The protective effect of *Hypericum origanifolium* in experimental renal ischemia/reperfusion injury in rats

Hakan SENTURK¹, Sahin KABAY², Hilmi OZDEN³, Gokhan BAYRAMOGLU¹, M. Cengiz USTUNER⁴, Nilgun OZTÜRK⁵, Gül GÜVEN³, Ali KUTLU¹, Gökçe BİLGİ¹, Derya USTUNER⁶ and H. Veysi GUNES⁴

¹Department of Biology, Faculty of Arts and Science, Eskisehir Osmangazi University, Eskisehir, Turkey.
²Department of Urology, Treating and Research Hospital, Dumlupınar University Kütahya, Kütahya, Turkey.
³Department of Anatomy, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey.
⁴Department of Medical Biology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey.
⁵Department of Pharmacognasy, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey.
⁶Department of Medical Genetic, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey.

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*Hypericum origanifolium* leaf extract (HOE) is a medicinal plant extract containing many polyphenolic compounds. Polyphenolic compounds have high antioxidant potential. Reactive oxygen metabolites (ROMs) play a role in the pathogenesis of ischemia/reperfusion injury (I/R) in the kidney. This study was designed to determine the possible protective effect of HOE on renal I/R injury. Twenty four adult female Wistar albino rats were evaluated in three groups. Group 1 (Control), group 2 (renal I/R injury+Saline), and group 3 (renal I/R injury+HOE 50 mg/kg) were designed to evaluate the effects of HOE in renal I/R injury on histopathological examinations. The malondialdehyde (MDA) levels, superoxide dismutase (SOD) and catalase (CAT) activities were determined. Plasma blood urea nitrogen (BUN), creatinine (Cr), uric acid (UAC), SGOT (alanine aminotransferase, ALT), SGPT (serum aspartate aminotransferase, AST) and lactate dehydrogenase (LDH) levels were measured. HOE treatments in renal ischemia-reperfusion decreased MDA in kidney and liver. SOD and CAT activities were increased with HOE treatment in kidney and liver. When all groups were compared histopathologically in kidney, HOE administration improved I/R-induced damages such as hyaline cast, tubular dilatation and parenchymal hemorrhagia. The plasma BUN values were increased in I/R group when compared with control; on the other hand after HOE administration, the BUN values decreased, but not significantly. The Cr, UAC, SGOT, SGPT and LDH levels increased in I/R group, but decreased after HOE administration when compared with I/R group. The findings imply that ROMs play a causal role in I/R-induced renal injury and HOE exerts renoprotective effects probably by the radical scavenging and antioxidant activities

Key words: Hypericum origanifolium, renal ischemia/reperfusion, rat, antioxidant.

INTRODUCTION

Acute renal failure (ARF) resulting from renal ischemia reperfusion injury (IRI) remains a major clinical problem...
encountered in many clinical situations: kidney transplantation, partial nephrectomy, renal artery angioplasty, cardiopulmonary bypass, aortic bypass surgery, accidental or iatrogenic trauma, sepsis, hydronephrosis, and elective urological operations. Although, the return of blood flow to ischemic tissue can result in the recovery of normal function, paradoxically the tissue may also be injured during the process of reperfusion (Sehirli et al., 2008; Yun et al., 2009). Renal ischemia initiates a complex and interrelated sequence of events, resulting in the injury and death of renal cells. Although, reperfusion is essential for the survival of ischemic renal tissue, it causes additional damage contributing to the renal dysfunction and injury associated with I/R of the kidney (Thandani et al., 1996; Lien et al., 2003). Especially, the cells of the proximal tubular epithelial are susceptible to I/R injury, leading to acute tubular necrosis, which plays a pivotal part in the pathogenesis of ARF (Lieberthal and Levine, 1996; Avlan et al., 2006). Reperfusion of the ischemic tissue produces reactive oxygen species, which are known to have deleterious effects such as increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration and necrosis. Oxidative stress is a relative excess of oxidants caused by increased free radical production and/or decreased antioxidant defense systems that impairs cellular function and contributes to the pathophysiology of many diseases (Zhao, 2005; Karimi et al., 2005). The antioxidant defense systems, non enzymatic free radical scavengers (e.g., vitamin E, vitamin C, uric acid, and bilirubin) and the antioxidant scavenging enzymes, (catalase [CAT], superoxide dismutase [SOD], and glutathione peroxidase [GPx]) protect cells and tissues against oxidative injury (Marubayashi and Dohi, 1996; Zhao, 2005). The genus Hypericum L., a member of the Guttiferae (Hypericaceae) family, contains about 400 species in the world. In Turkey, the genus is represented by about 80 species (Robson, 1967, 1988). It has traditionally been used on a widespread basis almost all over the world. Hypericum origanifolium extract (HOE) include naphthodiantronones (hypericin, pseudohypericin, proto-pseudohypericin, emodin, and frangulin), flavonoids (quercetin, myrcetin, and hyperoside), and xanthones (mangiferin and isomangiferin) (Mathis and Ourisson, 1964; Makovestška, 1999; Sirvent et al., 2002; Kitanov and Nedialkov, 1998). The polyphenolic compounds such as Hypericum perforatum have high antioxidant and preventive effect against damages of ischemia/reperfusion (I/R) injury (Abolfathi et al., 2011). There is increasing evidence to suggest that reactive oxygen metabolites (ROMs) play a role in the pathogenesis of I/R in the kidney.

We designed this study to determine the possible protective effect of HOE against oxidative stress during I/R injury of the kidney in rat via renal biochemical and histological and hepatic biochemical parameters.

**MATERIALS AND METHODS**

The experimental protocols were approved by the institutional animal ethics committee. Animals were obtained from the medical and surgical experimental research center of the institute and all experiments were carried out in the same center.

**Animals**

Twenty four adult female Wistar albino rats weighting 220 to 250 g were evaluated in three groups. All rats were housed in polycarbonate cages in a room with controlled temperature (22 ± 2°C), humidity (50 ± 5%), and a 12 h cycle of light and dark and were fed laboratory pellet chows and water was given ad libitum. The experiment was performed after a stabilization period in the laboratory for several days. All the rats used in the following experiments were subject to the Guiding Principles for the Care and Use of Laboratory Animals and the Recommendations of the Declaration of Helsinki.

**Experimental design**

Group 1 (Control), group 2 (renal I/R injury + saline), and group 3 (renal I/R injury + 50 mg/kg HOE) were designed to evaluate effects of HOE in renal I/R injury on the morphological changes (Öztürk, et al., 2009). Right nephrectomy was performed before 15 day renal I/R, except for group 1. HOE was administered (50 mg/kg, interperitoneally (i.p.) 15 min prior to ischemia. Renal I/R injury was induced with left renal pedicle occlusion for 45 min, followed with reperfusion for 6 h under anesthesia. After induction of I/R injury, left nephrectomies were performed for histopathological examinations.

**Surgical procedures and HOE administration**

**Drug administration**

Under xylazine (10 mg/kg) and ketamine (70 mg/kg) anesthesia, a right nephrectomy was performed and the rats were allowed to recover for 15 days before they were subjected to I/R injury. On the 15th day following nephrectomy, rats were fasted overnight. The renal pedicle was occluded for 45 min to induce ischemia and then subjected for 24 h of reperfusion (I/R groups). HOE was dissolved in serum physiologic as 2 ml/kg and was given as 50 mg/kg; I/R+ HOE groups. HOE or serum physiologic (I/R group) was administered intraperitoneally 15 min before ischemia and 12 h after reperfusion. The animals were decapitated after 24 h of reperfusion period. After induction of I/R injury, left kidney and liver were processed for histopathological examinations.

**Histopathological evaluation**

Left nephrectomy specimens and livers were processed routinely in 10% formalin solution, and embedded in paraffin. Tissue sections of 5 μm were obtained, and stained with hematoxylin and eosin (H and E). Histopathological examinations were performed under a light microscope (NIKON, Japan). A minimum of 10 fields for each kidney slides with minimum X50 magnification were examined to assign the severity of these morphological changes. The morphological changes were scored on a scale of none (−), mild (+), moderate (++), and severe (+++) damage in order to perform a
comparison between the groups.

**Biochemical analysis**

Blood samples were collected to determine blood urea nitrogen (BUN), creatinine (Cr) and uric acid (UAC) as indicators of kidney function, alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) to assess liver function. In addition, lactate dehydrogenase (LDH) was assayed in serum samples for the evaluation of generalized tissue damage. These were determined spectrophotometrically using an automated analyzer.

**Post mitochondrial supernatant preparation (PMS)**

After sacrificing the animals, isolated areas of the nephron of their kidneys were quickly removed and perfused immediately with ice-cold normal saline, and homogenized in chilled potassium chloride (1.17%) using a Potter Elvehjem homogenizer. The homogenate was centrifuged at 800 × g for 5 min at 4°C in a refrigerated centrifuge to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 × g for 20 min at 4°C to get the PMS which was used to assay malondialdehyde (MDA), catalase (CAT), and SOD activity.

**Protocols of lipid peroxidation and enzyme activities measurement**

MDA production is an end product of lipid peroxidation which reacts with thiobarbituric acid to form a red colored complex. The measurement of MDA levels by thiobarbituric acid reactivity is the most widely used method for assessing lipid peroxidation. 0.1 ml of homogenate, 3 ml of 1% phosphoric acid, and 0.5 ml of distilled water and 1.0 ml of 0.6% 2-thiobarbituric acid were added. The mixture was boiled in water bath for 45 min. Afterward, the mixture was cooled in on ice, followed by an addition of 4.0 ml of n-butanol to extract the cold thiobarbituric acid reactants. The optical density of the n-butanol layer was determined at 532 nm after centrifugation at 1,000 g for 5 min and was expressed as nmol MDA/g of tissue (Mihara and Uchiyama, 1978).

**Determination of SOD activity**

SOD activity was spectrophotometrically assayed with commercial kits. The Fluka SOD kit USA contains all reagents and solutions required for determining SOD activity in an indirect assay method based on xanthine oxidase and a novel color reagent. The homogenate SOD activity was determined by inhibition of Formosan dye (450 nm) employing the xanthin-xanthin oxidase enzymatic method to generate superoxide radicals and expressed as U/g.

**Determination of CAT activity**

One unit of CAT equals the enzyme activity that recognized 1 µmol of hydrogen peroxide in 60 s at 37°C. The three blank samples were prepared according to Goth (1991). CAT activity was measured with determination of absorbance of three blank samples at 405 nm in spectrophotometer. CAT activity (kU/L) was calculated as $271 \times \frac{\text{Abs}_{\text{blank1}} - \text{Abs}_{\text{blank3}}}{\text{Abs}_{\text{blank2}} - \text{Abs}_{\text{blank3}}}$ (Goth, 1991).

**Statistical analysis**

The statistical analysis was performed with the computer program “Statistical Package for Social Sciences for Windows” (SPSS Inc; Release 11.5; Sep 6, 2002). All of the data were expressed as means ± standard deviation (SD). Differences between groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison tests. The significance was tested at non significant (ns) $p > 0.05$, $p < 0.05$, $p < 0.01$, and $p < 0.001$.

**RESULTS**

The control group did not show any morphological changes. By contrast, hyaline cast was present in all the rats in group 2 (severe in 1, moderate in 4 rats, and mild in 3 rats). Tubular dilation and parenchymal hemorrhagia were detected in 7 rats (moderate in 4 rats and mild in 3 rats) and in 5 rats (moderate in 3 rats and mild in 2 rats) in group 2, respectively. In group 3 (renal I/R + 50 mg/kg HOE), mild in 1 parenchymal hemorrhagia and mild hyaline cast were present in 4 rats, and there were normal histopathological findings in 3 rats (Figures 1 and 2). We showed that MDA levels of group 2 (I/R) in renal tissue were significantly higher than control group. HOE administration had significantly decreased MDA levels in groups 3 when compared with group 2. When we compared the SOD levels, it significantly decreased in group 2 when compared with control group. But the increase of SOD levels was statistically significant in group 3 when compared with group 2. The CAT levels decreased significantly in group 2 when compared with control group, but after HOE administration CAT levels increased. The enzymatic activity changes in kidney are presented in Table 1. MDA levels of group 2 (I/R) in liver were significantly higher than control group, but it was lower in group 3 than in group 2. When we compared the SOD levels, it significantly decreased in group 2 when compared with the control group. The SOD levels in group 3 increased significantly when compared with group 2. The CAT levels decreased significantly in group 2 when compared with control group, but after HOE administration, CAT levels increased in group 3.

These results suggested that the other organs such as liver may be affected in renal I/R injury. HOE may be effective in preventing oxidative injury for other organs and tissues. The enzymatic activity changes in liver are demonstrated in Table 1. The plasma BUN values increased in I/R group when compared with the control; on the other hand after HO administration, the decrease of the BUN values were not significant. The Cr level increased in I/R group, but decreased after HO administration when compared with group 2. The UAC level were increased in I/R group, but decreased after HO administration when compared with group 2. The ALT, AST, and LDH levels increased in I/R group, but decreased after HO administration when compared with...
Figure 1. Parenchymal hemorrhagia (++) H&E, Scale bar: 15 μm.

Figure 2. Hyaline cast (+) and tubular dilatation (++) H&E, Scale bar: 15 μm.
Table 1. MDA, SOD, and CAT levels in kidney and liver tissue after I/R.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (mg/dl)</th>
<th>SOD (U/L)</th>
<th>CAT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Group 1</td>
<td>5.31 ± 0.59</td>
<td>4.81 ± 0.14</td>
<td>18.01 ± 0.96</td>
</tr>
<tr>
<td>Group 2</td>
<td>10.44 ± 1.20</td>
<td>4.15 ± 0.53</td>
<td>12.32 ± 1.09</td>
</tr>
<tr>
<td>Group 3</td>
<td>6.97 ± 0.72</td>
<td>5.38 ± 0.31</td>
<td>16.07 ± 0.65</td>
</tr>
</tbody>
</table>

P-values and multiple comparison of the groups
- G1 - G2***
- G1 - G3**
- G2 - G3***
- G2 - G3***
- G2 - G2***
- G2 - G3***
- G2 - G2***

*p > 0.05, *p < 0.05, **p < 0.01, and ***p < 0.001.

Table 2. The means and standard deviations (±SD) for biochemical analysis of groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>CRE (mg/dl)</th>
<th>UAC (mg/dl)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>LDH (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.65 ± 2.11</td>
<td>0.52 ± 0.056</td>
<td>1.22 ± 0.08</td>
<td>78.10 ± 2.53</td>
<td>66.61 ± 7.29</td>
<td>120.21 ± 13.11</td>
</tr>
<tr>
<td>I/R</td>
<td>108.22 ± 16.70</td>
<td>1.50 ± 0.22</td>
<td>2.80 ± 0.22</td>
<td>264.82 ± 9.45</td>
<td>94.61 ± 4.68</td>
<td>658.65 ± 47.79</td>
</tr>
<tr>
<td>HOE</td>
<td>98.37 ± 6.33</td>
<td>1.06 ± 0.29</td>
<td>2.36 ± 3.28</td>
<td>158.71 ± 11.68</td>
<td>73.98 ± 2.61</td>
<td>430.70 ± 25.90</td>
</tr>
</tbody>
</table>

group 2 (Table 2).

DISCUSSION

The acute renal failure produced by I/R is a clinical and experimental syndrome characterized by major reduction in glomerular filtration rate, extensive tubular damage, tubular cell necrosis, glomerular injury, and signs of tubular obstruction with cellular debris (Kabasakal et al., 2005). Renal I/R induced acute tubular necrosis is observed most frequently in patients after cardiac and aortic operations, trauma, severe dehydration, burns, and others. Because renal failure induced by these conditions is a devastating problem, evaluation of new therapeutic agents is essential (Avlan et al., 2006). Thus, to assess its protective effect in renal I/R injury, we investigated HOE. In our study, HOE treatments in renal ischemia-reperfusion decreased MDA in kidney and liver. SOD and CAT activities were increased with HOE treatment in kidney and liver. When all groups were compared histopathologically in kidney, HOE administration improved I/R-induced damages such as hyaline cast, tubular dilatation, and parenchymal hemorrhagia. The plasma BUN values increased in I/R group when compared with the control group; on the other hand after HOE administration, the BUN values decreased, but not significantly. The Cr, UAC, SGOT, SGPT, and LDH levels increased in I/R group, but decreased after HOE administration when compared with I/R group. Takahira et al. (2001) showed that treatment with dexamethasone did not ameliorate serum creatinine concentration, although dexamethasone attenuated enhanced neutrophil infiltration induced by renal I/R. Oztürk et al. (2001) showed that there are significant improvements in serum BUN and creatinine concentrations in the molsidomine treated and L-N^6^-nitroarginine methyl ester (L-NAME) treated animals. In addition, molsidomine improved renal damage; although, L-NAME did not improve as compared to I/R group. According to Takaoka et al. (2002), treatment with low dose Lipoic Acid (LA) tended to attenuate the histological damage, but its effects were not significant. The higher dose of LA significantly attenuated the development of all these lesions and suppressed BUN and creatinine elevation (Takaoka et al., 2002). Uz et al. (2009) found that the levels of urea, creatinine levels remained unchanged in ginger + I/R group as compared to I/R group. SOD enzyme activity was significantly increased by the treatment with ginger, but CAT activity and levels of MDA did not change. Histological examination of the kidneys subjected to I/R process showed the distinctive pattern of ischemic renal injury, which included widespread degeneration of tubular architecture, loss of brush border, sloughing tubular epithelial cells from the basement membrane, tubular cell necrosis, and intratubular cast formation, especially in the outer medulla. Ginger + I/R group demonstrated marked reduction of the histological features of renal injury (Uz et al., 2009). Yanarates et al. (2008) observed minimal tubular cell swelling, brush border loss, and nuclear
condensation in kidney section of plasminogen activator (PA) treated rat as compared to I/R rat. PA significantly reduced the I/R-induced increases in Cr, BUN, and AST. In addition, PA restored decreased antioxidant enzymes, and attenuated histological alterations. They emphasized that PA may serve as a potential therapeutic agent in protecting kidney and multiple target organs from I/R injury (Yanarates et al., 2008). Rhoden et al. (2001) used different times of reperfusion after the renal ischemia. The data revealed that the renal ischemia had significantly increased serum creatinine levels at 24 and 96 h after the surgical procedure, when compared with the control group. But it did not change at 192 h. Furthermore, alfa-tocopherol significantly protected renal function in rats subjected to renal ischemia and reduced the MDA concentration. Dietary deficiency of vitamin E seems to lead to greater structural and functional renal impairment and increased lipid peroxidation following renal ischemia (Rhoden et al., 2001). Sucu et al. (2002) investigated the effects of trimetazidine (TMZ) on tissue damage in kidney after hindlimb ischemia/reperfusion (I/R), to evaluate the distant organs after I/R. They observed that, there was a prominent tubulointerstitial injury with loss of prominent brush border. Although, there is no statistical significant difference, loss of Bowman’s space, increase in glomerular congestion, and bleeding in periglomerular and peritubular areas were observed in group I/R rather than TMZ-treated group. But leukocytic infiltration was decreased in TMZ group (Sucu et al., 2002). Unlü et al. (2003) observed decreased MDA levels and increased SOD activities in Daflon group. The histopathological evaluation showed that there was a prominent tubulointerstitial injury with loss of brush border and this degeneration was accompanied by segmental glomerular degeneration also for both control group and group D. But the leukocytic infiltration decreased in Daflon group (Unlü et al., 2003). Foglieni et al. (2006) showed that ethylenediaminetetraacetic acid (EDTA) preserved the architecture of kidneys submitted to I/R. In addition, serum urea and AST levels were decreased with trimapril treatment. I/R group showed significantly higher histopathological injury scores when compared with scores of other groups (Avlan et al., 2006). As seen, there are a lot of studies which examine the effects of the different antioxidants on renal I/R injury. They revealed different results for enzymatic activities and severe histopathological findings. Our results are in concordance with the result seen in some of them, while different from some of them. The findings of our study indicate that HOE may have protective role in I/R damage of organs such as kidney and liver by restoring antioxidants and then reducing lipid peroxidation in renal and hepatic tissue.

Conclusions

The findings imply that ROMs play a causal role in I/R-induced renal injury and HOE exerts renoprotective effects probably by the radical scavenging and antioxidant activities.

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