The protective effect of *Rosa canina* distilled water on ischemia-reperfusion injuries in the isolated rat heart

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**Abstract**

In recent years, postconditioning by natural pharmaceutical agents has been considered as a treatment for injuries due to ischemia/reperfusion (IR). Thus, in the present study, the effect of *Rosa canina* distilled water on ischemia and reperfusion experimental myocardial injury was investigated. Male Wistar rats were divided into five groups (n=7); one control IR group and four treated groups, with different doses of *Rosa canina* distilled water, 0.416%, 1.25%, 2.5% and 4.16% were added to perfusate at the final 5 minutes of ischemia and the first 15 minutes of reperfusion. The isolated rat hearts were fixed on the Langendorff system, and after a period of equilibrium were subjected to 30 min topical ischemia and 60 min reperfusion. Heart rate (HR), left ventricular developed pressure (LVDP) and rate-pressure product (RPP) were investigated. Myocardial infarct size was determined at the end of reperfusion. Compared with the IR group, 0.416% and 1.25 % decreased the HR at the end of ischemia significantly, the 1.25% and 2.5% groups significantly improved LVDP and RPP, and all treatment dilutions decreased myocardial infarct size. *Rosa canina* distilled water showed positive inotropic and negative chronotropic effects and induced cardioprotection in a dose-dependent manner.

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Introduction

Today, cardiovascular diseases (CVDs) are the main leading causes of death all over the world (Mathers and Loncar, 2006) and cause many disabilities and economic losses. Ischemic heart disease (IHD) is one of the most important sections of CVDs and its treatment has still preoccupied the specialist’s brain. In the last two decades, reperfusion therapy has become conventional for managing the acute myocardial infarction (AMI). But, restoration of the blood flow to the ischemic area results in the ischemia/reperfusion (IR)-injury (Moens et al., 2005).

Manipulation of the ischemic/reperfused heart during its reperfusion period (postconditioning) by physical (Tsang et al., 2004) (such as repeated short periods of IR) or pharmacological agents (Hu et al., 2013) can lead to cardioprotection (Buchholz et al., 2014).

Although new chemical agents are being introduced to the world, nowadays, physicians and people have turned to complementary or alternative medicine and natural products for treating many diseases because natural products are usually non-poisonous, have smaller side effects, and are available easily (Chandrasekaran et al., 2010). Furthermore, some of the biochemical products are of plant origin such as morphine (Papaver somniferum), Ephedrine (Ephedra vulgaris), Atropine (Atropa belladonna) (Lourenco et al., 2012). A variety of plants and their derivatives have been examined for their effects on heart and cardiac diseases such as grape, berries, pomegranate, barberry, saffron (Tiwari et al., 2009; Hassanpour Fard et al., 2011; Zhang et al., 2011; Sachdeva et al., 2012).

*Rosa canina* L. (Rosaceae) is a wild rose species native to Europe, northwest Africa and western Asia and is in the same family with *Rosa damascena*. The other well-known name of *Rosa canina* is Dog rose and its fruit is known as rosehip. This plant is called "Nasrin" or "Nastaran" in Iranian Traditional Medicine (ITM) and according to the traditional medical physicians’ opinion is a cardiotonic drug.

Moreover, it is recommended for treatment of some kinds of liver disorders, nausea, vomiting, ichter, tonsillitis, and catarrh; and reinforcing of liver, stomach, and gum in ITM (Aqili-Khurasani, 2001).

Petals, fruit and seeds of this plant are used for treatment. This plant (especially its fruit) is under consideration for its high antioxidant and vitamin C levels. In folk medicine dog rose fruit is used for disorders of the efferent urinary tract and the kidneys, kidney stones, rheumatic conditions such as rheumatism, and gout, cold, scurvy, and febrile conditions. No side effect is reported related to typical therapeutic dosages. Dog rose has been used as powder, the whole fruit and decoction (PDR, 2005). But, there is not any information or study about its efficacy in treatment of heart diseases.

Peplau established that rosehip decoction and ethanol extract could increase the tone of isolated frog cardiac muscle dose-dependently. Positive inotropic and negative chronotropic effects were determined for *Rosa canina* (Peplau, 1941). Of course, inotropic agents which support the contractility and sensitize the myofibrils to calcium are useful for reperfused stunned heart and don’t have any adverse effect on functional recovery (Verma et al., 2002). Also, it has been shown that rosehip and its seeds have antioxidative and anti-inflammatory effects (Chrubasik et al., 2008) and the phenolic part had main role in the antioxidative activity (Gao et al., 2000). On the other hand, it has been documented that antioxidants have a protective effect on myocardial IR injury and administration of antioxidants is a way to decrease these harms (Kutala et al., 2006; Yang and Liu, 2012; Testai et al., 2013). Since, there was not any investigation about effects of this drug on ischemic heart according to our knowledge; this study was designed to assess the postconditioning effect of *Rosa canina* distilled water on ischemia/reperfusion rat heart model. As, one of the widely prescribed medicine forms in ITM is distilled water, this preparation was considered for the investigation.
Materials and methods

Animals
Male Wistar albino rats (200 – 250 g) were used in this study. All animals were housed in standardized conditions 12-h light/dark cycle, 20–22°C ambient temperature and 40–50% humidity. They had free access to food and water. All animal care and experiments were conducted in accordance with the institutional guidelines of Tehran University of Medical Sciences (Tehran, Iran).

Treatment protocols and isolated Langendorff heart preparation
Wistar rats were anesthetized with sodium thiopental (60 mg/kg body weight, ip). The animal was placed in the supine position. The chest was rapidly excised and the heart was exposed to the air and washed with Krebs. After excising and lifting the pericardium and fatty tissue, the aorta was immediately cannulated with a cannula and connected to the Langendorff apparatus to start perfusion at a constant flow rate. This solution, equilibrated with 95% O₂ + 5% CO₂, was delivered to the aortic cannula with 37°C. A water-filled latex balloon, connected to a gauge, was inserted through the mitral valve into the left ventricle to record mechanical parameters; such as heart rate (HR), left ventricular developed pressure (LVDP, difference between left ventricular systolic and diastolic pressures) and rate-pressure product (RPP, multiplying HR and LVDP) using a Power Lab data system. LVDP and RPP changes during ischemia and reperfusion periods were reported as a percentage of baselines. Ischemia (Area at risk, AAR) and infarct size were measured at the end of experimental protocols. All hearts subjected to 30 minutes of regional ischemia (by ligation of left anterior descending artery) followed by 60 minutes of reperfusion. After heart isolation and prior to ischemia induction, periods of stabilization (30 minutes) and baseline (15 minutes) were established for all hearts.

Study Groups
Rats were assigned to five groups (n = 7); in ischemia/reperfusion group (IR), rat hearts were exposed to ischemia and reperfusion and were perfused with Krebs without treatment. In others, as treated groups, different doses of Rosa canina distilled water, 0.416%, 1.25%, 2.5% and 4.16% (diluted with Krebs) were injected to the hearts by use of a pump at the final 5 minutes of ischemia and the first 15 minutes of reperfusion. The doses were selected on the basis of the result of dose response examination in our pilot study.

Cardiac area at risk and infarct size determination
At the end of reperfusion, the coronary artery was ligated and 1 ml of Evans blue (2%) was injected to aorta. The Evans blue solution dyes the perfused myocardium, whereas the blocked vascular bed remains unstained. Then, both atria and the roots of the great vessels were removed. The heart was frozen for 1 hour and then cut into 2-mm transverse slices. All slices were incubated with a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC, in 0.1 M phosphate buffer, pH 7.4) stain for 20 min at 37°C. Then they were fixed in 10% formalin to enhance the contrast of the Evans blue and TTC staining. Both surfaces of each section were scanned using Photoshop program (Adobe Systems, version 7.0). Total area at risk was expressed as a percentage of the left ventricles (AAR/LV). Infarct size was expressed as a percentage of the area at risk (IS/AAR).

Materials
2,3,5-triphenyltetrazolium chloride and Evans blue were obtained from Sigma Chemical Co. The Rosa canina distilled water was purchased from Ghamsar Co (Kashan, Iran) with company registration number of 191180.

Statistical analysis
Statistical analysis of arterial hemodynamic parameters within the group was performed with repeated measures ANOVA followed by the Tukey’s post-hoc test. Comparison between the groups in hemodynamic parameters and infarct size were determined by one-way ANOVA followed by the Tukey’s post-hoc test. All data were expressed as...
mean ± SEM. Statistical significance was defined as p < 0.05.

Results

Hemodynamic functions

HR, LVDP, and RPP changes during the different periods of experiment are shown in Table 1.

*Rosa canina* distilled water (0.416% and 1.25%) significantly decreased the HR at the end of ischemia compared with their baseline and with IR group.

At the end of ischemia and reperfusion, LVDP of IR group was meaningfully reduced as compared with baseline. *Rosa canina* distilled water (1.25% and 2.5%) significantly prevented the decrease in LVDP at the end of both ischemia and reperfusion compared with their baseline. On the other hand, there were significant differences in LVDP at the end of ischemia and reperfusion between IR and 1.25% and 2.5%.

A significant decline in RPP during ischemia and reperfusion period was seen in IR group comparing with the baseline. Administration of 1.25% and 2.5% dilution of the drug significantly improved in sequence the reperfusion and ischemia RPP compared with their baseline and with IR group.

Table 1. Hemodynamic parameters. bpm, beat per minute; HR, heart rate; LVDP, left ventricular developed pressure; RPP, rate-pressure product. Data are presented as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Reperfusion</th>
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<tbody>
<tr>
<td></td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
</tr>
<tr>
<td>IR</td>
<td>126.2 ± 4.2</td>
<td>102 ± 3</td>
<td>104.7 ± 6.5</td>
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<tr>
<td>0.416%</td>
<td>124 ± 7.5</td>
<td>63.2 ± 4*</td>
<td>64.2 ± 4.6*</td>
</tr>
<tr>
<td>1.25%</td>
<td>114.4 ± 5.2</td>
<td>62 ± 8.3*</td>
<td>104 ± 4.7</td>
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<tr>
<td>2.5%</td>
<td>112.7 ± 6.9</td>
<td>77.5 ± 4.3</td>
<td>101.2 ± 9.1</td>
</tr>
<tr>
<td>4.16%</td>
<td>127 ± 3.7</td>
<td>98.5 ± 14.3</td>
<td>101.5 ± 6.4</td>
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<tr>
<td></td>
<td>LVDP (%of baseline)</td>
<td>LVDP (%of baseline)</td>
<td>LVDP (%of baseline)</td>
</tr>
<tr>
<td>IR</td>
<td>78.5 ± 3.3</td>
<td>63.5 ± 2.3*</td>
<td>54.7 ± 8*</td>
</tr>
<tr>
<td>0.416%</td>
<td>115.2 ± 5.4</td>
<td>58.2 ± 3.7*</td>
<td>79 ± 6.7*</td>
</tr>
<tr>
<td>1.25%</td>
<td>128.8 ± 6.7*</td>
<td>67.8 ± 5.2*</td>
<td>103 ± 4.1</td>
</tr>
<tr>
<td>2.5%</td>
<td>125 ± 7.2</td>
<td>82 ± 1.3*</td>
<td>92 ± 9.3</td>
</tr>
<tr>
<td>4.16%</td>
<td>99.5 ± 14.3</td>
<td>66.7 ± 5.5*</td>
<td>78.7 ± 5.8*</td>
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<td></td>
<td>RPP (%of baseline)</td>
<td>RPP (%of baseline)</td>
<td>RPP (%of baseline)</td>
</tr>
<tr>
<td>IR</td>
<td>63.5 ± 2.3*</td>
<td>104.7 ± 6.5</td>
<td>54.7 ± 8*</td>
</tr>
<tr>
<td>0.416%</td>
<td>115.2 ± 5.4</td>
<td>64.2 ± 4.6*</td>
<td>79 ± 6.7*</td>
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<tr>
<td>1.25%</td>
<td>128.8 ± 6.7*</td>
<td>104 ± 4.7</td>
<td>103 ± 4.1</td>
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<tr>
<td>2.5%</td>
<td>125 ± 7.2</td>
<td>105 ± 10.7</td>
<td>101.2 ± 9.1</td>
</tr>
<tr>
<td>4.16%</td>
<td>99.5 ± 14.3</td>
<td>101.5 ± 6.4</td>
<td>72.2 ± 10.9</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to their baselines.
*# p < 0.01 compared to their baselines.
*# p < 0.001 compared to their baselines.

Area at risk and infarct size measurements

The area at risk (AAR/LV) and the infarct size (IS/AAR) are presented in Fig.1. There were no significant differences in AAR/LV among all groups. Infarct size was 25.3 ± 1.5% in IR group, while treatment with different doses of *Rosa canina* distilled water (0.416%, 1.25%, 2.5%, and 4.16%) significantly reduced infarct size to 13.6 ± 1.8%, 8.8 ± 0.8%, 8 ± 0.6% and 11.7 ± 0.3% respectively vs. control group (p < 0.001).

Discussion

Our findings showed that applying *Rosa canina* distilled water at the end of ischemia and early reperfusion period induced cardioprotective actions, which reduced infarct size and improved the left ventricular developed pressure.

Pharmacological postconditioning is a practicable way for protection of the ischemic heart against ischemia/reperfusion injury and has too much therapeutic potential. (Ji et al., 2008). It can be induced by administration of drugs at the end of ischemia and early reperfusion (Schipke et al., 2006) and simply applied to all clinical situations of myocardial ischemia-reperfusion (Yellon and Opie, 2006). Zhao et al proposed that the early minutes of reperfusion had an important role in the pathogenesis of post-ischemic injury and management of this phase could decrease harmful outcomes of ischemia–reperfusion injury (Zhao et al, 2003). It has been revealed that reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide (H_2O_2), and hydroxyl radicals involve in myocardial tissue damages due to ischemia and reperfusion (Jeroudi et al., 1994). According to Schipke et al, limitation of oxygen radicals has an important role in cardioprotection. Postconditioning appears to
decrease the oxidative stress, by decrease of the superoxide load (Schipke et al., 2006). Moreover, free radical scavengers and antioxidants can improve ischemia reperfusion injuries (Das and Maulik, 1994).

According to our study, Rosa canina distilled water exerted postconditioning effect in a dose-dependent manner. Studying dose response is vital to defining safe and dangerous levels and dosages for drugs and finding concentrations with smaller side effects. Rosa canina distilled water changed infarct size in a U-shaped style and the optimum dose which had this property was 1.25%.

Administration of Rosa canina distilled water improved hemodynamic functions. LVDP in both doses of 1.25% and 2.5% preserved at the baseline extent in reperfusion period; recommending that 1.25% is the minimum dose necessary to raise the recovery of contractile function. Thus, Rosa canina distilled water acted as a positive inotropic agent by increasing LVDP and myocardial contractility force in dose of 1.25%.

Rosa canina was a negative chronotropic agent because of decreasing heart rate after administration of diluted drug before inducing reperfusion. This effect was transient and did not continue to the end of reperfusion stage. Anyway, we could result that decrease of HR had a protective effect on heart because it led to decrease of oxygen demand.

The infarct size in all treated groups was significantly reduced and this was the indicator of cardioprotection in all doses especially 1.25% and 2.5%. It looks that reduction of infarct size by some doses of Rosa canina distilled water has resulted in improving left ventricular function with enhanced LVDP.

To our knowledge, this is the first report to demonstrate that Rosa canina can induce cardioprotective effects against ischemia reperfusion injuries. The existing reports are about the effects of various extracts of Rosa canina; including, the hepatic ameliorative effect of Rosa canina in renal IR injury (Gholampour and Owji, 2013), strong antioxidant activity against lipid oxidation (Ganhão et al., 2010), strong free radical and hydrogen peroxide scavenging dependent to the concentration (Serteser et al., 2008), controlling the development of carrageenan-induced edema (Lattanzio et al., 2011), anti-inflammatory property in vitro (Wenzig et al., 2008), and reducing the chemoluminescence of neutrophils and chemotaxis of leukocytes and monocytes (Kharazmi and Winther, 1999; Larsen et al., 2003). On the other hand, ROS production and inflammation have important role in IR injury.
According to the Kilicgun’s study, *Rosa canina* had dual effects dependent on its concentration. He demonstrated that high concentration of the drug led to prooxidant agent production and low concentration had an antioxidant property (Kilicgun and Altiner, 2010). Similarly, in our study the high dilution of *Rosa canina* distilled water could not improve LVDP and RPP in comparison with low dilutions.

Nitric oxide (NO) has a critical role in the cardioprotection against IR injury, in the control of cardiac contractility and heart rate, and in the remodeling due to ischemia reperfusion. Myocardial contractility is improved by low concentrations of NO (Rastaldo *et al*., 2007). Moreover, *Rosa canina* exerted its protective effect on cartilages with osteoarthritis through diminishing NO production and via this effect probably postpone or prevent early stages of the disease (Schwager *et al*., 2011).

Eugenol, which is a member of the phenylpropanoids, is the highest among the other components of *Rosa canina* aromatic or distilled water (Hosni *et al*., 2010). Fouad and Yacoubi demonstrated that eugenol treatment in rats with acute doxorubicin cardiotoxicity ameliorated cardiac tissue injuries significantly, decreased lipid peroxidation, and attenuated the elevations in cytosolic Ca(2+) and nitric oxide levels in cardiac tissue. (Fouad and Yacoubi, 2011). It has been shown that eugenol exerted its vasorelaxant activity on the activation of the NO/cGMP pathway (Luna-Vázquez *et al*., 2013). Also, it was indicated that eugenol had beneficial effects on preventing cardiomyocyte apoptosis and reversal of oxidative stress (Choudhary *et al*., 2006).

In conclusion, administration of *Rosa canina* distilled water before regional myocardial ischemia and reperfusion reduced the extent of myocardial injuries; but more research and examination are needed to determine the exact mechanism.

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**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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