Liver Ameliorative Effects of the Hydroalcohol Extract of *Rosa canina* L. Fruit against Ischemic Acute Renal Failure-induced Hepatic Damage in Wistar Rats

F. Gholampour and S.M. Owji

Department of Biology, School of Sciences, Shiraz University, Shiraz, Iran
Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding Author: F. Gholampour, Department of Biology, School of Sciences, Crossroad of Adabiat, Shiraz, Iran

ABSTRACT

Recent studies have shown remote effects of renal Ischemia/Reperfusion (I/R) injury on the liver. Furthermore, I/R injury is correlated with the generation of Reactive Oxygen Species (ROS). This study investigated the effect of *Rosa canina* on the hepatic dysfunction and histological damage induced by renal ischaemic reperfusion at an early stage. There were three groups (n = 10), in which rats received orally 7 days before induction of ischemia, extract of *Rosa canina* (500 mg kg⁻¹) in RC+I/R group and distilled water in I/R group. In sham group, the renal arteries were not occluded and distilled water was administered orally 7 days before surgery. Renal ischemia was induced by both renal arteries occlusion for 45 min followed by 24 h of reperfusion. Blood samples were collected for biochemical analysis and finally liver samples were preserved for future histological examination. The renal ischaemic challenge resulted in major histological damages of the liver (p<0.001), which were associated with increased levels of creatinine, Blood Urea Nitrogen (BUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) during reperfusion period (all p<0.001). In RC+I/R group, the histological damage to the liver was improved (p<0.001) along with increased in plasma creatinine, BUN, ALT and AST being smaller than those of the non-treated rats (p<0.001). *Rosa canina* exhibited a hepatoameliorative effect against renal ischemic/reperfusion-induced lesions.

Key words: *Rosa canina*, ischaemia/reperfusion, creatinine, liver, congestion

INTRODUCTION

Acute Renal Failure (ARF) is a syndrome characterized by a deterioration of kidney function over a short period of time, resulting in its insufficiency to excrete nitrogenous waste products and to maintain fluid and electrolytes homeostasis (Edelstein and Schrier, 2007).

Both Liver and kidney have roles in the regulation of body homeostatic responses, metabolism and excretion of toxic products and drugs. According to recent studies there is cross-talk between the kidneys and liver. A number of studies have shown Ischemia/Reperfusion (I/R)-induced local response in kidney tissue (Kadkhodaei et al., 1996, 2008). Recently, remote effects of renal I/R injury on the liver has been investigated. There is high mortality due to liver injury associated with renal disease in clinic. I/R injury induces an inflammatory response, which results in the formation of Reactive Oxygen Species (ROS). The excess ROS can inhibit the normal function of cellular proteins, lipids, or DNA due to damaging them (Sudha et al., 2013; Noori et al., 2009; Almansour, 2008; Ahmed, 2006). ROS besides to augmenting local tissue damage, affects organs remote from the site of I/R.
Exposure of organisms to free radicals has been led to develop a series of defence mechanisms (Cadenas, 1997; Hajipour et al., 2010). One of the defence mechanisms against free radical-induced oxidative stress includes antioxidant defence. One division of non-enzymatic antioxidants that minimizes the harmful effect of ROS, is nutrient antioxidants. Nutrient antioxidants cannot be produced in the body but they are provided through diet or supplements (Pham-Huy et al., 2008; Montaz and Abdollahi, 2012). Recent studies have shown that the antioxidants of plant origin with free-radical scavenging properties are important as therapeutic agents in several oxidative stress induced diseases (Ramehoun et al., 2009; Nwaejukor et al., 2012; Hussein and Abu-Zinadah, 2010).

In practice, the fruits of *Rosa canina* L. have been used traditionally for the prevention and therapy of various ailments, including diabetes mellitus, arthritis, sciatica, colds, rheumatism, gout, hemorrhoids, influenza and gallstones (Rein et al., 2004; Orhan et al., 2007; Wenzig et al., 2008).

So far, none of these indications clinical effectiveness has been demonstrated except for osteoarthritis (Winther, 2008), nephrolithiasis (Tayefi-Nasrabadi et al., 2012) and ischemic acute renal failure (Gholampour et al., 2012).

The present study aims to examine the preventive and curative effects of *R. canina* on ARF-induced liver injury.

**MATERIALS AND METHODS**

**Plant material**: Collective samples of *R. canina* that were found to be growing wild in the southwest region of Iran were collected during the postflowering phases (September, 2011) from Meimand (in the Fars province). The material was dried in the dark at room temperature before extraction.

**Extraction of the plant material**: Seventy grams of finely powdered dried fruit was submitted to extraction with 300 mL of methanol and water at a ratio of 1:1 in a Soxhlet apparatus for 10 h. After extraction, the solvent was filtered and then evaporated via a rotovap evaporator at 40°C. The dried extract weighed 21.5 g and, therefore, achieved a 30.71% yield. The dried extract was reconstituted to prepare a solution of 65 mg mL⁻¹ in distilled water just before the start of the experiments (Tayefi-Nasrabadi et al., 2012). Hydroalcohol extract of *R. canina* L. fruit was prepared in pharmacology laboratory (Shiraz University of Medical Sciences).

**Experimental procedure**: Male Wistar rats (240-280 g) were obtained from Razi institute, Shiraz, Iran. The animals were grouped and housed in polyacrylic cages and maintained under standard laboratory conditions (temperature, 25±2°C) with a 12:12 h light/dark cycle. They were allowed free access to a standard pellet diet and water ad libitum. The local ethics committee approved the study. The rats were divided into three groups: control (n = 10), I/R (n = 10), *R. canina*+I/R (*R. canina*, 500 mg kg⁻¹ during 7 days; n =10). After 7 days, the both renal arteries were occluded for 45 min followed by 24 h of reperfusion in I/R performed groups. In control group, the renal arteries were not occluded and animals received distilled water for 7 days before surgery. Rats were anesthetized with ketamine (60 mg kg⁻¹, i.p.) and xylazine (5 mg kg⁻¹, i.p.) before I/R operation. At the end of reperfusion period, blood sample was collected from heart ventricles under anesthesia and rats were sacrificed and the liver was quickly isolated and preserved.
Biochemical analysis: Plasma samples were assayed for creatinine and urea nitrogen in milligram per deciliter by means of an autoanalyser (RA 1000; Technicon Instruments, NY, USA). ALT and AST activities in plasma samples were measured by commercially available kits.

Histopathological examinations: Liver samples were embedded in paraffin and 5 μm sections were obtained by microtome (Erma, Japan). Routine staining with hematoxylin and eosin was done for each liver section. In a blinded fashion, each section was examined in at least 10 randomly selected non-overlapping fields under light microscope. In each section, the degree of the presence of congestion and cellular degenerative changes was examined. The level of each pathological manifestation was graded according to the changes involving: none with 0, less than 20% with 1, 21-40% with 2, 41-60% with 3, 61-80% with 4 and greater than 80% with 5. The sum of all numerical scores in each group was taken as the total histopathological score.

Statistical analysis: Data are presented as Mean±SEM. They were assessed by one-way analysis of variance followed by Duncan’s post hoc for between-group comparisons. The histopathological scores were statistically compared between groups by nonparametric Kruskal-Wallis multiple comparison test. All data analyses were performed using SPSS ver. 11.5 software (SPSS Software, Chicago, IL, USA) and significance was taken at p<0.05.

RESULTS

Plasma levels of creatinine (1.37±0.08) and Blood Urea Nitrogen (BUN) (37.1±2.35) of I/R group were statistically higher than the sham group (0.62±0.02 and 24.9±2.00, respectively) (p<0.001). R. canina-treated group showed significant reduction (p<0.001) in creatinine (0.86±0.04 vs. 1.37±0.08) and BUN (26.2±1.74 vs 37.1±2.35) level in comparison to I/R group (Table 1).

In the I/R group plasma levels of ALT (255.1±15.78) and AST (437.8±14.59) were statistically higher than the sham group (125.4±8.09 and 254.2±7.56, respectively). R. canina-treatment reduced the levels of ALT (170.90±14.29 vs. 255.1±15.78) and AST (320.70±15.09 vs. 437.8±14.59) in R. canina+I/R in comparison to I/R group (p<0.001) (Table 1).

Histology: Results from the histological studies were in agreement with the measured activities of plasma enzymes. There were no abnormalities or histological changes in the livers of normal rats (Fig. 1a, 1b). In the I/R group, vascular congestion was severe (grade 5) in comparison to the sham group (b1 and b2 in Fig. 1); while this manifestation had lower grade of 1 in the R. canina+I/R group (c1 and c2 in Fig. 1). In the I/R group, apoptosis of the hepatic cells was identified with a grade of 3; it had lower grade of 1 in the R. canina+I/R group (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine</th>
<th>BUN</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.62±0.02</td>
<td>24.9±2.00</td>
<td>254.2±7.56</td>
<td>125.4±8.09</td>
</tr>
<tr>
<td>I/R</td>
<td>1.37±0.08**</td>
<td>37.1±2.35**</td>
<td>437.8±14.59**</td>
<td>255.1±15.78**</td>
</tr>
<tr>
<td>RC+I/R</td>
<td>0.86±0.04***</td>
<td>26.2±1.74**</td>
<td>320.70±15.09**</td>
<td>170.90±14.29***</td>
</tr>
</tbody>
</table>

Data was analysed by ANOVA followed by Duncan multiple comparison test. Values are expressed as Mean±SEM, **p<0.001 vs. Sham
***p<0.001 Vs I/R
**Fig. 1 (a-c):** Representative light microphotographs of the liver obtained from sham group (a1, a2) and I/R group (b1, b2), or RC+I/R group (c1, c2). (haematoxylin–eosin staining; a1, b1 and c1 ×250; a2, b2 and c2 ×400)

**Table 2: Histopathological score in sham, I/R and RC+I/R groups (each n = 10) at the end of reperfusion period of ischemia/reperfusion-(I/R)-induced acute renal failure (MeansSEM)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Histopathological score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td>I/R</td>
<td>7.8±0.20**</td>
</tr>
<tr>
<td>RC+I/R</td>
<td>2.8±0.49***</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. **p<0.001 vs. Sham. ***p<0.001 vs I/R

**DISCUSSION**

Acute renal ischemic injury continues to be associated with a high mortality rate. Renal I/R injury occurs in many clinical situations, such as transplantation, partial nephrectomy, or sepsis. Although most research in this area has focused on the renal response to this injury, recent work has suggested that renal injury affects the liver (Kelly, 2003). In the present study, the changes in hepatic function and histology were examined at the early phase of reperfusion. In addition, it was determined that *Rosa canina* pretreatment could modulate ARF-induced liver dysfunction.

In the rats subjected to I/R and receiving *Rosa canina* (*R. canina*+I/R group), the reduction in GFR was attenuated as indicated by decrease of plasma level of creatinine and urea nitrogen.
Plasma AST and ALT levels were measured mainly as a marker of hepatic parenchymal cell injury. The results showed that the plasma AST and ALT levels increased significantly after renal I/R (45 min /24 h). In the present study, Rosa canina prevented the alterations in the ALT and AST enzyme activities. Furthermore, histological structure were examined in order to determine the injury, which occurred in the liver of rats after renal I/R.

Light microscopy for I/R group showed vascular congestion and apoptosis of the hepatic cells. Thus, the combination of these two factors were likely to be responsible for the reduced liver function following the ischaemia in I/R group. A number of animal studies have shown that ischemia/reperfusion is correlated with the generation of Reactive Oxygen Species (ROS) (Bhalodia et al., 2010). ROS are associated with the inflammatory response and frequently they contribute to the tissue damaging effects of inflammatory reactions (Pawliczak, 2003; Cuzzocrea et al., 2000; Leiro et al., 2004). On the other hand they are important mediators of programmed cell death induced by TNF (Los et al., 2002; Lin et al., 2004). Furthermore, at intermediate concentrations ROS induce apoptosis whereas at higher concentrations it induce necrotic cell death (Takeda et al., 1999; Renz et al., 2001). Neutrophils play a crucial role in the development and manifestation of inflammation and they are the major source of free radicals at the site of inflammation. Lipid peroxidation mediated by free radicals might yield a large number of reactive aldehydes and also lipid peroxides which are causally involved in pathophysiological changes associated with oxidative stress in cells and tissues (Bhalodia et al., 2010; Dabrowski et al., 1999; Wolfeys and Oliveira, 1997). On the other hand, Park et al. (2011) showed that both ischemic and non-ischemic renal injury initiates IL-17A generation in the small intestine resulting in small intestinal and liver inflammation, apoptosis and necrosis. They demonstrated crucial roles for TNF-α, IL-17A and IL-6 in generating these injuries. Also, they provided evidence that small intestine derived IL-17A causes further cytokine generation to induce hepatic injury and systemic inflammation. Thus, it is suggested that renal I/R-induced liver injury may be induced by the generation of ROS. In the rats subjected to I/R and receiving Rosa canina (R+I/R group), vascular congestion and apoptosis were attenuated. Since Rosa canina serves as an anti-inflammatory and antioxidant as well ROS scavenger agent (Orhan et al., 2007; Kharazmi and Winther, 1999; Larsen et al., 2003; Serteser et al., 2008; Kilciogun and Dehen, 2009), it is concluded that the anti-inflammatory and anti-oxidant properties of Rosa canina are important in alleviation of tissue damage in the liver.

In conclusion, 45 min of ischemia and 24 h of reperfusion induced ischemic acute renal failure (IARF) and hepatic damage in rats. However, administration of Rosa canina for 7 days before ischemia improved GFR and liver function and decreased the hepatic vascular congestion and apoptosis of the hepatic cells. The inhibition of post-ischaemic inflammatory events plays an important role regarding the renal and hepatic ameliorative effects of Rosa canina. These findings suggest that Rosa canina may be useful in preventing organ injury after renal ischemia/reperfusion.

ACKNOWLEDGMENTS

We wish to acknowledge the Research Council of Shiraz University, Shiraz, Iran, for the financial support of this study (grant no. 88-GR-SCST-117). We also thank Mrs Jabeendarbashi H. (Department of Pathology, Shiraz University of Medical Sciences) for her cooperation in preparing tissue samples for light microscopy.
REFERENCES


