocardial ischemic-reperfusion injury.

It was found from the present research that the chloroform and ethyl acetate extracts of O. falcata Bunge were much more effective than the ethanol extract in reducing
myocardial ischemic-reperfusion injury. We have concluded flavonoids of *O. falcata* Bunge have major role against myocardial ischemic-reperfusion injury.

**REFERENCES**


**ACKNOWLEDGMENTS**

This work was supported by the Qinghai Province Science and Technology Office (NO. 2014-ZJ-914).