

Effects of Total Extract of *Dracocephalum moldavica* on Ischemia/Reperfusion Induced Arrhythmias and Infarct Size in the Isolated Rat Heart

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Abstract

Objective(s)

Dracocephalum moldavica (*D. moldavica*) have been traditionally used as a cardiogenic agent in the folk medicine of some regions of Iran. In the present study, effects of total extract of *D. moldavica* on ischemia/reperfusion induced arrhythmias and infarct size investigated in isolated rat heart.

Materials and Methods

The isolated hearts were mounted on a Langendorff apparatus then perfused during 30 min regional ischemia and 120 min reperfusion, either by a modified Krebs-Henseleit solution as the control group or by enriched Krebs solution with total extract of *D. moldavica* (25-200 µg/ml) as the treatment groups. The ECGs recorded and analyzed to determine cardiac arrhythmias. At the end of the reperfusion, the hearts stained by Evans blue solution then incubated by triphenyltetrazolium chloride. The volume of infarcted tissue and percentage of infarct size determined by computerized planimetry.

Results

The results demonstrated that total extract of *D. moldavica* caused a significant reduction in the number of ventricular tachycardia (VT), total ventricular ectopic beats (VEBs) and VT duration in ischemic and reperfusion periods. The incidence of ischemic VT reduced from 93% in the control group to 0, 50 and 50% in the treatment groups. The infarct size was 37±1% in the control group, however, perfusion of the extract (25, 50, 200 µg/ml) reduced it to 13±2, 8±1 and 18±2%, respectively ($P<0.001$). In addition, the extract remarkably lowered volume of infarcted tissue compared to the control group ($P<0.05$).

Conclusion

Our findings showed cardioprotective effects of total extract of *D. moldavica* against ischemia/reperfusion injuries in the isolated rat heart.

Keywords: Arrhythmia, *Dracocephalum moldavica*, Ischemia, Myocardial infarction, Rat, Reperfusion,

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Introduction

Dracocephalum moldavica (*D. moldavica*) is a perennial herb belongs to the Lamiaceae (Labiatae) family. In Iran, the plant known as *Badershoo* has traditionally been used for its culinary properties plus treatment of stomach and liver disorders, headache and congestion (1). *D. moldavica* is also, used as a cardiogenic agent in the folk medicine of Iran (2). *In vitro* evaluation of *D. moldavica*'s total extract showed anti-*Helicobacter pylori* activity (3). Other reported pharmacologic properties of this plant are sedative, carminative, skin anti-inflammatory (as a topical preparation) (4, 5), spasmolytic, anti-microbial (6) and fungicidal effects (7).

During myocardial ischemia, depressed oxygen supply results in the uncoupling of oxidative phosphorylation, leading to the accumulation of beta-hydroxy fatty acid intermediates and acyl CoA molecular species. The accumulation of fatty acids and their intermediates during ischemia can be deleterious to the recovery of myocardial function in the reperfused heart (8).

Most of the previous studies have focused on analyzing and detecting the phytochemical compounds of *D. moldavica*. Dastmalchi *et al* (2007) reported the presence of polar compounds including hydroxycinnamic acids and flavonoids, with caffeic and ferulic acids, luteolin and apigenin in the total extract of *D. moldavica* (9). In another study, two important compounds (syringaresinol 4-O-beta-D-monoglucoside and beta-daucosterol) were identified in the extract (10). Some compounds in the total extract demonstrated antioxidant activity (9, 11) and daucosterol showed potent protective effects against cellular oxidative damages in comparison with lovastatin (10). The presence of apigenin in the extract decreased the platelet aggregation by reduction of cyclic AMP response to prostacyclin (12). In addition, syringaresinol 4-O-beta-D-monoglucoside produced calcium channel blocking activity (13) which may probably protect heart from stress damage (14).

Despite the potential protective effects of some chemical compounds of *D. moldavica* (antioxidant activity, protection against

oxidative damages and platelet aggregation, etc.), there is no report regarding cardioprotective effects of total extract of *D. moldavica* against ischemia/reperfusion injuries. Therefore, in the present study, the effects of total extract of *D. moldavica* on ischemia/reperfusion (I/R) induced cardiac arrhythmias and infarct size were investigated in the isolated rat heart.

Materials and Methods

Total extract preparation of the plant

Aerial parts of *D. moldavica* were freshly collected from open field (Maragheh- East Azerbaijan, Iran) in June 2006 and a voucher specimen (No. D151-TBZ) was deposited at herbarium of School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. Hundred g of the air dried, powdered aerial parts of the plant were extracted with MeOH-H₂O (70:30) (3×1 liter) by maceration in room temperature for 12 hr each time. The extracts were combined and concentrated *in vacuo* to yield a dried extract. The dried extract residue was kept at 4 °C for experiments (15).

Animals and surgical procedure

Male Wistar rats weighing 270-330 g used in this study. The rats pretreated with i.p. injection of 300 IU heparin then anaesthetized with sodium pentobarbital (50–60 mg/kg, i.p.) (16). The hearts excised rapidly and mounted on a non-recirculating langendorff apparatus under 100 mmHg pressure at 37 °C and perfused throughout the experiments with modified Krebs-Henseleit (K/H) solution which was previously equilibrated with 95% O₂-5% CO₂. A fluid filled balloon introduced into the left ventricle and inflated to give a preload of 8–10 mmHg (16, 17). After 20 min stabilization, the hearts subjected to 30 min regional ischemia and 120 min reperfusion. In control group (n=10), the hearts perfused only by normal K/H solution throughout the experiment, while in the treatment groups (3 groups, n=10 in each group), they perfused with enriched K/H solution with the total extract of *D. moldavica* (25, 50 and 200 µg/ml, respectively) during 30 min regional ischemia followed by 120 min reperfusion. Induction of regional ischemia achieved by temporary

occlusion of left anterior descending (LAD) coronary artery followed by 120 min reperfusion. An epicardial ECG and hemodynamic factors including left ventricular developed pressure (LVDP) and the heart rate (HR) recorded by a polygraph during the experiment. Based on the Lambeth conventions, the ECGs analyzed to determine the total number of ventricular ectopic beats (VEBs), the number of beats occurring as ventricular tachycardia (VT), the incidence and duration of VT, ventricular fibrillation (VF) during ischemia and the first 30 min of reperfusion time (18,19).

Measurement of myocardial infarct size

To determine the infarct size, at the end of 120 min reperfusion period, the ligature around the LAD artery re-tied and the heart slowly perfused with 2-3 ml of saline solution containing 0.25% Evans blue dye (w/v) via the side arm of the aortic cannula (20).

The hearts frozen, and then the ventricles of the frozen hearts sliced transversely in a plane perpendicular to the apico-basal axis into 2 mm thick sections. The slices incubated with 1% (w/v) triphenyltetrazolium chloride (TTC) solution in phosphate buffer for 15 min at 37 °C to dye the non-infarcted region (20, 21). This procedure resulted in the normally perfused tissue being stained blue, non-infarcted, non-perfused tissue stained brick red, infarcted tissue remaining unstained and appeared pale (22).

Statistical analysis

Except for the incidence of VT and VF that indicated as percentage, all results expressed as mean±SEM. To compare the number of VT, VEBs and duration of VT, VF between groups, the Mann-Whitney non-parametric

U-test employed. Analyzing the incidence of VT and VF accomplished by Fisher Irwin test (Chi-square with Yates correction). Hemodynamic changes, infarcted volume and percentage of infarct size analyzed, using one-way ANOVA and then considerable differences examined by LSD post hoc range test (18, 20). Differences between groups were considered significant at a level of $P < 0.05$.

Results

During stabilization, perfusion of total extract of the *D. moldavica* caused large reduction in LVDP (Figure 1). LVDP in the control group was 98 ± 3 mmHg at 15th min of stabilization, however, the extract (25, 50 and 200 µg/ml) reduced this figure to 86 ± 1 , 60 ± 6 and 48 ± 3 mmHg, respectively ($P < 0.001$ for all). At the same time, HR was also decreased from 300 ± 16 beats/min (control) to 203 ± 12 , 190 ± 6 and 153 ± 3 beats/min in the treatment groups, ($P < 0.001$).

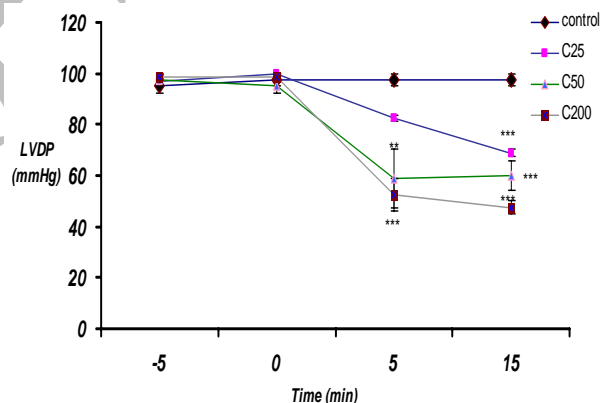


Figure 1. Effects of total extract of *D. moldavica* on left ventricular developed pressure (LVDP) during stabilization time in isolated rat hearts. $**P < 0.01$, $***P < 0.001$ versus control group. n=10 rats in each group.

The effects of total extract of *D. moldavica* on ischemic and reperfusion arrhythmias are summarized in Table 1.

Table 1. Effects of total extract of *D. moldavica* (25-200 µg/ml) on cardiac arrhythmias during 30 min ischemia and 30 min reperfusion in isolated rat hearts.

Groups	Ischemia time			Reperfusion time		
	VF Duration (sec)	VT Duration (sec)	VT incidence (%)	VF Duration (sec)	VT Duration (sec)	VT incidence (%)
Control	44±29	19±5	93	101±41	23±8	80
<i>D. moldavica</i> (25 µg/ml)	1±1*	0 ***	0***	5±4*	3±3 **	20 **
<i>D. moldavica</i> (50 µg/ml)	47±26	10±4	50*	21±14	2±1 **	40
<i>D. moldavica</i> (200 µg/ml)	113±100	18±7	50*	229±43	10±5	50

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group. n=10 rats in each group.

During ischemia, the incidence of VT in the treatment groups with 25-200 $\mu\text{g}/\text{ml}$ of the extract, reduced in order of 93% (control) to 0 ($P<0.001$), 50 ($P<0.05$) and 50% ($P<0.05$), (Table 1). In addition, the extract decreased VT duration in comparison with the control group but the effect was only notable in the 25 $\mu\text{g}/\text{ml}$ group (Table 1). At the same time, 25 $\mu\text{g}/\text{ml}$ of the extract significantly lowered the duration of Rev VF ($P<0.01$). The extract also, decreased the number of VT and total VEBs during the ischemic period (Figure 2).

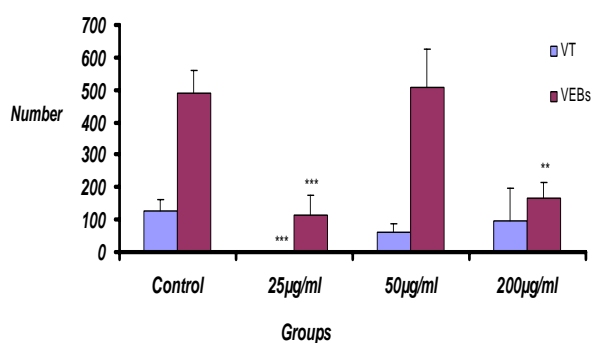


Figure 2. The total number of ventricular ectopic beats (VEBs) and ventricular tachycardia (VT) in the control and isolated rat hearts receiving total extract of *D. moldavica* during 30 min ischemia. Data are represented as mean \pm SEM. ** $P<0.01$, *** $P<0.001$ versus control group. n=10 rats in each group.

At reperfusion time, VT incidence was lowered by all concentrations of the extract, however, the treated group with 25 $\mu\text{g}/\text{ml}$ had significant ($P<0.01$) reduction (Table 1). Similar to the ischemic phase results, the extract reduced duration of VT throughout reperfusion period with much effect at the concentrations of 25 and 50 $\mu\text{g}/\text{ml}$ (Table 1). Except for the 25 $\mu\text{g}/\text{ml}$ concentration of the extract, the higher concentrations did not produce any remarkable changes in duration of VF versus the control during the reperfusion time (Table 1). The low concentrations of the extract also, decreased the number of VT and total VEBs during the reperfusion period (Figure 3).

As shown in Figure 4, the infarct size was $37\pm 2\%$ in the control group while the perfusion of the total extract of *D. moldavica* (25, 50 and 200 $\mu\text{g}/\text{ml}$) decreased it to 13 ± 2 , 8 ± 1 and $18\pm 2\%$, respectively ($P<0.001$). In addition, the extract at the concentrations of

25 and 50 $\mu\text{g}/\text{ml}$ largely lowered the volume of infarcted tissue, from $109\pm 12 \text{ mm}^3$ to 61 ± 10 and $33\pm 9 \text{ mm}^3$, compared to the control group, ($P<0.05$).

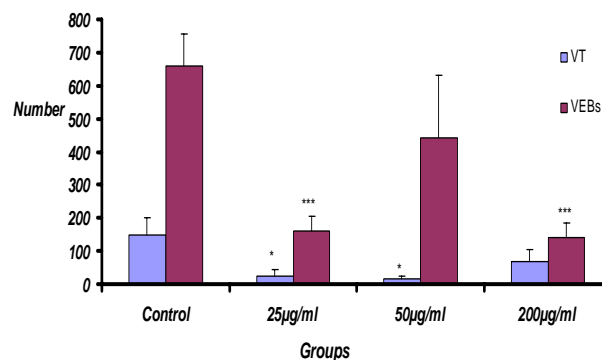


Figure 3. The total number of ventricular ectopic beats (VEBs) and ventricular tachycardia (VT) in the control and isolated rat hearts receiving total extract of *D. moldavica* during 30 min reperfusion. Data are represented as Mean \pm SEM. * $P<0.05$, *** $P<0.001$ versus control group. n=10 rats in each group.

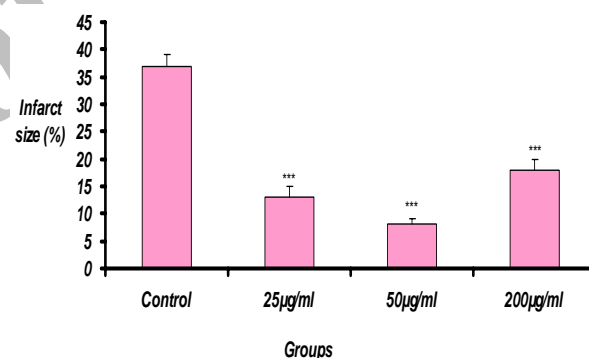


Figure 4. Myocardial infarct size in the control and isolated rat hearts receiving 25-200 $\mu\text{g}/\text{ml}$ of total extract of *D. moldavica* during 30 min ischemia followed by 120 min reperfusion. Data are represented as mean \pm SEM. *** $P<0.001$ versus control group. n=10 rats in each group.

Discussion

The most important causes of mortality in the course of cardiac surgery and myocardial infarction are ventricular arrhythmias such as VT and VF (23). For the first time, the results of present study showed that total extract of *D. moldavica* produces antiarrhythmic effects against I/R-induced arrhythmias such as VT and VF when it is used during 30 min ischemia and 30 min reperfusion.

Perfusion of low concentrations of the extract (especially 25 $\mu\text{g}/\text{ml}$) produced significant

reduction in the number of VT and VEBs, duration of VT, VF and incidence of VT during both ischemia and reperfusion time. The higher concentrations of the extract did not produce considerable change in duration of VT, VF or number of VEBs. The discrepancy between the inhibitory effect by low concentrations and the ineffectiveness of higher concentrations of the extract might be explained by the hypothesis that some of the active constituent(s) of *D. moldavica* at high concentration may probably exhibit pro-arrhythmic properties. It is also likely that the total extract may have components with different anti or pro-arrhythmic effects.

Our findings also demonstrated that the total extract of *D. moldavica* caused marked and potent protective activity against I/R injuries with resulting reduction of infarct size and infarcted volume in this model of study.

Total extract of *D. moldavica* principally contains polar compounds including hydroxyl cinnamic acids and flavonoids, with caffeic and ferulic acids, luteolin-7-O-glucoside, rosmarinic acid, luteolin and apigenin (9). Other isolated chemical compounds of the plant are syringaresinol 4-O-beta-D-monoglucoside, syringaresinol-4,4'-O-bis-beta-D-glucoside, 2''-p-coumarylstragalol, kaempferol-3-O-beta-D-(6''-O-p-coumaroyl)-galactopyranoside, takakin-8-O-beta-D-glucopyranoside and beta-daucosterol (10). Total extract of *D. moldavica* has demonstrated activity in all antioxidant assay methods (9, 14, 24-25). Some epidemiological reports have demonstrated that people may have lower incidence of heart diseases if they use a high dietary intake of flavonoids (26). The presence of flavonoids, antioxidants and compounds releasing nitric oxide (NO) from endothelium (27) in the extract may probably have important roles in preventing I/R induced injuries such as arrhythmia and infarction (28). A NO releasing agent can also mimic ischemic preconditioning and by reducing infarct size and lowering incidence of various dysrhythmias (such as VT and VF) protects the myocardium against both stunning and infarction (29). In addition, the presence of compounds with calcium channel blocking

activity such as citral and syringaresinol 4-O-beta-D-monoglucoside may be responsible for antiarrhythmic effects of the extract (13, 30). This action probably is conducted by blocking the cardiac calcium currents and therefore, by slowing the conduction and increasing the refractory period in calcium-dependent tissues such as the AV node (31). In addition, at the stabilization time, application of different concentrations of the extract also significantly decreased LVDP and HR. Maybe the reduction of LVDP and HR in the isolated hearts at stabilization time, was related to some of the above-mentioned properties of the total extract such as calcium channel blocking and vasodilatory effects. These effects may probably involve in cardioprotective effects of the extract. Moreover, the total extract contains compound with anti-stressing effect, such as syringaresinol 4, 4'-O-bis-beta-D-glucoside, which can protect heart from stress during this damage (14). This effect may probably provide another potential protective mechanism by *D. moldavica* during I/R condition.

In summary, regarding the presence of some important phytochemical compounds in the total extract of *D. moldavica* and their recognized roles discussed above, it seems that the extract may protect the isolated hearts against I/R induced injuries such as arrhythmia and infarction via different mechanisms.

Conclusion

Our findings demonstrated that perfusion of the total extract of *D. moldavica* can decrease significantly the number of VT and VEBs in ischemic and reperfusion periods in the isolated hearts. In addition, the extract caused significant reduction in duration and incidence of VT during the ischemic time. Moreover, the infarct size showed significant reduction in all treatment groups compared to the control.

By considering the data, it may be concluded that the total extract of *D. moldavica* could recover ischemic-reperfused isolated rat hearts and consequently has anti-arrhythmic and anti-infarct activity. Future studies are required to determine the exact cardioprotective mechanism(s) of the extract.

Acknowledgment

This study was supported by the Research Affairs of Tabriz University of Medical

Sciences, Tabriz, Iran. The authors declare that they have no conflict of interests.

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