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# Protective Effects of Total Flavonoids from *Dracocephalum moldavica* L. on Myocardial Ischemia / Reperfusion Injury in the Isolate Rats Heart

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**The purpose of this study was to examine the cardioprotective effects of total flavonoids from *Dracocephalum moldavica* L. (DML) on ischemia/reperfusion injury (I/R) using an isolated Langendorff rat heart preparation. Hearts were perfused with Krebs-Henseleit (K-H) solution containing DML (100 ug/L) for 10 min before 20-minute global ischemia, then reperfusion was begun with K-H solution for 40 min. The left ventricular developed pressure (LVDP) and maximum up/down rate of left ventricular pressure (±dp/dtmax) were recorded by physiological recorder as the myocardial function. And the level of superoxide dismutase (SOD), malondialdehyde (MDA) and GSH/GSSG ratio were analyzed to determine the oxidative stress status in myocardial tissue. Compared with I/R injury group, pre-incubation with DML (100 ug/L) significantly improved LVDP and ±dp/dtmax during reperfusion. Notably, the level of MDA significantly decreased ($P<0.01$) in DML group compared to I/R injury group, as well as, the SOD activity ($P<0.01$) and total glutathione ($P<0.05$) increased. The results implied that the DML has obviously protective effects on myocardial reperfusion injury.**

**Key words:** Myocardial Ischemia/Reperfusion, SOD, MDA, GSH/GSSG.

In [1]ischemia-reperfusion, disturbances in cellular homeostasis threaten the life of myocardial cells. Although recent notions have implicated myocardial cells renewal to be of possible clinical importance after myocardial infarction (Nadal-Ginard *et al*., 2003; Beltrami *et al*., 2001), cardiac tissue has limited ability to replace dead cells (Ahuja *et al*., 2008). Therefore prevention of cell death bears therapeutic implications during ischemia-reperfusion.

As generation of reactive oxygen species is a primary reason of ischemia-reperfusion injury (Murphy and Steenbergen, 2008). One of the preventive strategies is to reduce the magnitude of oxidative stress. Reduction of oxidative stress can be achieved by administration of exogenous antioxidants, although administration of individual antioxidant vitamins has not always produced favorable results (Dhalla *et al*., 2000).

Flavonoids are the most potent and versatile biologically active compounds in plant and have been known as outstanding antioxidant and neuroprotection (Petr *et al*., 2002; Vauzour *et al*., 2008). Flavonoids were known to be major active chemical component isolated from medicinal plants. Dracocephalum moldavica L .(DML) is an important traditional Chinese medicine which has been used to treat heart and brain diseases, activate blood circulation, dissipate blood stasis, relieve pain and detoxicate for thousands of years (Feng and Li, 2003).

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Pharmacological studies showed that DML was able to ease angina, improve myocardial ischemia, decrease blood viscosity and inhibit platelet aggregation (Granule *et al*., 2009). Based on the anti-myocardial ischemia/reperfusion effect of flavonoids, in addition to its antioxidant activity, it might be possible for DML to prevent myocardial ischemia/reperfusion injury. Thus, we performed the present experiment to examine the protect effects of DML against myocardial ischemia/ reperfusion and understand the mechanisms potentially.

## METHODS AND MATERIALS

### Chemicals and reagents

Total flavonoids (puritye"80%) was extracted from shade dried whole plant in Xinjiang Institute of Meteria Medica Research. All other chemicals and reagents were of analytical grade.

### Ischemia/Reperfusion (IR)

Rats were anesthetized with chloraldurat (350 mg/kg). Hearts were excised quickly and immediately immersed in ice-cold Krebs-Henseleit buffer containing 118 mM NaCl, 1.2 mM KH2PO4, 4.7 mM KCl, 1.7 mM CaCl2, 1.2 mM MgSO4, 20 mM sodium acetate and 10 mM glucose, pH 7.4. The buffer was kept at 37 °C and bubbled continuously with Oxygen. Then the excised hearts, were cannulated through the aorta by a Langendorff apparatus, and perfuse in retrograde with K-H buffer containing 95% O2-5% CO2 throughout the experiment. Perfusion pressure was maintained at 75 mmHg. A water-filled latex balloon, coupled to a pressure transducer (Statham), was inserted into the left ventricular cavity via the left auricle for pressure recording. Ventricular end-diastolic pressure (LVEDP) was adjusted between 5-12 mmHg. The hearts were stabilized for 30 min, and then global ischemia and reperfusion were established for 15 min and 45 min, respectively. Control group hearts were perfused for 90 min stabilization period. During the experiment left ventricular developed pressure (LVDP), left ventricular end diastolic pressure (LVEDP), heart rate (HR) and rate of developed pressure during contraction and relaxation (±dP/dtmax) were monitored continuously using 4S AD Instruments biology polygraph. The heart effluents were collected for 1-min intervals at selected times for determination of coronary flow.

### Animals and Experimental Groups

Male Wistar rats weighing 250~300 g were randomly assigned to one of the group of the control, IR, or DML (Dracocephalum moldavica L.). Control group hearts were perfused for the 90 min stabilization period. IR group hearts were stabilized for 30 min, and then global ischemia and reperfusion were established for 15 min and 45 min, respectively. DML group hearts were stabilized for 20 min, instead K-H buffer with DML (100 ug/L) and then global ischemia and reperfusion were established for 15 min and 45 min, respectively.

### Assay of lipide oxidative damage

At the end of the perfusions, the hearts were harvested and kept at -70 °C for later analysis. The frozen ventricles were crushed to a powder by liquid nitrogen-chilled tissue pulverizer. For tissue analyses, weighed amounts of the frozen tissues were homogenized in appropriate buffer using micro-centrifuge tube homogenizer.

Then the GSSH, GSG, SOD and MDA were analyzed spectrophotometrically according to the instruction of the assay kits.

## RESULTS

### Myocardial function index

As shown in Fig 1-4 and Table 1, compared with control group, ischemia/Reperfusion significantly decreased the myocardial diastole and constriction function. Meanwhile preconditioning with DML, myocardial diastole and constriction function index was obviously increased, compared with Ischemia/Reperfusion group.

### Effect of DML on GSH/GSSG ratio

As shown in Fig 5, compared with control group, the ratio of GSH/GSSG significantly decreased after Ischemia-Reperfusion. In DML pretreatment group, the ratio of GSH/GSSG keeps at higher level.

### Effect of DML on SOD, MDA activities

To identify anti-oxidative capacity of DML, we determined the level of SOD and MDA in myocardial tissue after Ischemia/Reperfusion pre-treatment with DML. The activity of SOD significantly increase, while MDA content significantly decrease compared with I/R group (Table 2).

**Table 1.**Effect of DML on CF in rats subjected to Ischemia-Reperfusion. ($\bar{x} \pm x1\%$)

| Groups | Reperfusion | | |
|---|---|---|---|
| | 15 min | 30 min | 45 min |
| Control | 85.00±2.31 | 81.50±3.22 | 80.00±3.1 |
| I/R | 55.61±13.05 ## | 51.30±10.21 # | 47.01±7.19 ## |
| DML | 65.12±7.79* | 61.84±6.12** | 56.98±6.24** |

Results were presented as means ±SEM (n=8). *#P<0.05, ##P<0.01* compared with control group. **P<0.05, **P<0.01* compared with I/R group.

**Table 2.**Effect of DML on SOD, MDA level in rats subjected to Ischemia-Reperfusion ($\bar{x} \pm s$)

| Groups | Dose μg/ml | Reperfusion | |
|---|---|---|---|
| | | SOD (U/mg·Prot) | MDA(μmol/kg·Prot) |
| Control | - | 696.32±30.55 | 0.11±0.01 |
| I/R | - | 382.92±28.32## | 0.36±0.03## |
| DML | 0.1 | 649.08±43.21** | 0.17±0.02** |

Results were presented as means ±SEM (n=8). *#P<0.05* compared with control group. *##P<0.01, *P<0.05* compared with control group. ***P<0.01* compared with I/R group.

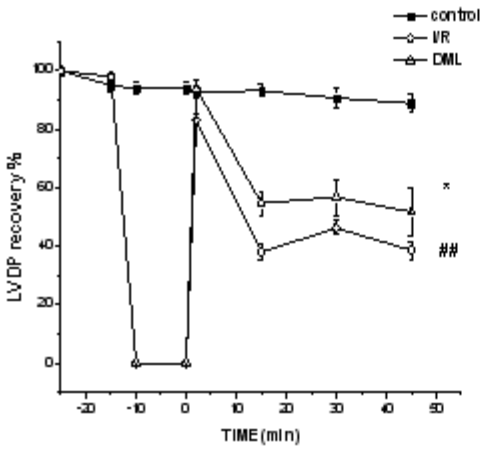



**Fig 1 .** The variation curve of comparing the value of LVDP each time point after ischemia-reperfusion with 10min before ischemia

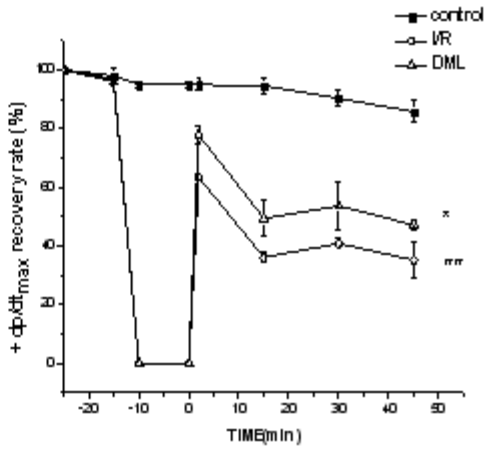



**Fig 2 .** The variation curve of comparing the value of +dp/dtmax each time point after ischemia-reperfusion with 10min before ischemia

## DISCUSSION

A lot of flavonoid compounds, such as tilianin, agastachoside, acacetin, apigenin, luteolin, kaempferol, isorhamnetin and syringaresinol, were identified from the whole plant of Dracocephalum moldavica L.(Li and Ding, 2001; Gu *et al*., 2004).

The present study for the first time demonstrated that Dracocephalum moldavica L. total flavonoids educed neuroprotection by

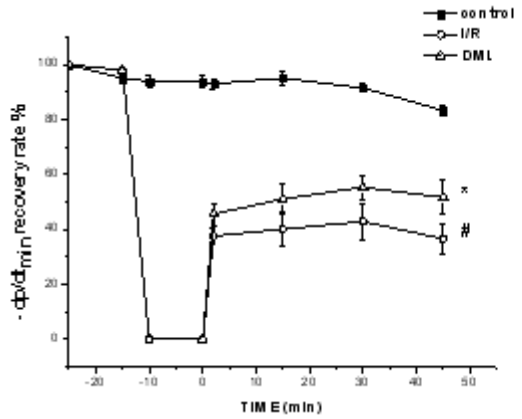



**Fig 3 .** The variation curve of comparing the value of -dp/dtmin each time point after ischemia-reperfusion with 10min before ischemia

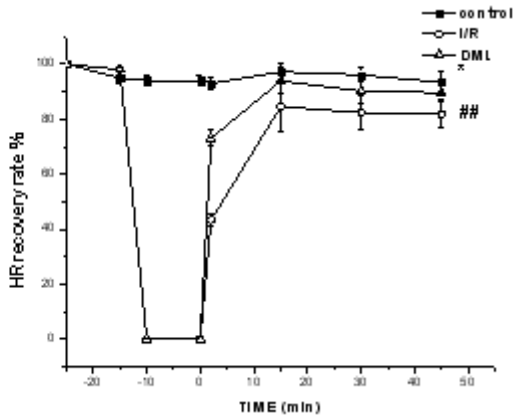
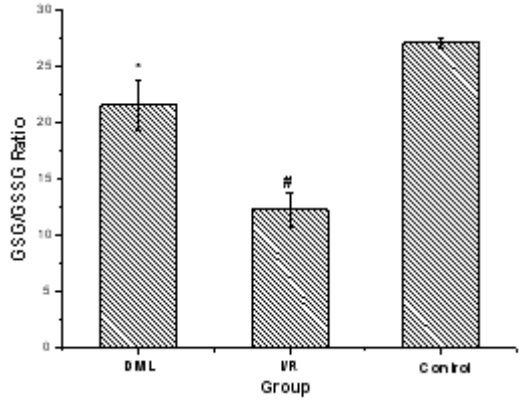



**Fig 4 .** The variation curve of comparing the value of HR each time point after ischemia-reperfusion with 10min before ischemia Results were presented as means ±SEM (n=8). #P<0.05 compared with control group. ##P<0.01, *P<0.05 compared with control group.. **P<0.01 compared with I/R group.




Results were presented as means ±SEM (n=8). #P<0.05 compared with control group. ##P<0.01, *P<0.05 compared with control group. **P<0.01 compared with I/R group.

**Fig. 5.** Effect of DML on GSH/GSSG ratio in rats subjected to Ischemia-Reperfusion

attenuating oxidative stress in Ischemia/ Reperfusion rats, which was consistent with previous reports.

The present study showed that, Ischemia/ Reperfusion significantly decreased the myocardial diastole and constriction function, LVDP, CF, HR, +dP/dtmax and -dP/dtmin ,which was consistent with previous reports(Zheng *et al*., 2008), while preconditioning with DML, myocardial diastole and constriction function index was obviously increased, compared with IR group.

Oxygen radicals and reactive oxygen species (ROS) are known to be generated in large amounts under inflammatory conditions and in the first few minutes of postischemic organ reperfusion (Becker *et al*., 2008).

GSH (glutathione), a tripeptide comprised of glutamate, cysteine and glycine, plays key roles as antioxidant and neuromodulator in the central nervous system (Aoyama *et al*., 2008). The reduced glutathione/ oxidized glutathione (GSH/GSSG) ratio serves as an index of redox state (Jones, 2002). In the present study, decreased in GSH/GSSG ratio after myocardial ischemia-reperfusion indicates that ischemia/reperfusion altered redox state in heart tissue. DML ameliorated the GSH/GSSG ratio suggesting that it might modulate myocardial antioxidant defense by elevating tissue redox status.

When ROS production exceeds antioxidant defense system capacity, oxidative stress occurs. Primary antioxidant enzymes, such as SOD, work in parallel with nonenzymatic antioxidants to protect tissue from oxidative damage induced by ROS (Wu *et al*., 2009). Previous study have shown that pretreatment with DML could increase antioxidant enzyme activities after heart ischemia/reperfusion.

MDA, carbonyl and 8-OHdG have been proposed as the most frequently monitored markers

of oxidative damage during ischemia/reperfusion injury. Consistent with earlier reports, to observed obvious increase in oxidative damage, as evident from increased MDA contents in model group (Briyal *et al*., 2009; Khan *et al*., 2009; Koumura *et al*., 2009). DML treatment significantly reduced these oxidative damage product contents indicates that DML was able to protect heart from oxidative injury.

In summary, the present study indicated that DML preconditioning reduced ischemia-reperfusion injury in rat heart through improving myocardial function and reducing peroxidation, which might be contributed to the alleviation of lipid peroxidation. Further studies are also needed to investigate whether other mechanisms play roles on DML's protect ischemia-reperfusion Injury effect.

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