Indian Journal of Experimental Biology
Vol. 51, August 2013, pp. 653-660

Cardioprotective effect of methanolic extract of *Marrubium vulgare* L. on isoproterenol-induced acute myocardial infarction in rats

Keyvan Yousefi[1], Hamid Soraya[2], Fatemeh Fathiazad[3], Arash Khorrami[2], Sanaz Hamedeyazdan[3], Nasrin Maleki-Dizaji[2] & Alireza Garjani[2,*]

[1]Student Research Committee; Tabriz University of Medical Sciences, Tabriz, Iran

[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

[3]Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

Received 28 February 2012; revised 20 May 2013

Isoproterenol injection (100 mg/kg; sc) produced changes in ECG pattern including ST-segment elevation and suppressed R-amplitude. The methanolic extract of *M. vulgare* at doses of 10, 20, and 40 mg/kg significantly amended the ECG changes. A severe myocardial necrosis and edematous along with a sharp reduction in the arterial blood pressure, left ventricular contractility ($LVdP/dt_{max\ or\ min}$), but a marked increase in the left ventricular end-diastolic pressure (LVEDP) were seen in the isoproterenol group. All parameters were significantly improved by the extract treatment. The extract (10 mg/kg) strongly increased $LVdP/dt_{max}$. Similarly, treatment with 40 mg/kg of *M. vulgare* lowered the elevated LVEDP and the heart to body weight ratio. In addition to *in vitro* antioxidant activity, the extract suppressed markedly the elevation of malondialdehyde levels both in serum and in myocardium. The results demonstrate that *M. vulgare* protects myocardium against isoproterenol-induced acute myocardial infarction and suggest that the effects could be related to antioxidant activities.

Keywords: Electrocardiography, Isoproterenol, *Marrubium vulgare*, Myocardial infarction

Marrubium vulgare L. (Lamiaceae), known as horehound, is a perennial herb with stems covered by woolly hairs[1]. The plant is a popular medicinal herb which is being used traditionally in many countries for the treatment of flu, headache and inflammatory, respiratory and gastrointestinal disorders[2,3]. The essential oil and the extract obtained from the aerial parts of *M. vulgare*[4] as well as *M. deserti*[5,6] have been shown to have strong antimicrobial and antioxidant activities. The main active ingredient that is produced and accumulated in the aerial parts of the plant is a diterpenoid known as marrubiin[7]. A substantial antioxidant, anticoagulant, antiplatelet and anti-inflammatory effects have been attributed to marrubiin[8]. *M. vulgare* also contains marrubenol and phenylpropanoid esters which have been shown to exhibit L-type calcium channel blocking[9] and cyclooxigenase (COX) inhibitory[10] activities, respectively. Further, phenylpropanoids have been proved to protect cardiomyocyte against hypoxia-induced death[11]. Acute myocardial infarction is an important ischemic heart disease and a leading cause of morbidity and mortality worldwide. Isoproterenol is a synthetic β-adrenoceptor agonist that its subcutaneous injection induces myocardial infarction in rats[12], which results in irreversible cellular damage and ultimately infarct-like necrosis[13,14]. The acute phase of myocardial necrosis induced by isoproterenol mimics changes in blood pressure, heart rate, electrocardiogram (ECG) and left ventricular dysfunction similar to that occurs in patients with myocardial infarction. The rat model of isoproterenol-induced myocardial infarction offers a reliable non-invasive technique for studying the effects of various potential cardioprotective agents[15]. Amongst various mechanisms proposed to explain isoproterenol induced cardiac damage, generation of highly cytotoxic free radicals through autooxidation of catecholamines has been implicated as one of the important causative factor[16]. Although *M. vulgar*e, as an important medicinal plant, shows strong

*Correspondent author
Telephone: +98-411-3341315; +98 914 301 4818
Fax: +98-411-3344798
E-mail: garjania@tbzmed.ac.ir; garjania2002@yahoo.com

antioxidant properties due to presence of flavonoids, terpenes, and phenols[17,18] its cardioprotective activity against isoproterenol induced myocardial infarction has not been elucidated. The aim of the present study is to investigate the effect of oral administration of methanolic extract of aerial parts of *M. vulgar*e on isoproterenol induced myocardial injury. Left ventricular dysfunction, oxidative stress, and histopathological changes induced by isoproterenol were examined and their modulation with different doses of *M. vulgar*e extract was evaluated.

Materials and Methods

Plant material—The aerial parts of *M. vulgare* were collected during flowering stage during June 2011 from Kiasar, Mazandaran, Iran. The botanical identification was made by Dr. M. Mazandarani (Department of Biology, Azad Islamic University, Gorgan, Iran). Voucher specimens (712 Tbz-Fph) were deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Extract preparation and TLC analysis—Dried aerial parts (200 g) were grounded and extracted with methanol (2L × 4) by maceration at room temperature and the solvent was removed at 40 ºC using a rotary evaporator. A greenish residue weighing 17.8% (w/w) was obtained and kept in air tight bottle in a refrigerator until use. To identify the chemical constituents, the obtained methanolic extract was subjected to preliminary phytochemical screening. Thin-layer chromatography (TLC) was performed on silica gel GF_{254} with benzene-acetone (17:3). Detecting the spots with spraying H_2SO_4 10% in EtOHrevealed the presence of Marrubiin at R_f = 0.63 as a major compound in the extract[19].

Determination of in vitro total antioxidant activity—1,1-diphenyl-2-picryl-hydrazil (DPPH; Sigma-Aldrich Quı´mica S.A., Madrid, Spain) was used to determine free radical-scavenging potential of the methanolic extract. IC_{50} (50% inhibitory concentrations) of methanolic extract was calculated versus MeOH as a negative control and Quercetin was used as a positive control[23]. Briefly, a stock solution of the methanolic extract was prepared in MeOH to achieve the concentration of 1 mg/mL. Dilutionswere made to obtain concentrations of 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6} and 5×10^{-7} mg/mL. Two mL of each solution was added to 2 mL of DPPH solution (in MeOH; 80 g/mL). The absorbance was measured at 517 nm after 30 min of reaction at 25 ºC. The experiments were performed in duplicate and the average absorption was noted for each concentration.

Animals—Male albino wistar rats (260-280 g) were purchased from institute of Pasture (Tehran-Iran). Rats were housed at constant temperature of 20±1.8 ºC with 50±10% RH in standard polypropylene cages, six per cage, under a 12:12 h light/dark cycle, and were allowed food and water freely. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz-Iran (National Institutes of Health publication No. 85-23, revised 1985).

Induction of acute myocardial infarction — Isoproterenol (Sigma Co., USA) was dissolved in normal saline and injected subcutaneously to rats (100 mg/kg) for two consecutive days at an interval of 24 h to induce acute myocardial infarction. Animals were sacrificed 48 h after the first dose of isoproterenol.

Experimental protocol—The animals were randomized into 6 groups consisting of 6 rats each. Rats in group 1 (normal control) received a subcutaneous injection of normal saline (0.5 mL) and were left untreated for the whole period of the experiment. In group 2 (sham) rats received a subcutaneous injection of normal saline and *M. vulgare* extract was given orally (40 mg/kg) two times per day for two days. Rats in group 3 (isoproterenol group) received a subcutaneous injection of isoproterenol (100 mg/kg) for two consecutive days at an interval of 24 h and normal saline as vehicle (0.5 mL) was given orally concurrent with and 8 h after each isoproterenol injection. Rats in groups 4 to 6 received a subcutaneous injection of isoproterenol (100 mg/kg) for two consecutive days at an interval of 24 h, *M. vulgare* extract was given orally (10, 20 and 40 mg/kg) concurrent with and 8 h after each isoproterenol injection.

Hemodynamic measurements—Animals were anaesthetized by intraperitoneal injection of ketamin, xylazin, and acepromazin mixture 48 h after the first dose of isoproterenol. When the rats no longer responded to external stimuli, the systemic arterial blood pressure was recorded from a catheter inserted into the left carotid artery. A standard limb lead II ECG was monitored continuously throughout the experimental period. The mean arterial blood pressure was calculated from the systolic and diastolic blood pressure traces. The heart rate was calculated from the

ECG. To evaluate the cardiac left ventricular function, a Mikro Tip catheter transducer (Millar Instruments, Inc.) was advanced into the lumen of the left ventricle. This helped to measure the left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum and minimum rates of developed left ventricular pressure ($LVdP/dt_{max}$ and $LVdP/dt_{min}$) and the rate of pressure change at a fixed right ventricular pressure ($LVdP/dt/P$)[20]. All the parameters were continuously recorded using a Powerlab system (AD Instruments, Australia).

Tissue weights—After the hemodynamic measure–ments, blood samples were collected from inferior vena cava in centrifuge tubes and centrifuged to obtain serum for measurement of MDA and then the animals were killed by an overdose of the anesthetic and the hearts were removed and weighed. The wet to body weight ratios were calculated to assess the degree of congestion.

Histopathological examination—The hearts were fixed in 10% buffered formalin. The tissues were embedded in paraffin, sectioned at 5 µm thickness and stained with hematoxylin and eosin (H&E) for evaluation of histology. Myocardial necrosis was evaluated in each section of the heart tissue using a morphometric point-counting procedure[21]. Two persons graded the histopathological changes as 1, 2, 3, and 4 for low, moderate, high, and intensive pathological changes, respectively.

Determination of lipid peroxidation in serum and myocardium—Malondialdehyde (MDA), a thiobarbiturate reactive substance, was measured as a marker for oxidative stress in serum and myocardial homogenates as per Satoh[22]. To prepare the homogenates, another set of experiment (n=6) including all groups was repeated and heart tissues were homogenized in a ratio of 1:10 in 0.15% KCl by means of a homogenizer. The lipid peroxide expressed as nanomole MDA production/g heart tissue and nanomole/mL serum, were measured spectrophotometrically.

Statistics—Data were presented as mean±SE. One-way-ANOVA was used to make comparisons between the groups. If the ANOVA analysis indicated significant differences, a Student–Newman–Keuls post test was performed to compare the mean values between the treatment groups and the control. Any differences between groups were considered significant at $P<0.05$.

Results

Preliminary phytochemical screening of methanolic extract of *M. vulgar*e indicated the presence of diterpenoids. The TLC of the extract showed a major band related to *marrubiin* with R_f value of 0.63. The methanolic extract of the plant was tested for its free radical scavenging effect on DPPH. The IC_{50} values for methanolic extract of *M. vulgare* and quercetin were found to be 8.24 and 3 µg/mL, respectively.

The normal control group and the rats that had received the extract alone (40 mg/kg; sham) showed normal patterns of ECG (recorded from limb lead II), whereas the rats treated with isoproterenol exhibited a marked ($P<0.001$) reduction in R-amplitude along with a significant ($P<0.001$) elevation of ST-segment, indicative of myocardial infarction (Fig. 1). Treatment with all doses of the extract demonstrated a profound reduction in the ST-segment elevation and a marked increase in the R-amplitude ($P<0.01$ and <0.001)

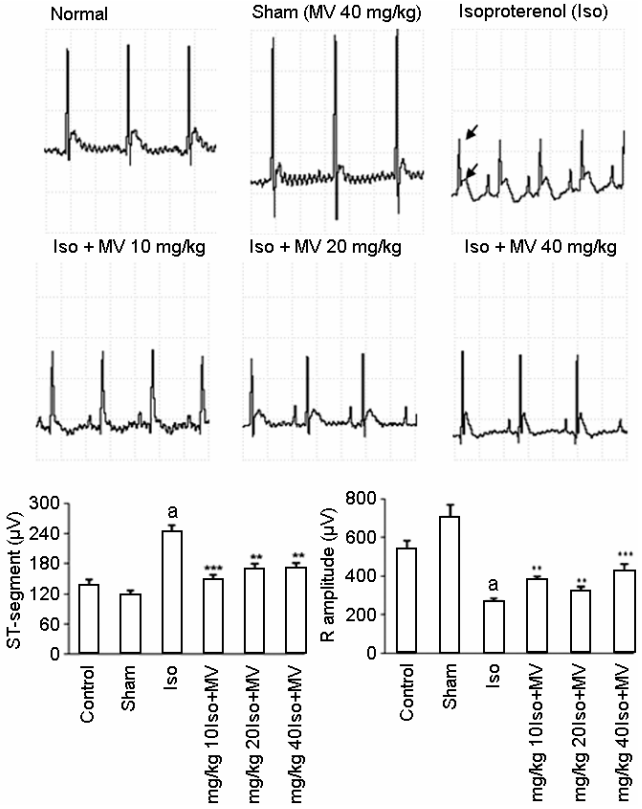

Fig. 1—Effect of the methanolic extract of *M. vulgare* (MV) on electrocardiographic pattern and changes (recorded from limb lead II) in normal control, isoproterenol alone injected (MI) and treated rats. The ST-segment elevation and R wave have been indicated by arrows. Values are mean±SE from 6 animals in each group. *P* values: [a]<0.001 from respective control value; [**]<0.01, [***]<0.001 as compared with isoproterenol treated group using one way ANOVA with Student-Newman-Keuls post hoc test.

as compared to the isoproterenol alone treated rats (Fig. 1).

The mean arterial blood pressure was significantly decreased from 107±6.3 mmHg in normal control to 66±6 mmHg in the isoproterenol treated group (P<0.001; Table 1). There was a considerable increase in the mean arterial blood pressure to 92±2.3, 98±6.2, and 108±7 mmHg, respectively after treatment with 10, 20, and 40 mg/kg of *M. vulgare* extract. Heart rate was increased from 255±5 bpm in control groups to 323±15 by injection of isoproterenol (P<0.05). The extract reduced the heart rate but this reduction did not reach a significant level. The intraventricular pressure was measured to determine the degree of left ventricular responses to the isoproterenol injection. Isoproterenol reduced the left ventricular systolic pressure (LVSP) from 125±7 in the normal control group to 72±9 mmHg (P<0.001). All doses of the extract caused a significant increase in LVSP. However, the increase produced by low dose of 10 mg/kg was much higher (168±11mmHg; P<0.001) than that of high dose of 40 mg/kg (97±10 mmHg). There was almost a 3 fold elevation in the left ventricular end diastolic pressure (LVEDP) of isoproterenol alone treated rats, thereby indicating left ventricular dysfunction. All three doses of the extract considerably (P<0.01; <0.001) improved the left ventricular function by lowering LVEDP from 19±1.2 mmHg in the rats with myocardial infarction to 8.2±2.1, 7.7±2.9, and 6.4±1.7 mmHg, respectively (Table 1). When compared with the normal control, the rats with left ventricular dysfunction (isoproterenol group) demonstrated a fall in the values of the left ventricular maximal and minimal rates of pressure (LV dP/dt_{max}; LV dP/dt_{min}, P<0.001; <0.05; Fig. 2) as well as a lower rate of pressure change at a fixed ventricular pressure (LV dP/dt/P, <0.05; Table 1). Comparable with the LVSP changes, the indices of myocardial contractility also showed a marked improvement (P< 0.001; <0.01) by all doses of the extract.

In order to assess the extent of heart weight gain developed by injection of isoproterenol, the heart weight to body weight ratio was determined (Fig. 3). The ratio was significantly higher in the isoproterenol treated rats (4.16±0.05) compared with the normal control group (2.56±0.02; P<0.001). Oral treatment with 20 and 40 mg/kg of the *M. vulgare* extract produced a substantial (P<0.01 and <0.001, respectively) reduction in the heart to body weight ratio (Fig. 3).

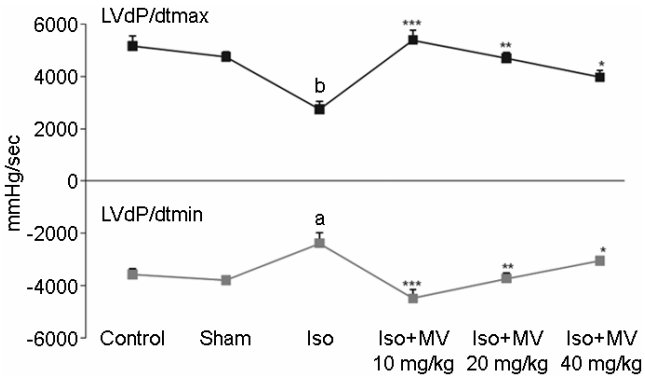

Fig. 2—Left ventricular maximal and minimal rates of pressure increase (LV dP/dt_{max}; LV dP/dt_{min}) in the control group and in the rats treated with isoproterenol alone (rats with myocardial infarction), *M. vulgare* extract alone (sham), and isoproterenol plus *M. vulgare* extract. Iso: isoproterenol; MV: *M. vulgare* extract. Values are mean±SE from 6 animals in each group. P values [a]< 0.05; [b]<0.001 from respective control value; *<0.05, **<0.01, ***< 0.001 as compared with isoproterenol treated group using one way ANOVA with Student-Newman-Keuls post hoc test.

Table 1—Effects of the methanolic extract of *M. vulgare* on hemodynamic and left ventricular function in rats with myocardial infarction.

[Values are mean±SE from 6 animals in each group]

Groups	MAP (mmHg)	Herat rate (bpm)	LVSP (mmHg)	LVEDP (mmHg)	LV dP/dt/P (1/sec)
Control	107±6.3	255±5	125±7	6.3±1.1	64±2.3
Sham	106±5.8	262±10	117±4	5.6±0.9	60±1.4
Isoproterenol	66±6[c]	323±15[a]	72±9[b]	19±1.2[c]	38±9.1[a]
M. vulgare (10 mg/kg) + isoproterenol	92±2.3[d]	315±19	168±11[e]	8.2±2.1[e]	66±2.5[d]
(20 mg/kg) + isoproterenol	98±6.2[e]	292±11	113±13[d]	7.7±2.9[e]	61±3.6[d]
(40 mg/kg) + isoproterenol	108±7[f]	302±16	97±10	6.4±1.7[f]	56±1.2[d]

MAP= Mean arterial pressure; LVSP= left ventricular systolic pressure; LVEDP= left ventricular end diastolic pressure
P values: [a,d]<0.05; [b,e]<0.01; [c,f]<0.001 compared with respective control (a, b, c) and isoproterenol group (d, e, f) using one way ANOVA with Student-Newman-Keuls post hoc test

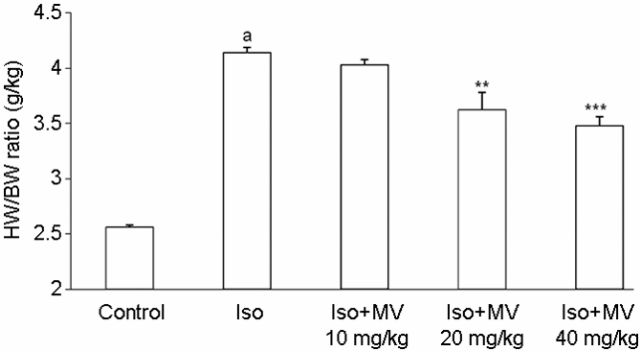

Fig. 3—Heart weight (HW) to body weight (BW) ratio in the control group and in the rats treated with isoproterenol alone (rats with myocardial infarction), and with isoproterenol plus *M. vulgare* extract. Iso: isoproterenol; MV: *M. vulgare* extract. Values are mean±SE from 6 animals in each group. *P* values:[a]<0.001 from respective control value; **<0.01; ***<0.001 as compared with isoproterenol treated group using one way ANOVA with Student-Newman-Keuls post hoc test.

In the normal control group, myocardial fibers were arranged regularly with clear striations. No apparent degeneration or necrosis was observed (Fig. 4). Histological sections of the isoproterenol treated hearts showed widespread subendocardial necrosis and abundant hyperplasia along with increased edematous intramuscular space (Fig. 4.). *M. vulgare* treatment to isoproterenol groups showed a significant protection against myocardial injury. The *M. vulgare* extract with doses of 10, 20, and 40 mg/kg reduced the isopreterenol-induced necrosis and edematous dose dependently by 32% ($P<0.05$), 48% ($P<0.001$), and 60% ($P<0.001$), respectively as shown in Fig. 5.

To determine the lipid peroxidation, MDA levels were measured in serum and myocardial homogenates. Both serum and heart MDA levels were considerably increased ($P<0.001$) in isoproterenol

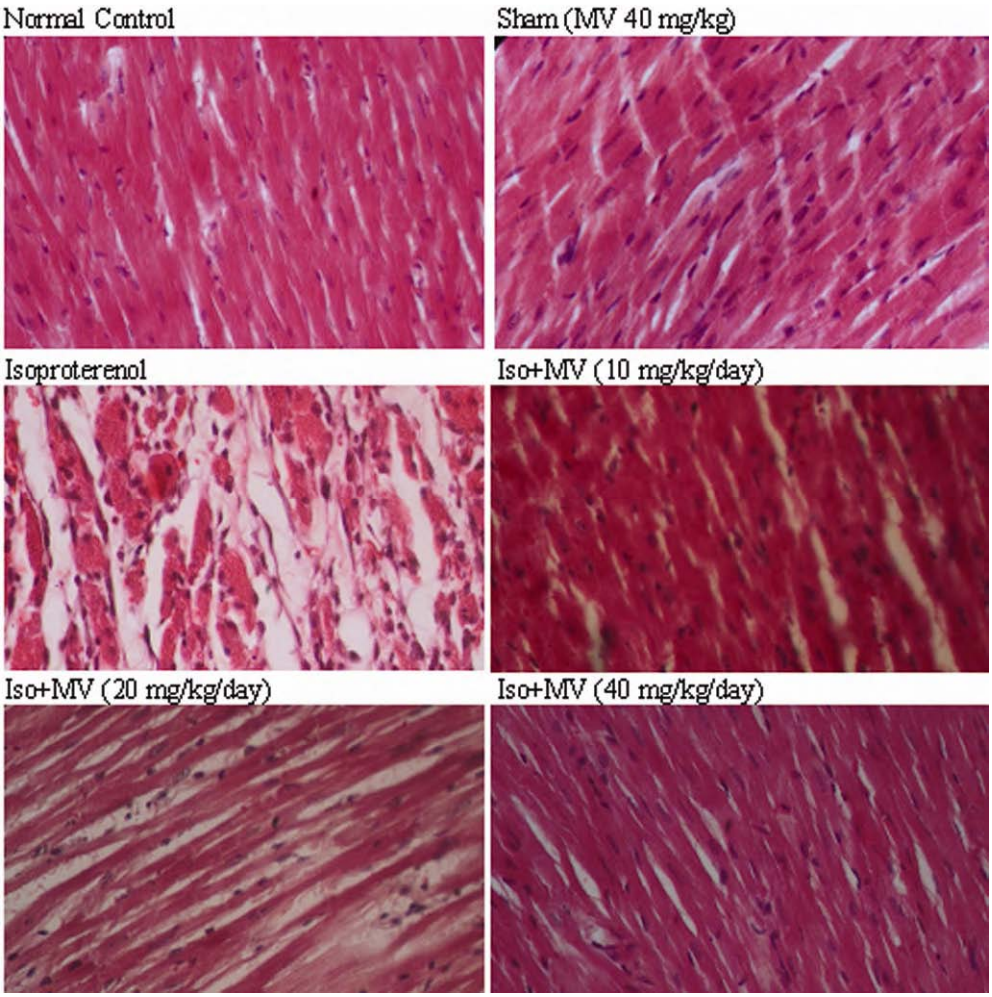

Fig. 4—Photomicrographs of sections of rat cardiac apexes. Heart tissue of a rat subcutaneously injected with isoproterenol alone shows intensive cardiomyocyte necrosis and increased edematous intramuscular space. Treatment with *M. vulgare* extract demonstrates a marked improvement. Iso=isoproterenol; MV=*M. vulgare* extract. H&E (40X).

injected rats (MI group) in comparison with normal control (Table 2). Treatment with *M. vulgare* extract markedly diminished the myocardial and serum MDA levels by almost 50-65% (P<0.001; Table 2).

Discussion

Acute myocardial infarction remains a leading cause of morbidity and mortality worldwide. The aerial parts of *M. vulgare* are being used traditionally in many countries in the treatment of inflammatory, respiratory, and gastrointestinal disorders[2,3]. However, little is known about its cardioprotective actions in cardiovascular diseases. In the present study, the therapeutic efficacy of the methanolic extract of the aerial parts of the plant was investigated in rats with acute myocardial infarction induced by isoproterenol. The ECG is considered as the most important clinical test for diagnosis of myocardial infarction. A subcutaneous injection of isoproterenol (100 mg/kg/day) for 2 consecutive days exhibited a ST-segment elevation (diagnostic of myocardial infarction) and suppressed R-amplitude. The ST-segmen televation reflects the potential difference in the boundary between ischemic and non-ischemic zones and the consequent loss of cell membrane function, whereas the decreased R-amplitude is may be due to the onset of myocardial edema following isoproterenol administration[12]. The animals on *M. vulgare* treatment showed an obvious improvement in their ECG pattern, indicating its protective effects on cell membrane functions.When administrated for a period of more than 24 h, high-dose β-agonists results in myocardial edematous and histological changes that include myocyte necrosis, myofibrilar degeneration, and leukocyte infiltration[24]. The dose of isoproterenol used in the present study was sufficient to yield a considerable myocardial edematous and cardiotoxicity. Oral administration of the extract in doses of 10, 20, and 40 mg/kg for two days clearly and dose dependently prevented myocardial injury and edematous in the isoproterenol treated rats. The subcutaneous injection of isoproterenol also significantly decreased the arterial pressure indices, left ventricular contractility ($LVdP/dt_{max}$) and relaxation ($LVdP/dt_{min}$), and increased the left ventricular end-diastolic pressure (LVEDP). These changes were very similar to the homodynamic changes seen in human acute myocardial infarction. In the treatment of post myocardial infarction heart failure it is very important to improve the contractility of the heart and at the same time decrease the left ventricular end diastolic pressure. In the present study, the beneficial effects of the methanolic extract of *M. vulgare* were clearly observed with respect to a variety of indices including improvement of arterial blood pressure, increase in myocardial contractility force, and parallel decrease in the left ventricular end diastolic pressure. The preliminary phytochemical finding of the present study revealed the presence of marrubiin as a major constituent in the methanolic extract obtained from *M. vulgare* aerial parts. Reactive oxygen species have been implicated in the progression of ventricular remodeling to myocardial infarction.The results of the present study show a considerable *in vitro* (IC_{50}= 8.24 µg/mL) and *in vivo* antioxidant activity of *M. vulgare* extract, so that the extract diminished the myocardial and serum lipid peroxidation by 50-65%. Marrubiin is a diterepe and

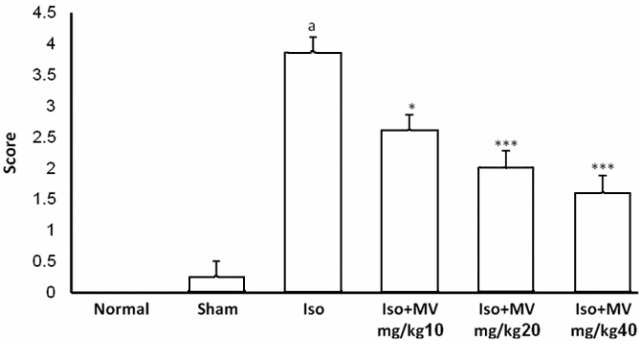

Fig. 5—Grading of histopathological changes in the rat's cardiac apex tissues. Grades 1, 2, 3, and 4 show low, moderate, high and intensive pathological changes, respectively. Iso=isoproterenol; MV=*M. vulgare* extract. Values are mean±SE from 4 animals in each group. P values: [a]<0.001 *vs* normal control, *<0.05, **<0.01, ***<0.001 as compared with isoproterenol treated group using one way ANOVA with Student-Newman-Keuls post hoc test.

Table 2—The effects of oral administration of methanolic extract of *M. vulgare* aerial parts on serum and myocardial malondialdehyde (MDA) levels.

[Values are mean±SE from 6 animals in each group]

Groups	Serum (nmol/mL)	Myocardium (nmol/mg)
Control	5.4±0.48	1.6±0.13
Isoproterenol	13.7±0.2[a]	3.9±0.36[a]
M. vulgare (10mg/kg) + Iso	4.7±0.2[c]	1.8±0.02[c]
(20mg/kg) + Iso	7.4±0.5[b]	1.9±0.05[c]
(40mg/kg) + Iso	7.8±0.6[b]	2.1±0.13[c]

Iso= Isoproterenol. P values: [a]<0.001 from respective control value; [b]<0.01, [c]<0.001 as compared with isoproterenol treated group using one way ANOVA with Student-Newman-Keuls post hoc test.

plant diterpens are antioxidant and scavenge oxygen free radicals, therefore suggesting their role in preventing of isoproterenol induced damages in this study. Moreover, marrubin has been found to have some interesting biological and pharmacologic activities including anticoagulant, anti-inflammatory, and antiplatelet effects[8]. Similar to the effect of the extract on the left ventricular function, a low dose of the extract (10 mg/kg) produced the strongest reduction on the level of MDA both in serum and in myocardium. The discrepancy that low dose of the extract had greater favorable effects on the left ventricular functions than the high dose, might be explained by this hypothesis that some of the active constituent(s) of *M. vulgare* at high concentration exhibit diverse effect such as cardio-depressive activity. In fact, as mentioned above, El-Bardai *et al*.[9] have found that marrubenol extracted from *M. vulgare* blocks L-type calcium channels. Calcium antagonists in general have anti-ischemic effects but high doses of the cardio-selective drugs can exaggerate myocardial suppression.

Myocardial healing was seen with different dose of the methanolic extract of *M. vulgare* in isoproterenol treated rats suggesting a potent protective effect of the extract on myocardial infarction.The potent cardioprotective effect of methanolic extract of *M. vulgare* L. in isoproterenol induced acute myocardial infarction could be related toits antioxidant activities.

Acknowledgment

The present study was supported by a grant from the Research Vice Chancellors of Tabriz University of Medical Sciences; Tabriz, Iran.

References

1 Appleton RA, Fulke JWB, Henderson MS & Mc Crindle R, The stereochemistry of marrubiin, *J ChemSoc C,* (1967) 1943.

2 Lorenzi H & Matos FJA, *Plantas medicinais do Brasil: nativas e exóticas* (Nova Odessa, Instituto Plantarum) 2002.

3 Newal CA, Anderson LA & Philipson JD, *Herbal medicines: a guide for health care professionals* (The Pharmaceutical Press, London) 1996.

4 Firuzi O, Javidnia K, Gholami M, Soltani M & Miri R, Antioxidant activity and total phenolic content of 24 Lamiaceae species growing in Iran, *Nat Pro Commun*, 5 (2010) 261.

5 Laouer H, Yabrir B, Djeridane A, Yousfi M, Beldovini N & Lamamra M, Composition, antioxidant and antimicrobial activities of the essential oil of *Marrubium deserti*, *Nat Prod Commun*, 4 (2009) 1133.

6 Petrović S, Pavlović M, Maksimović Z, Milenković M, Couladis M, Tzakouc O & Niketić, M, Composition and antimicrobial activity of *Marrubium incanum Desr.* (*Lamiaceae*) essential oil, *Nat Prod Commun,* 4 (2009) 431.

7 Piccoli PN & Bottini R, Accumulation of the labdane diterpene marrubiin in glandular trichome cells along the ontogeny of *Marrubium vulgare* plants, *Plant Growth Regul*, 56 (2008) 71.

8 Mnonopi N, Levendal RA, Davies-Coleman MT & Frost CL, The cardioprotective effects of marrubiin, a diterpenoid found in Leonotisleonurus extracts, *J Ethnopharmacol,* 31 (2011) 67.

9 El-Bardai S, Wibo M, Hamaide MC, Badiaa L, Quetin-Leclercq J, Moreal N, Fischer GP, McGregor A & Saria B, Characterization of marrubenol, a diterpene extracted from *Marrubium vulgare*, as an L type calcium channel blocker, *Br J Pharmacol,* 140 (2003) 1211.

10 Sahpaz S, Garbacki N, Tits M & Bailleul F, Isolation and pharmacological activity of phenylpropanoid esters from Marrubium vulgare, *J Ethnopharmacol,* 79 (2002) 389.

11 Zhang J, Liu A, Hou R, Zhang J, Jia X, Jiang W& Chen J, Salidroside protects cardiomyocyte against hypoxia-induced death: A HIF-1α-activated and VEGF-mediated pathway, *Eur J Pharmacol,* 607 (2009) 6.

12 Patel V, Upaganlawar A, Zalawadia R & Balarman R, Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histoarchitectural evaluation, *Eur J Pharmacol,* 644 (2010) 160.

13 Rona G, Catecholamine cardiotoxicity, *J Mol Cell Cardiol,* 17 (1985) 291.

14 Chagoya de Sánchez V, Hernández-Muñoz R, López-Barrera F, Yañez L, Vidrio S, Suárez J, Cota-Garza MD, Aranda-Fraustro A & Cruz D, Sequential changes of energy metabolism and mitochondrial function in myocardial infarction induced by isoproterenol in rats: a long-term and integrative study, *Can J Physiol Pharmacol,* 75 (1997) 1300.

15 Korkmaz S, Radovits T, Barnucz E, Hirschberg K, Neugebauer P, Loganathan S, Veres G, Páli S, Seidel B, Zöllner S, Karck M&Szabó G, Pharmacological activation of soluble guanylatecyclase protects the heart against ischemic injury, *Circulation*, 120 (2009) 677.

16 Singal PK, Kapur N, Dhillon KS, Beamish RE & Dhalla NS, Role of free radicals in catecholamine induced cardiomyopathy, *Can J Physiol Pharmacol,* 60 (1981) 1390.

17 Schinella GR, Tournier HA, Prieto JM, Mordujovich de Buschiazzo P& Rios JL, Antioxidant activity of anti-inflammatory plant extracts, *Life Sci,*70 (2002) 1023.

18 Orhan IE, Belhattab R, Senol FS, Gülpinar AR, Hosbas S & Kartal M, Profiling of cholinesterase inhibitory and antioxidant activities of *Artemisia absinthium*, *A. herba-alba*, *A. fragrans*, *Marrubium vulgare*, *M. astranicum*, *Origanum vulgare* subsp. glandulossum and essential oil analysis of two Artemisia species, *Industrial Crops Products*, 32 (2010) 566.

19 Wagner H & Bladt S, *Plant drug analysis: A thin layer chromatography atlas* (Springer-Verlag, Berlin) 1996, 349.

20 Garjani A, Andalib S, Biabani S, SorayaH, Doustar Y, GarjaniA & Maleki-Dizaji M, Combined atorvastatin and coenzyme Q10 improve the left ventricular function inisoproterenol-induced heart failure in rat, *Eur J Pharmacol,* 666 (2011)135.

21 Benjamin IJ , Jalil JE, Tan LB, Cho K, Weber KT & Clark WA, Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis, *Circ Res,* 65 (1989) 657.

22 Satoh K, Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method, *Clin Chim Acta,* 90 (1978) 37.

23 Nahar L, Russell WR, Middleton M, Shoeb M & Sarker SD, Antioxidant phenylacetic acid derivatives from the seeds of *Ilex aquifolium, Acta Pharmaceutica,* 55 (2005) 187.

24 PiperRD, Li FY, Myers ML & Sibbald WJ, Effects of isoproterenol on myocardial structure and function in septic rats, *J Appl Physiol,* 86 (1999) 993.