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Research Article

Cardioprotective Effects of Rosmarinic Acid on Isoproterenol-Induced Myocardial Infarction in Rats

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ABSTRACT

Background: Rosmarinic acid is a polyphenolic compound with considerable antioxidant activities. We aimed to investigate its cardioprotective effects against isoproterenol-induced myocardial infarction (MI) in rats.

Methods: Male Wistar rats were assigned to 5 groups of control, isoproterenol, and treatments with 10, 20, 40 mg/kg of rosmarinic acid. Myocardial infarction was induced by subcutaneous injection of isoproterenol (100 mg/kg) once daily for 2 days. Rosmarinic acid was injected intraperitoneally once daily for 4 days, from the day of isoproterenol injection. In the fifth day the animals were anesthetized and hemodynamic and electrocardiographic parameters were recorded. After collecting the blood samples, the hearts were removed, weighed immediately to measure the cardiac enlargement, and kept for further histological studies. Lactate dehydrogenase and malondialdehyde were measured in the heart tissues for evaluating the damages and lipid peroxidation, respectively.

Results: Rosmarinic acid revealed a considerable antioxidant activity in vitro, with IC50 of 6.43µg/ml. Isoproterenol induced cardiac arrhythmias, myocardial damage and cardiac enlargement. Rosmarinic acid significantly reduced peripheral neutrophil percentage and inhibited isoproterenol-induced ST-segment elevation and R-amplitude depression in the infarcted hearts. It also significantly increased the mean arterial pressure and heart rate and decreased the left ventricular end diastolic pressure. The ventricular contractility was considerably improved by rosmarinic acid. Histopathological evaluations showed that rosmarinic acid significantly diminished the post-MI necrosis and fibrosis in the myocardium and inhibited the cardiac edematous.

Conclusion: It is deducible from the results that rosmarinic acid improves the cardiac performance and inhibits post-MI myocardial depression, probably due to its anti-oxidative activity.

Introduction

Coronary artery diseases are the leading cause of wide range of clinical syndromes. Myocardial ischemia is a result of imbalance between coronary blood supply and myocardial demand. The myocardial infarction is the common presentation of ischemic heart disease and occurs when the myocardial ischemia surpasses the critical point for extended time.[1] The hypoxia during the ischemia results in various negative alterations in myocardium including the release of proteolytic enzymes, respiratory chain damage, and reduction in the endogenous antioxidant capacity.[2] The main treatment of acute myocardial infarction is known to be the reperfusion of un-perfused area, which in fact leads to several undesirable sequels such as myocyte death, dysrhythmia, myocardial stunning, endothelial and microvascular dysfunction.[3] During the reperfusion, restoration of oxygenated blood flow to the ischemic area lead to sudden massive increase in oxygen concentration causing an imbalance between oxidant and anti-oxidative processes.[2] Thus, resultant excess production of reactive oxygen species (ROS) initiates lipid peroxidation in the cell membrane and

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consequently loss of cardiac contractility. Numerous studies have confirmed a promising role of natural antioxidants in preventing of morbidity and mortality of ischemic heart disease. Accordingly, plenty of investigations on cardio-protective effects of natural products with free radical scavenging properties have been done, providing more evidences about the role of oxygen reactive species in generation of post MI injuries and benefits of antioxidant administration in myocardial infarction.[4,5]

Results of a previous study on protective effects of ethanolic extract of *Ocimum basilicum L.* demonstrated obvious cardio-protective properties against isoproterenol-induce oxidative stress and cardiac hypertrophy and necrosis. Administration of the extract significantly improved ECG pattern and cardiac contractility by reducing the lipid peroxidation and cell damage.[6] It has been reported that the main content of the *Ocimum basilicum* extract is rosmarinic acid, thus we hypothesized that the observed protective effects could be mainly due to the presence of rosmarinic acid in the plant.

Rosmarinic acid is an ester of caffeic acid and 3,4-dyhydroxyphenyllatic acid. It is the most abundant polyphenol in species of *Lamiaceae* subfamily such as *Melissa officinalis* (balm), *Prunella vulgaris*, *Rosmainus officinalis* (rosmary), *Ocimumbasilicum* (basil).[7-9]

There are plenty of studies on several biological properties of rosmarinic acid such as anti-inflammatory, photo-protective, anti-cancer, anti-depressive, anti-viral, anti-bacterial and anti-angiogenesis effects, and its protection in neurodegenerative diseases.[8] In addition, rosmarinic acid exhibits a distinct free radical scavenging activity in the biological systems which makes it a suitable candidate in the treatment of ischemic heart disease. The aim of the present study was evaluating the cardio-protective effects of rosmarinic acid in myocardial infarction and its efficacy in preventing the post MI injuries.

Methods and Materials

Quantitative DPPH radical-scavenging assay

Free radical scavenging activity of rosmarinic acid was assessed on 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) with the following method. 2 ml of methanolic solutions of rosmarinic acid with 10 different concentrations from 0.5 mg/ml to 1 µg/ml were mixed with 2 ml of DPPH solution (0.004% w/v) and after 30 min, the absorbance of the solutions at 517 nm were recorded against methanol. The DPPH radical reduction (R%) was calculated via the following equation:

$$R\ \% = [(A_0 - A_{sample})/A_0] \times 100 \qquad \text{Eq.(1)}$$

Where, A_0 is the absorbance of the control, and A_{sample} is the absorbance of the tested sample. The IC50, which represents the concentration of the extract that inhibited 50% of radical, was calculated according to the R%-concentration graph. Quersetin was used as a positive control.

In vitro nitric oxide radical (NO.) scavenging assay

To determine the NO. radical scavenging activity of rosmarinic acid, 10 different concentrations of rosmarinic acid from 0.5 mg/ml to 1 µg/ml were prepared and 0.5 ml of each solution, separately, were mixed with 0.5 ml of phosphate saline buffer (pH=7.4) and 2 ml of sodium nitroproside 10 mM and the mixtures were kept in room temperature for 150 min. Then, 0.5 ml of mixtures was added to 1 ml of sulfanilamide solution (0.33%) and after 5 min, 1 ml of N-(1-Naphthyl)ethylenediamine dihydrochloride 0.1% was added to the samples. After 30 min, the absorbances of solutions were recorded at 540 nm, for calculating the NO. radical reduction.

Animals

Male albino Wistar rats (280-310g) were used in this study. Rats were housed at constant temperature (20±2°C) and relative humidity (50±10%) in standard polypropylene cages, six per cage, under a 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 which is in line with National Institutes of Health publication, 8th edition, and revised 2011.

Experimental protocol

The animals were randomly allocated into 5 groups. To induce myocardial infarction, isoproterenol dissolved in normal saline and was injected subcutaneously to rats (100 mg/kg) twice with 24 h intervals. Treatment groups received intraperitoneal (IP) injection of rosmarinic acid (Sigma-Alderich, USA) in 0.2% hydroxypropyl methyl cellulose (HPMC) solution (at doses of 10, 20 and 40 mg/Kg) 30 min prior to isoproterenol injection and once daily for the next 2 days. The control and MI group received IP injection of normal saline.

Hemodynamic measurement

Seventy two hours after the second injection of isoproterenol, the animals were anesthetized by ketamine (0.5 ml) and xylazin (0.3 ml) mixture. The standard limb lead II electrocardiogram was recorded for evaluating the heart rate (HR), ST-segment elevation and R-amplitude, using POWERLAB system (AD instruments, Australia). Then a small cut in the mid line of the throat was performed and systemic arterial blood pressure was recorded by inserting a Micro Tip catheter (Millar

instruments, INC) through the right carotid and the mean arterial blood pressure (MAP) was calculated from the systolic and diastolic blood pressure traces. For assessment of cardiac left ventricular function, the catheter was advanced into the lumen of the left ventricle via the carotid, and the left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), maximum and minimum of developed left ventricular pressure ($LVdP/dt_{max}$ and $LVdP/dt_{min}$) were measured.[5]

Sample collection and tissue weight

After recording the hemodynamic parameters, the blood sample was collected from the hepatic vein and the heart was harvested quickly and washed in the ice cold normal saline and weighed, then the heart weight to body weight ratio was calculated to assess the degree of heart congestion and weight gain. Then the hearts were kept in -70°C for further studied. The collected blood samples were centrifuged (3000 rpm, 10 min, 4°C) to separate the serum. The serum was kept in -70°C for further studies.

Peripheral neutrophil counting

After collecting the blood samples from the hepatic vein, before centrifuging, small amounts of the samples were smeared on clean lams and fixed with methanol and stained with Gimsa solution and the percent of neutrophils was determined by counting the white blood cells at 100x zooming optical microscope.

Determination of lipid peroxidation in heart tissue

Malondialdehyde (MDA) level in myocardium, as an indicator of lipid peroxidation, was measured by the following procedure according to our previous study.[10] The hearts were homogenized in a ratio of 1/10 in 1.15% (w/v) cold KCl solutions and o.5 ml of the homogenate was shaken with 3 ml of 1% ortho-phosphoric acid in a 10 ml centrifuge tube. 1 ml of 0.6% TBA was added to the mixture, shaken, and warmed for 45 min in a boiling water bath. After cooling, 4 ml of n-butanol was added to the tubes and mixed vigorously. Then tubes were centrifuged for 15 min at 5000 rpm and MDA content in the serum was determined from the absorbance at 535 by spectrophotometer against n-butanol.

Lactate dehydrogenase assay in serum

The collected serum samples were used for LDH assay according to the optimized method of DGKC.[11] The monoreagent procedure was used. Ten microliter of serum was added to 1000 µl of the mixture of LDH assay standard solutions, including solution 1 (pyruvate 0.60 mmol/l in phosphate buffer, pH=7.5) and solution 2 (NADH 0.18 mmol/l in Good's buffer, pH=9.6), mixed with 4:1 proportion few minute before adding the sample. The absorbance (A) of the final solution at 340 nm was recorded with spectrophotometer 1, 2 and 3 minutes after mixing. The LDH activity was calculated as fallowed:

$$\text{Activity (U/l)} = \Delta A/\text{min} \times \text{factor (f)} \qquad \text{Eq.(2)}$$

The factor (f) in monoreagent procedure at 340 nm is 16000.

Histopathological examinations

In another set of experiment, the harvested hearts were excised and fixed in 10% buffered formalin. Then the tissues were embedded in paraffin, sectioned at 5 µm thick slices and stained with Hematoxylin and Eosin (H&E) for evaluating the necrosis and edematous level in myocardium, and Gomeri Trichrom for distinguishing the fibrotic tissue. Two persons graded the histopathological changes as 0, 1, 2, 3 and 4 for none, low, moderate, high and intensive pathologic changes, respectively.

Statistics

Data were presented as mean±sem. One-way-ANOVA was used to make comparisons between the groups. If the ANOVA analysis indicated significant difference, a Student-Newman-Keuls post hoc 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

In vitro antioxidant activity of rosmarinic acid

According to the radical scavenging test on DPPH, the IC50 value of rosmarinic acid and Quersetin as a standard were calculated as 6.43 and 3µg/ml, respectively. However, the NO. radical scavenging test revealed no significant reduction in NO. radicals ($p>0.05$).

Effect of rosmarinic acid on electrocardiogram parameters

Injection of isoproterenol exhibited obvious changes in ECG pattern. Particularly it caused ST-segment elevation ($p<0.001$) from 123.67±5.05 in control to 197.35±6.1 µV (Figure 1), and as it is shown in Figure 2, R-amplitude was markedly declined to 225.2±60.3 µV with isoproterenol compared to 415.75±60.6 µV in the normal group ($p<0.05$). However, rosmarinic acid with all three doses notably lowered the ST-segment elevation ($p<0.01$, $p<0.01$, $p<0.001$ with 10, 20 and 40 mg/kg, respectively) and the concentrations of 10 and 20 mg/kg significantly improved the R-amplitude up to 402.8±50.2 and 363.6±16.65 µV, respectively ($p<0.05$, $P<0.05$).

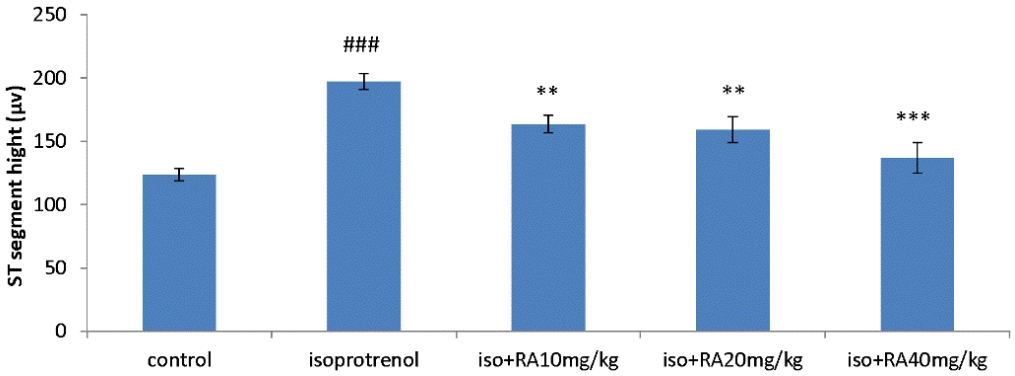

Figure 1. The effects of rosmarinic acid on ST segment height (recorded from limb lead II). Iso: isoproterenol; RA: Rosmarinic acid. Values are expressed as mean±sem (n=6). ###p<0.001 from respective control value; **p<0.01 and ***p<0.001 as compared with isoproterenol group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

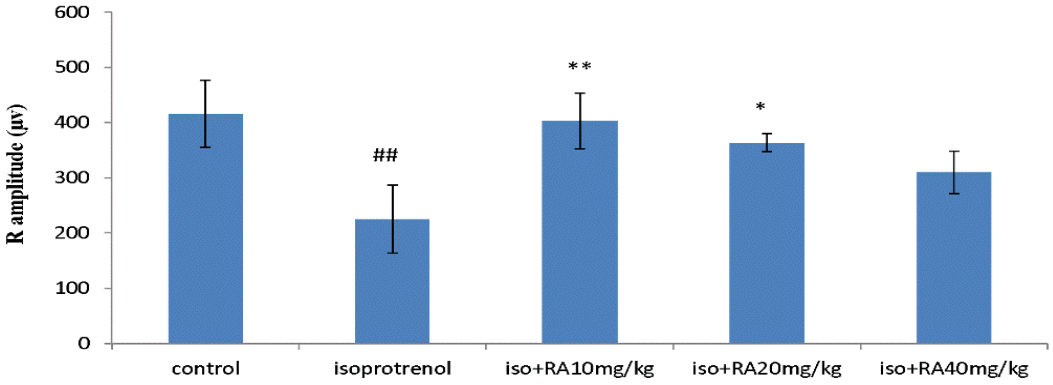

Figure 2. The effects of rosmarinic acid on R amplitude (recorded from limb lead II). Iso: isoproterenol; RA: Rosmarinic acid. Values are expressed as mean±sem (n=6). ##p<0.01 from control value; *p<0.05, **p<0.01 as compared with isoproterenol group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

Table 1. The effects of rosmarinic acid on hemodynamic parameters in rats injected isoproterenol.

Group (n=6)	MAP (mmHg)	Heart rate (bpm)	LVSP (mmHg)	LVEDP (mmHg)
Control	122± 8.3	300± 8.6	137± 6.3	12± 2.2
Isoproterenol (iso)	93± 3.8#	212± 16.6##	120± 2.1#	18± 1.2#
Rosmarinic acid (10mg/kg) +iso	104± 4.6	247± 9.5*	136± 4.7*	9± 0.6***
Rosmarinic acid (20mg/kg) +iso	125± 7.9**	230± 10.4	154± 8.2**	13± 0.6*
Rosmarinic acid (40mg/kg) +iso	126± 6.6***	281± 12.6**	123± 5.9	13± 1.9*

Data are expressed as mean±sem (n=6). MAP: mean arterial pressure; LVSP: left ventricular systolic pressure; LVEDP: left ventricular end diastolic pressure. #p<0.05, ##p<0.001 vs. respective control value; *p<0.05, **p<0.01 and ***p<0.001 as compared with isoproterenol (MI) group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

The effects of rosmarinic acid on hemodynamic parameters

The hemodynamic data analysis showed that the mean arterial pressure (MAP) was significantly increased from 93±3.8 mmHg in isoproterenol group to 125±79 and 126±6.6 mmHg, respectively with 20 and 40 mg/kg of rosmarinic acid (P<0.01 and P<0.001; Table 1). Isoproterenol reduced the heart rate (HR) from 300±8.6 bpm in normal group to 212±16.6 bpm (P<0.001); but it was increased to 247±9.5 at 10 mg/kg (P<0.05) and 281±12.6 bpm by 40 mg/kg of rosmarinic (P<0.01). The normal left ventricular systolic pressure (LVSP) in control group was 137±6.3 mmHg which was decreased to 120±2.1 mmHg by isoproterenol injection; however, rosmarinic acid increased LVSP close to the normal value at 10 mg/kg (P<0.05), and even improved it to 154±8.2 mmHg at the dose of 20 mg/kg (P<0.01). The left ventricular end diastolic pressure (LVEDP) was increased by isoproterenol injection (18±1.2 mmHg, P<0.05), but it was decreased by half with 10 mg/kg of rosmarinic acid (P<0.001). The concentrations of 20 and 40 mg/kg of rosmarinic acid also decreased the LVEDP significantly, comparing to the isoproterenol group (P<0.05; Table 1). Analyzing the left ventricular maximal and minimal rates of pressure (LV dP/dt_{max} and LV dP/dt_{min}) showed that

isoproterenol causes a reduction in LV dP/dt_{max} ($P<0.001$) and elevation in LV dP/dt_{min} ($P<0.01$). In the groups receiving 10 and 20 mg/kg of rosmarinic acxid, the parameters remarkably improved to the normal value, as LV dP/dt_{max} was increased ($P<0.001$ and $P<0.01$) and LV dP/dt_{min} was significantly decreased ($P<0.05$; Figure 3).

The effect of rosmarinic acid on wet heart weight to body weight ratio

In order to assess the extent of cardiac post-MI weight gain and edematous, the heart weight to body weight ratio (w/w %) was calculated. Analyzing the heart weight to body weight ratio (Figure 4), revealed a significant increase from 0.22±0.01% in the control group to 0.37±0.01% in isoproterenol group ($p<0.01$). However, all three doses of rosmarinic acid were able to reduce the ratio significantly ($p<0.01$, $p<0.05$ and $p<0.01$).

The effect of rosmarinic acid on the peripheral neutrophil count

As shown in Figure 5, the percentage of neutrophils in the peripheral blood, which is an indicator of systemic inflammation, was significantly increased from 8.75±0.47% in the control to 22.77±1.9% in the isoproterenol group ($p<0.001$). However, administration of rosmarinic acid with all three doses were able to reduce the neutrophil percentage remarkably to 14.62±1.4%, 10±0.66% and 16.14±1.4%, respectively ($p<0.01$, $p<0.01$ and $p<0.05$).

The effect of rosmarinic acid on MDA in the heart tissue

MDA level in myocardium, as an indicator of lipid peroxidation, was measured. The injection of isoproterenol significantly increased the MDA level in the heart tissue from 5.5±0.2 nmol/mg in control group to 7.5±0.8 nmol/mg ($p<0.05$). However, rosmarinic acid at 20 mg/kg notably decreased the tissue level of MDA close to the normal level (5.4±0.7 nmol/mg, $p<0.05$; Table 2).

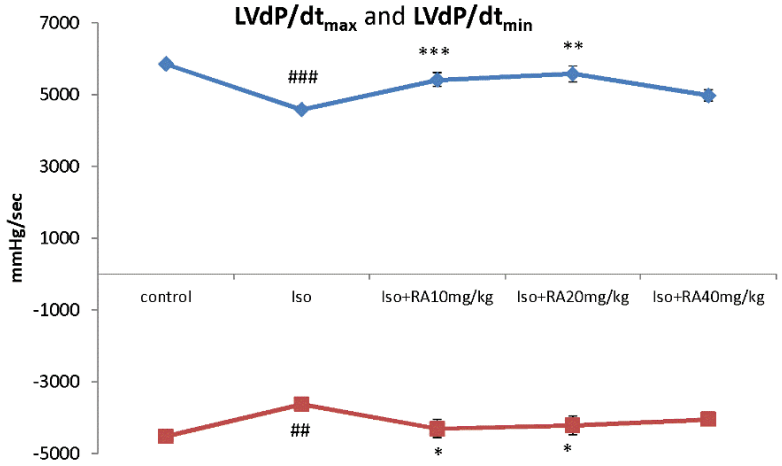

Figure 3. The effects of rosmarinic acid on left ventricular maximal and minimal rates of pressure (LVdP/dtmaxand LVdP/dtmin). Iso: isoproterenol; RA: Rosmarinic acid. Values are expressed as mean±sem (n=6). ##p<0.01, ###p<0.001 from respective control value; *p<0.05, **p<0.01 and ***p<0.001 as compared with isoproterenol group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

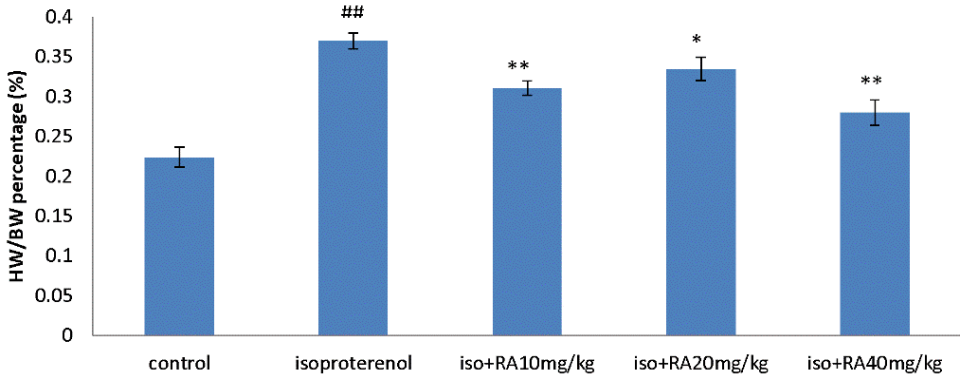

Figure 4. The effects of rosmarinic acid on wet heart weight to body weight ratio. Iso: isoproterenol; RA: Rosmarinic acid. Values are expressed as mean±sem (n=6). ##p<0.01 from respective control value; *p<0.05, **p<0.01 as compared with isoproterenol group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

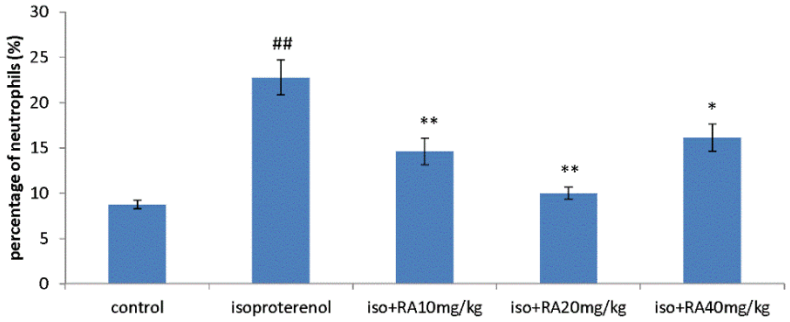

Figure 5. The effects of rosmarinic acid on peripheral neutrophil count. Iso: isoproterenol; RA: Rosmarinic acid. Values are expressed as mean±sem (n=6). ##p<0.01 from respective control value; *p<0.05, **p<0.01 as compared with isoproterenol group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

Table 2. The effects of rosmarinic acid on serum level of lactate dehydrogenase (LDH) and heart tissue content of malondialdehyde (MDA).

	control	Isoproterenol	Iso+RA (20 mg/kg)
Myocardial MDA (nmol/mg)	5.6 ± 0.2	7.5 ± 0.8 #	5.5 ± 0.7 *
Serum LDH level (U/l)	76.2 ± 7.5	112.2 ± 9.2 #	64.1 ± 10.1 *

Iso: isoproterenol; RA: Rosmarinic acid. Values are expressed as mean±sem (n=4). #p<0.05 from respective control value; *p<0.05 as compared with isoproterenol group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

The effect of rosmarinic acid on MI biomarkers LDH in the serum

In the group receiving SC injection of isoproterenol the LDH level in serum was considerably high (112.21±9.25 U/l vs. 76.14±76 U/l, p<0.05). However, in the group treated with 20 mg/kg of rosmarinic acid, the LDH level was significantly decreased to 64.12±10 U/l (p<0.05; Table 2).

The effects of rosmarinic acid on histopathological changes

According to photomicrographs of myocardial tissue stained with Hematoxylin and Eosin (H&E), there was no degeneration and necrosis in the heart tissues obtained from the control group, but injection of isoproterenol led to a noticeable level of subendocardial necrosis and edematous in intramuscular space along with hyperplasia (Figure 6). Treatment with rosmarinic acid, especially with the high dose, remarkably prevented the necrosis and edematous, and the scores were significantly lessened, compared to isoproterenol group (p<0.01). The evaluation of results from Gomeri Trichrom staining of myocardial sections revealed that injection of isoproterenol caused a sever grade of fibrosis which is recognizable as blue dyed dots in Figure 7. All three doses of rosmarinic acid, dose-dependently and significantly, diminished the fibrotic tissue in the myocardium in comparison with the isoproterenol group (p<0.01).

Discussion

The in vitro radical scavenging tests on DPPH radicals revealed that rosmarinic acid has a high antioxidant activity with a considerably low IC50. However, the results from in vitro NO radical scavenging test suggests that the antioxidant activity of rosmarinic acid could be due to mechanisms other than inhibition of NO free radicals production, thus, more researches are needed to the determine the exact pathway of free radical scavenging property of the compound.

The ECG is considered as an essential clinical test for diagnosis of myocardial infarction. The most significant marker of prevalent MI in ECG is ST-segment elevation, which is due to the potential difference between ischemic and non-ischemic area and the cell membrane dysfunction.[1] The results of this study show that rosmarinic acid lowered the ST-segment elevation induced by isoproterenol. This observation suggests that rosmarinic acid may have some cell membrane protective effects. The other altered parameter in ECG is R-amplitude, which is decreased by isoproterenol due to the consecutive myocardial edema.[1] However, intraperitoneal injection of rosmarinic acid was able to improve the R-amplitude by reducing the cell injury and edema.

Injection of isoproterenol, a beta-adrenergic receptor agonist, causes myocardial hyperactivity and arterial hypotension,[12] which leads to cardiac ischemia. After the ischemia and infarction due to the necrosis and myocyte injuries, the mean arterial pressure (MAP), the heart rate, and cardiac contractility decrease and left ventricular systolic pressure (LVSP) declines which are associated with left ventricular end diastolic pressure (LVEDP) increase. All of these changes are considered as markers of post MI heart failure. Rosmarinic acid protected the heart against post MI heart failure by improving cardiac function and hemodynamic parameters.

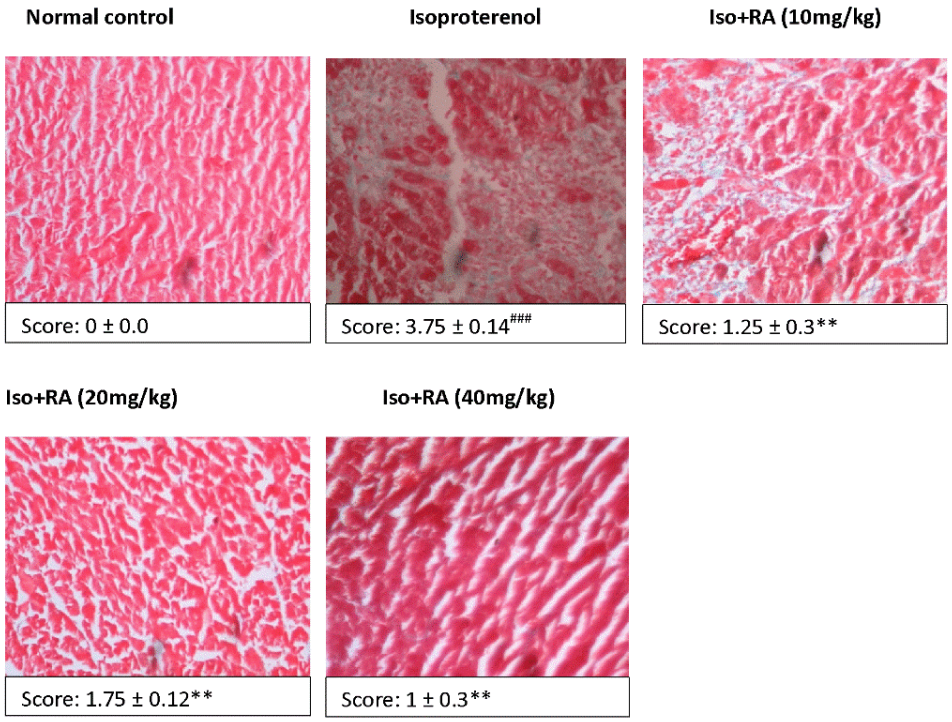

Figure 6. Photomicrographs of sections of the apex of rat heart stained with Gomeri Trichom. Severe cardiomyocyte fibrosis (dyed blue) with increased edematous in intramuscular space is observed in hearts of rats receiving subcutaneous isoproterenol. Rosmarinic acid injection obviously reduced the fibrosis. Iso: isoproterenol; RA: Rosmarinic acid. GomeriTrichom (40 M). Grades 0, 1, 2, 3 and 4 respectively show none, low, moderate, high and intensive pathologic changes. Values are expressed as mean±sem (n=6). ###p<0.001 from respective control value; **p<0.01 as compared with isoproterenol group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

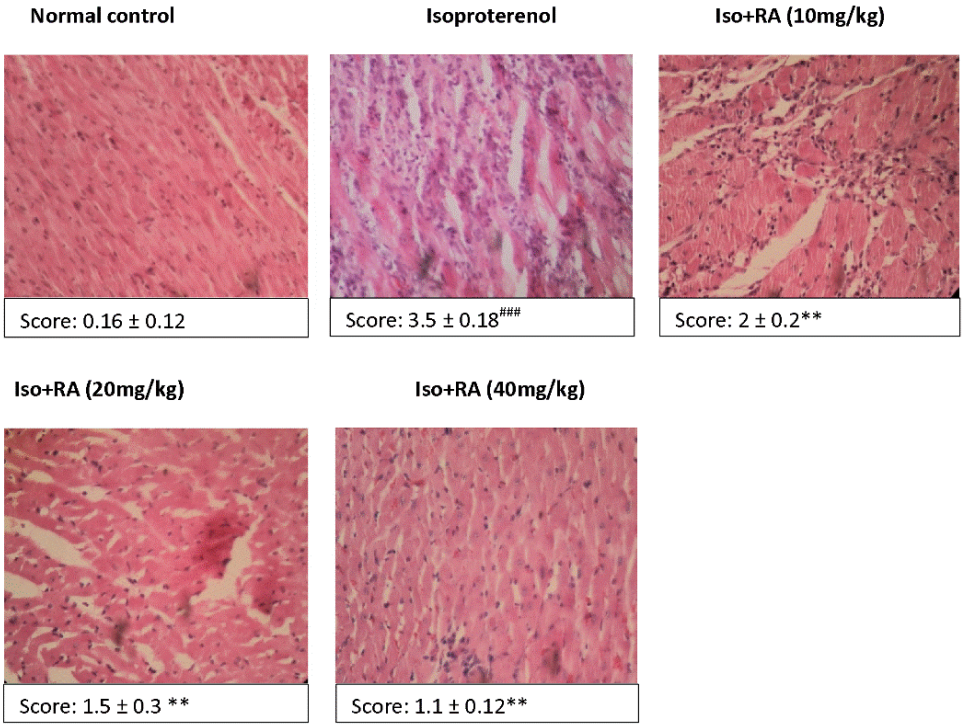

Figure 7. Photomicrographs of sections of the apex of rat heart stained with H&E. severe cardiomyocyte necrosis with increased edematous in intramuscular space is observed in hearts of rats receiving subcutaneous isoproterenol. Rosmarinic acid injection obviously improved the necrosis. Iso: isoproterenol; RA: Rosmarinic acid. H&E (40 M). Grades 0, 1, 2, 3 and 4 respectively show none, low, moderate, high and intensive pathologic changes. Values are expressed as mean±sem (n=6). ###p<0.001 from respective control value; **p<0.01 as compared with isoproterenol group. One way ANOVA with Student-Newman-Keuls post hoc test is used.

The results of this study showed that it improved MAP and heart rate and systolic pressure, and more importantly decreased the LVEDP which is an essential parameter of congestion degree of the heart. All these effects could be due to the anti-oxidant properties of rosmarinic acid, which prevents further myocardial necrosis. In addition, reduction of LVEDP helps to increase the blood flow to the sub-endocardial area and as a result, diminishes the necrosis. Lower doses of rosmarinic acid also helped maintain the cardiac contractility, as it improved the $LVdP/dt_{max}$ and $LVdP/dt_{min}$ values (markers of myocardial contractile and relaxation, respectively). So it showed positive inotropic activity, probably because of its effect on reducing the myocardial necrosis and restoring the blood flow. However, high dose of rosmarinic acid had reverse effect on contractility parameters, which could also be related to the anti-oxidative properties of the compound. Studies showed that anti-oxidant consumption is a double-edged sword, since the overdose of these reactive compounds may cause some toxic effects.[13]

Isoproterenol injection dose-dependently causes cardiotoxicity and results in histological changes such as myocyte necrosis and degeneration, edema and leucocyte infiltration.[14] One of the involved mechanisms, as discussed before, is production of oxidants resulting from catecholamine autoxidation.[14] Cardiac hypertrophy also results from exposure to high doses of beta-adrenergic receptor agonists and consequent increase in heart work. It has been proposed that a 1% increase in myocardial water content (due to edema) could lead to a possibly 10% reduction in cardiac function.[15] The involved mechanisms could be the myocyte hyperplasia and interstitial fibrotic tissue replacement, beside the edema and increase in collagen accumulation.[16] It has been shown that myocardial edema stimulates fibrosis within the myocardium, which affects the cardiac function.[15]

The results of this study revealed a significant decrease in heart weight to body weight ratio with all three concentration of rosmarinic acid, so it was able to prevent the cardiac weight gain and hypertrophy. In addition, histopathological evaluation with H&E staining showed an obvious reduction in necrosis and myocyte degeneration degree with rosmarinic acid. Gomeri trichrom staining of the cardiac tissue slices also revealed a considerable diminishes in edematous intermuscular and fibrotic tissue generation in myocardium in groups receiving rosmarinic acid. All this results could be related to the preventive effects of rosmarinic acid against oxidant induced myocyte injuries, by scavenging the free radicals produced in the ischemic tissue.

The infiltration of poly-morphonuclear neutrophils (PMN) into the ischemic tissue upon reperfusion period mediates the tissue destructive events linked to the release of toxic agents like oxygen reactive species from neutrophils.[17,18] The percentage of peripheral neutrophil reflects the inflammatory response to the myocardial infarction so assessment of neutrophil infiltration into the blood can be considered as an important factor in determination of severity of inflammation in the myocardium.[19] Results of this study demonstrated that rosmarinic acid reduced the neutrophil percentage in the peripheral blood after MI. This confirms the anti-inflammatory and anti-oxidant properties of rosmarinic acid and its preventive effect on stimulation of PMN infiltration probably by reducing the oxidant-induced inflammation in myocardium and suppressing the release of inflammatory mediators.

The interaction of free radicals with cellular elements such as lipids, forms oxidative products like lipid peroxides within the infarcted myocardium. These products later decompose to several final products such as malodialdehyde (MDA). In the present study MDA was measured as an indicator of lipid peroxidation level and free radical activity in the heart tissue.[20] The results showed that rosmarinic acid reduced the MDA level in the myocardium. The effect of rosmarinic acid on MDA level confirms its anti-oxidative and preventive properties against lipid peroxidation. Lactate dehydrogenase (LDH), an enzyme involved in anaerobic metabolism, is abundant in ischemic myocardium and is released to the blood after myocardial infarction due to myocyte injuries and necrosis. it becomes detectable 8-12 h after myocardial infarction and gets to the peak concentration 24-72 h post MI and has a sensitivity of 90-99% for retrospective diagnosis of MI.[21] In this study, measurement of LDH level in serum sample of animals demonstrated that rosmarinic acid prevented LDH level elevation, most probably by suppressing oxidative stress and consequent myocardial necrosis.

Conclusion

The present study demonstrates that rosmarinic acid restores cardiac function following myocardial infarction by enhancing the cardiac contractility, improvement of hemodynamic parameters, and amending cardiac electrical activity. The compound also prevents MI-induced myocardial necrosis and fibrosis. The results of the present study suggest that the main mechanism behind the protective effects of rosmarinic acid against isoproterenol-induced myocardial infarction and injuries can be due to its antioxidative and free radical scavenging properties. This natural compound can be considered as an antioxidant in improving cardiac function following myocardial infarction.

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Conflict of interests

Prof. Alireza Garjani is the Editor-in-Chief of *Pharmaceutical Sciences*. The peer-review process of the submission was supervised by another member of the editorial board. The authors claim no other competing interests.

References

1. Patel V, Upaganlawar A, Zalawadia R, Balaraman R. Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histoarchitectural evaluation. Eur J Pharmacol. 2010;644(1-3):160-8. doi:10.1016/j.ejphar.2010.06.065
2. Muzakova V, Kandar R, Vojtisek P, Skalicky J, Cervinkova Z. Selective antioxidant enzymes during ischemia/reperfusion in myocardial infarction. Physiol Res. 2000;49:315-22.
3. Broskova Z, Drabikova K, Sotnikova R, Fialova S, Knezl V. Effect of plant polyphenols on ischemia-reperfusion injury of the isolated rat heart and vessels. Phytother Res. 2013;27(7):1018-22. doi:10.1002/ptr.4825
4. Priscilla DH, Prince PSM. Cardioprotective effect of gallic acid on cardiac troponin-t, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in wistar rats. Chem Biol Interact. 2009;179(2-3):118-24. doi:10.1016/j.cbi.2008.12.012
5. Yousefi K, Soraya H, Fathiazad F, Khorrami A, Hamedeyazdan S, Maleki-Dizaji N, et al. Cardioprotective effect of methanolic extract of marrubium vulgare l. On isoproterenol-induced acute myocardial infarction in rats. Indian J Exp Biol. 2013;51(8):653-60.
6. Fathiazad F, Matlobi A, Khorrami A, Hamedeyazdan S, Soraya H, Hammami M, et al. Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of ocimum basilicum l.(basil) against isoproterenol induced myocardial infarction in rats. Daru. 2012;20(1):87. doi:10.1186/2008-2231-20-87
7. Lamaison JL, Petitjean-Freytet C, Carnat A. [Medicinal lamiaceae with antioxidant properties, a potential source of rosmarinic acid]. Pharm Acta Helv. 1991;66(7):185-8.
8. Petersen M, Simmonds MS. Rosmarinic acid. Phytochemistry. 2003;62(2):121-5. doi:10.1016/s0031-9422(02)00513-7
9. Wang H, Provan GJ, Helliwell K. Determination of rosmarinic acid and caffeic acid in aromatic herbs by hplc. Food Chem. 2004;87(2):307-11. doi:10.1016/j.foodchem.2003.12.029
10. Garjani A, Andalib S, Biabani S, Soraya H, Doustar Y, Garjani A, et al. Combined atorvastatin and coenzyme q10 improve the left ventricular function in isoproterenol-induced heart failure in rat. Eur J Pharmacol. 2011;666(1-3):135-41. doi:10.1016/j.ejphar.2011.04.061
11. Shahsavani D, Mohri M, Kanani HG. Determination of normal values of some blood serum enzymes in acipenser stellatus pallas. Fish Physiol Biochem. 2010;36(1):39-43. doi:10.1007/s10695-008-9277-3
12. Yeager JC, Iams SG. The hemodynamics of isoproterenol-induced cardiac failure in the rat. Circ shock. 1981;8(2):151-63.
13. Bouayed J, Bohn T. Exogenous antioxidants—double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxid Med Cell Longev. 2010;3(4):228-37. doi:10.4161/oxim.3.4.12858
14. Piper RD, Li FY, Myers ML, Sibbald WJ. Effects of isoproterenol on myocardial structure and function in septic rats. J Appl Physio. 1999;86(3):993-1001.
15. Laine GA, Allen SJ. Left ventricular myocardial edema. Lymph flow, interstitial fibrosis, and cardiac function. Circ Res. 1991;68(6):1713-21. doi:10.1161/01.res.68.6.1713
16. Ozaki M, Kawashima S, Yamashita T, Hirase T, Ohashi Y, Inoue N, et al. Overexpression of endothelial nitric oxide synthase attenuates cardiac hypertrophy induced by chronic isoproterenol infusion. Circ J. 2002;66(9):851-6. doi:10.1253/circj.66.851
17. Epstein FH, Weiss SJ. Tissue destruction by neutrophils. N Engl J Med. 1989;320(6):365-76. doi:10.1056/nejm198902093200606
18. Romson JL, Hook BG, Kunkel SL, Abrams G, Schork M, Lucchesi B. Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. Circulation. 1983;67(5):1016-23. doi:10.1161/01.cir.67.5.1016
19. Yousefi K, Fathiazad F, Soraya H, Rameshrad M, Maleki-Dizaji N, Garjani A. Marrubium vulgare l. Methanolic extract inhibits inflammatory response and prevents cardiomyocyte fibrosis in isoproterenol-induced acute myocardial infarction in rats. Bioimpacts. 2014;4(1):21-7. doi:10.5681/bi.2014.001
20. Raghuvanshi R, Kaul A, Bhakuni P, Mishra A, Misra M. Xanthine oxidase as a marker of myocardial infarction. Indian J Clin Biochem. 2007;22(2):90-2. doi:10.1007/bf02913321
21. Rosenblat J, Zhang A, Fear T. Biomarkers of myocardial infarction: Past, present and future. UWOMJ. 2012;81(1):23-5.