Original Article

Pretreatment with Olive Leaf Extract Improves Renal and Liver Antioxidant Systems Following Renal Ischemia-Reperfusion Injury in Rats

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Abstract

Background and Aim: The effect of Olive leaf extract (OLE) as a natural antioxidant was investigated on renal and liver antioxidant enzyme activities, after renal ischemia-reperfusion (IR) in a rat model.

Materials and Methods: Forty male rats divided into 5 groups: Sham (no IR), 3 "OLE+IR" groups (25mg/kg, 50mg/kg and 100mg/kg oral OLE once daily for 2 weeks, prior to renal IR) and "water+IR" group (oral gavage of water prior to IR). In the last 4 groups, both renal pedicles clamped for 45 minutes followed by 24 hours of reperfusion. Clearance of creatinine was measured. Renal and liver catalase and glutathione peroxidase activities and glutathione level were assessed as well. Volume densities of the renal proximal convoluted tubules were measured stereologicaly.

Results: Glutathione level, catalase and glutathione peroxidase activities, clearance of creatinine and volume density of the proximal convoluted tubules decreased in "water+IR" compared to the sham group. Pretreatment with OLE in 3 different doses significantly increased renal antioxidant enzymes, creatinine clearance and volume density of the proximal convoluted tubules in comparison with "water+IR" group. Pretreatment with only one dose (25mg/kg) of OLE significantly increased liver glutathione and GPx compared to the "water+IR" group. Pretreatment with 2 doses (25mg/kg, 50mg/kg) increase liver catalase compared to the water+IR group.

Conclusions: Two weeks of oral pretreatment with different doses of OLE decreased renal injury couesd by renal IR. This was associated with increased renal catalase and glutathione peroxidase activities. Some doses of OLE were also associated with increased liver antioxidant system parametes.

Keywords: Ischemia, Reperfusion, Olive leaf extract, Renal, Liver

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Introduction

About five percent of hospitalized persons are patients with acute renal failure (ARF) and nearly ten percent of them need medical attempts to somehow compensate renal dysfunction. Renal ischemia-reperfusion is among the most important causes of acute renal failure (1). Ischemia usually occurs during hemorrhagic shock, cardiac arrest, heart failure and also during renal transplantation, heminephrectomy and repair of suprarenal aneurysm surgeries (2, 3). Reactive oxygen species (ROS) produced during renal ischemia and particularly subsequent reperfusion causes protein, lipid, DNA and mitochondrial injuries which lead finally to cellular apoptosis and organ injury (4, 5). Dysfunction of protective antioxidants in the time of reperfusion is considered to be one of the causes of ischemia-reperfusion injury (6) and with increase in the time of ischemic period ,more advanced injuries can be observed in protective antioxidant mechanisms of the kidneys (7). Liver injury is one of the distant organ damages caused by renal IR (8). It has been demonstrated that renal IR injury not only may induce liver oxidative stress but also enhance lipid proxidation in liver tissue (9). It is well demonstrated in rats that after renal IR, the liver tissue antioxidant enzymes activities diminish (10).

It seems beneficial to use antioxidants to decrease IR-induced free radical injuries. Some synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and hydroquinon have harmful biological effects, so the use of natural antioxidants has been considered progressively (11). Medicinal plants have a special place in traditional medicine and have been used for many years to treat some diseases. Olive leaf extract (OLE) with both antibacterial and antiviral characteristics has some other documented therapeutic effects like decreasing blood pressure and sugar (12-15). Olive leaf extract has well known natural antioxidant and among different sections of olive tree its leaf is the richest source of phenolic compounds with high antioxidant characteristics (16). Many antioxidants such as ETS-GS (5), mesna (17) and vitamin C (18) have been recognized as effective materials in decreasing IR-induced renal injuries, probably by decreasing intracellular ROS content. With regard to the considerable antioxidant characteristic of OLE as a natural product, in present study, we investigate the effects of this herbal extract on a lipid peroxidation marker (Malondialdhyde) level and antioxidant systems in kidney and liver following Renal Ischemia-Reperfusion in a rat model.

Materials and Methods

All animal experiments carried out in the present study were approved by the ethics committee of Lorestan University of Medical Sciences.

Preparation of Ethanolic olive leaf extract and Oleuropein content analyzing method

The olive leaves (*Olea europaea*, variety of sevillano) were collected from Khorramabad in Lorestan province, Iran. After collection, they were weighed and washed. The air-dried leaves were grinded into fine powder and then powdered leaves were extracted with 300 ml of 80% ethanol for 16 hours at a temperature of 40°C. This extract was kept at 40°C, until use. The extract was dissolved in a required amount of water with regard to its dose, to be administered orally to animals.

The oleuropein amount of this extract was measured by high-performance liquid chromatogram procedure (HPLC) the method which was described previously (19). Briefly, HPLC analysis of the samples was conducted by a Shimadzu (model L-10AD, Japan) instrument consisting of two reciprocating pumps, quaternary pump (LC-10ATvp), UV-Vis detector (SPD-M10Avp) and vacuum degasser and system controller (SCL-10Avp). A manual injector with a 20 µL sample loop was applied to load the sample. Class VP-LC workstation was employed to acquire and process chromatographic data. A reversed-phase C_{18} analytical column (Shim-Pack VP-ODS, 250 mm × 4.6 mm i.d., Shimadzu, Japan) was used. The UV detector was set at 254 nm. For the HPLC separation of oleuropein an isocratic elution was used. The mobile phase consisted of distilled water (pH=2.9, adjusted with orthophosphoric acid) and acetonitrile (70:30, v/v). The amount of oleuropein in this extract was reported as 13.57%.

Experimental groups

Forty male Sprague-dawley rats weighing 200-250 g were kept at a temperature of 24±1°C with 12 hour light/dark cycle in the animal lab of Razi Herbal Medicines Research Center. The rats were randomly divided into 5 equal groups with 8 rats in each group as follows: 1. Sham group (once daily gavage of 0.5 ml water for 2 weeks prior to surgical procedure without induction of ischemia). 2. "OLE25+IR" group treated with once daily gavage of 25 mg/kg OLE for 2 weeks prior to IR induction. 3. "OLE50+IR" which was similar to the previous group except for oral administration of 50 mg/kg OLE. 4) "OLE100+IR" group which was like groups 2 and 3 with daily gavage of 100mg/kg OLE 5) Control or "water+IR" group which received daily 0.5 ml water by gavage for 2 weeks prior to ischemic insult.

Surgical procedure

Two weeks after treatment with OLE or water, the animals were anesthetized by intraperitoneal injection of 60 mg/kg ketamine and 2.5 mg/kg diazepam and then the abdominal region of the rats were shaved and sterilized with povidone-iodine solution. A midline laparotomy incision was made from a point superior to the pubic symphysis to the tip of xiphoid process and the abdomen was exposed as described previously (20, 21).

To observe the kidneys in the retro peritoneal region, the intestines were pushed aside gently. Then both left and right renal pedicles were occulted bilaterally with two non-traumatic microvascular clamps for 45 min. During this ischemic period, the color of the kidneys changed and this was considered as a landmark for confirmation of renal ischemia (21). After 45 min of ischemia, the clamps were removed from the renal pedicles to consider the kidneys for 5 min until their color turned again. This color change, confirmed kidney reperfusion. During 45 min of ischemia, both intestines and kidneys were conserved with humid and sterilized gauze. After abdominal suture, each rat was kept in a metabolic cage and 24-hour urine was collected.

Biochemical analysis

Twenty four hours after ischemic period, the rats were sacrificed and liver and left kidney tissue samples were removed and kept in -8°C freezer. In the time of chemical assessments, tissue samples were then homogenized with Phosphate Buffered Saline (PBS) and centrifuged in 5000rpm at 4°C for 15 minutes. The supernatant was used to measure parameters such as MDA, catalase, glutathione (GHS) and glutathione peroxidase (GPx).

Renal function parameters

Serum creatinine, blood urea nitrogen (BUN) and urine creatinine levels were measured by an autoanalyzer (Biotecnica instruments, BT 1000, Italy) with commercial standard kits (Pars Azmon, Tehran, Iran). Creatinine clearance was calculated as mL/min as follows:

Creatinine clearance = Urine Creatinine × Urine Flow / Serum Creatinine

and normalized according to the body weight (kg) and reported as mL/min.kg

Renal biochemical parameters

The amount of MDA was measured by the method described by Rungby and Ernst (22). Glutathione (GSH) was measured by Sedlak and Lindsay method (23). MDA and GSH levels were expressed as nmol/mg protein.

The activity of catalase was measured by spectrophotometric method at 240nm as described by Hfaiedh et al (24). The activity of GPx was detected spectrophotometrically at 340nm as was described by Floh and Gunzler (25).

Stereological Study on Renal Tissue

After the 24h period of reperfusion, the right kidney tissue samples were fixed in formalin 10% for histological assessments. Paraffin-embedded kidney specimens were sectioned at 5 mm slices by a microtome and stained using periodic acide schiff (PAS) method. 50 areas corresponding to renal cortex were analyzed in each kidney for assessment. The point counting rules was used to estimate the volume density of proximal convoluted tubule per cortex. Sections of kidney slices cone section from each part were utilized. Video projector by power point software, a point probe was designed (15×16 cm square with 360 +) at total magnifaction × 400, points that that hit epithelium of proximal tubules were counted. For point counting, researchers only considered the tubular profiles which fell inside the probe and did not cross the lower and left lines of the probe. The below equation was used to estimate the volume density of proximal convoluted per cortex.

∑ Pp/ ∑ Pt.

Where ∑ Pp equals to the sum of points which hit the proximal convoluted tubule epithelium and ∑ Pt equals to points that fall on probe (26).

Statistical analysis

Data were expressed as Median (Min-Max). The group differences were analyzed by Mann-Withney U test, and statistical significance was considered at the 5% level (p value≤0.05). Statistical analyses were performed using the SPSS software version 16.

Results

The effect of olive leaf extracts on renal function parameters

Serum creatinine and BUN levels were significantly increased in "water+IR" (control) group compared to sham group (p= 0.002 and 0.028 respectively). In three OLE-treated groups the amount of Cr and BUN levels were less than the control group, however the differences in comparison with control group were statistically significant only in "OLE50+IR" group (p= 0.021 and 0.01 respectively) (Fig.2). In comparison with sham group, the creatinine clearance of "water+IR" group was significantly decreased (p< 0.001). The creatinine clearance in all OLE-treated groups was significantly higher than "water+IR" group (p< 0.05). The amount of creatinine clearance was not significantly different between sham and 3 OLE-treated groups (Fig2).

Liver and renal MDA levels

The amount of liver was significantly different among the 5 experimental groups. The amount of renal MDA was not significantly different among groups except "water+IR" and Sham groups (p≤0.05). (Fig 3).

Liver and renal Glutathione levels

Ischemia-reperfusion led to decreased renal and liver glutathione activities (p≤0.05). Pretreatment with OLE largely prevented the decrease in this antioxidant enzyme activity in kidney tissue. Liver glutathione in "OLE25+IR" group was significantly higher than "water+IR" group (P≤0.05) (Fig 4).

Liver and renal GPx activity levels

Activity of GPx in the liver and Kidney in the "water+IR" group significantly decreased compared to the sham group (p≤0.05). Pretreatment with OLE prevented the decrease of GPx activity in the renal tissue of all OLE treated groups, but GPx activity in the liver tissue, significantly increased only in "OLE25+IR" group compared to "water+IR" group (p≤0.05) (Fig. 5).

Liver and renal Catalase activity levels

Ischemia-reperfusion decreased catalase activity in liver and renal tissues in "water+IR" group. Pretreatment with OLE enhanced this enzyme activity in the renal tissue of all OLE treated groups and in the liver tissue of "OLE25+IR" and "OLE50+IR" groups compared to the control group (P≤0.05) (Fig 6).

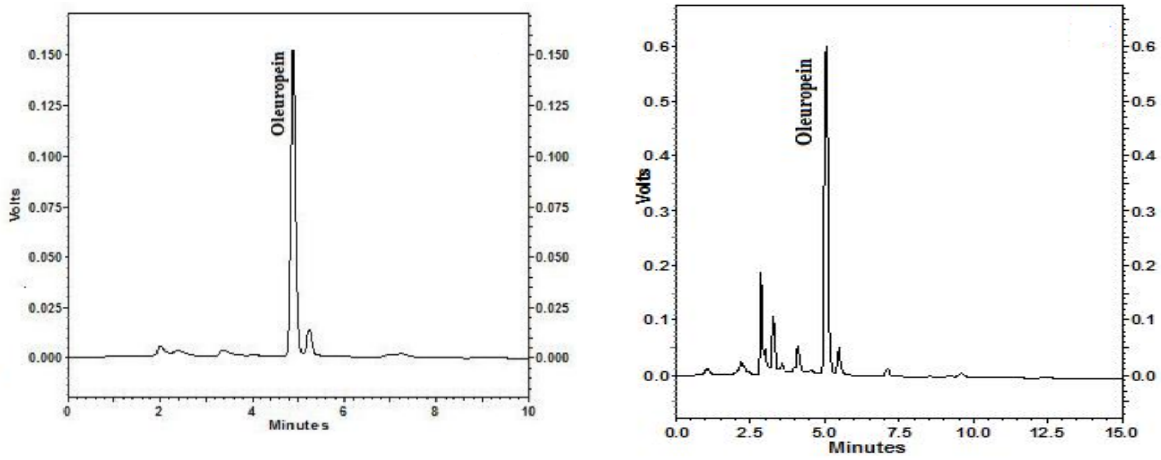

Fig. 1. HPLC chromatograms of oleuropein standard sampale (left) and olive leaf extract sample.

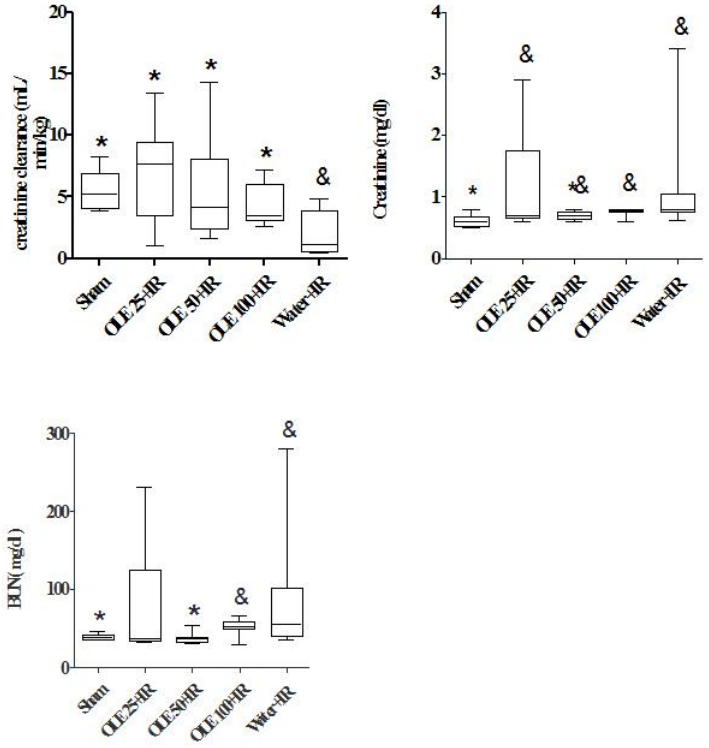

Fig. 2. Renal function parameters in various experimental groups. Values are expressed as the Median (Min-Max).
P≤ 0.05 compared to Sham group
* P≤ 0.05 compared to "Water+IR" (control) group.

The Effect of olive leaf extract on Volume density of proximal convoluted tubules

Volume density of proximal convoluted tubules significantly decreased in "Water+IR" group compared to the sham group (p≤0.05). Treatment with three doses of OLE significantly increased proximal convoluted tubule volume density compared to the "water+IR" group (p≤0.05). In none of the OLE pretreated groups, the volume density of the proximal convoluted tubules maintained at the same level of the sham group (p≥0.05) (Fig. 7).

Discussion

The present study like similar ones indicated that 45 minutes of bilateral renal ischemia followed by 24 hours reperfusion causes an acute renal dysfunction in rats. Two weeks pretreatment of rats with oral OLE gavage in three different doses (25mg/kg, 50mg/kg and 100mg/kg) could ameliorate kidneys function and volume density of the proximal convoluted tubules. Renal ischemia-reperfusion led to reduction of catalase and GPx activities and GSH level in both kidney and liver tissues and OLE pretreatment at least partially preserved these antioxidant parameters in both tissues.

Renal ischemia-reperfusion and possible resulted acute tubular necrosis (ATN) may occur after suprarenal vascular surgeries, kidney transplantation, renal and cardiavascular surgeries, hemorrhagic shock, cardiac arrest and acute myocardial infarction and accompanies with high mortality and morbidity rates (2, 3). Although reperfusion is necessary for saving the organs' cells from ischemia-induced death, it enhances the injuries caused by ischemia (27). It has been determined that ROS plays an essential role in pathogenesis of IR-induced injuries and free radicals

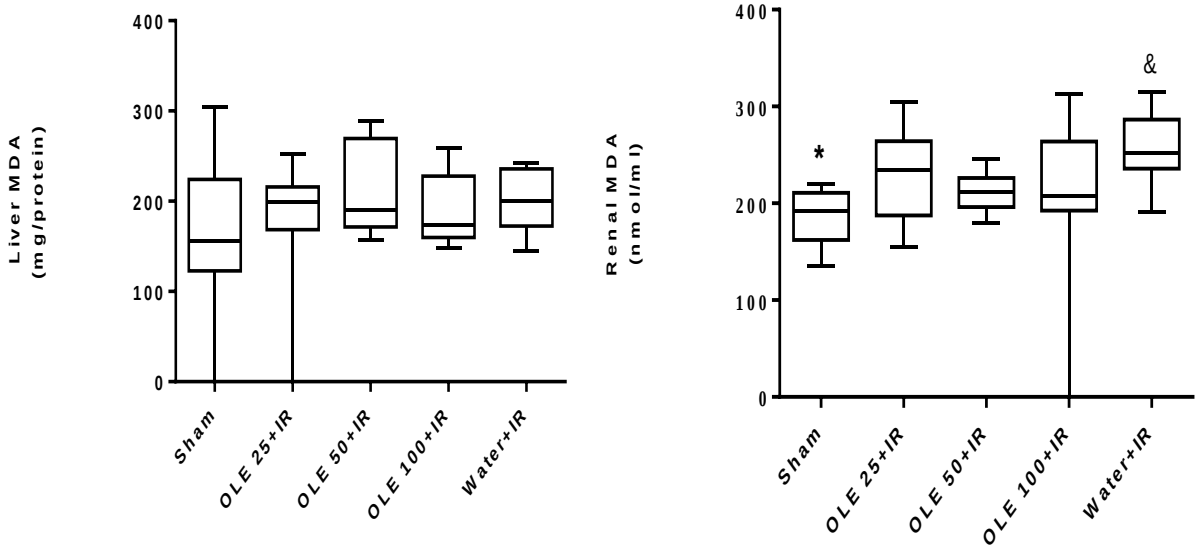

Fig. 3. Liver and Renal MDA levels in various experimental groups. Values are expressed as the Median (Min-Max).
P≤ 0.05 cpmpared to Sham group
* P≤ 0.05 compared to "Water+IR" (control) group.

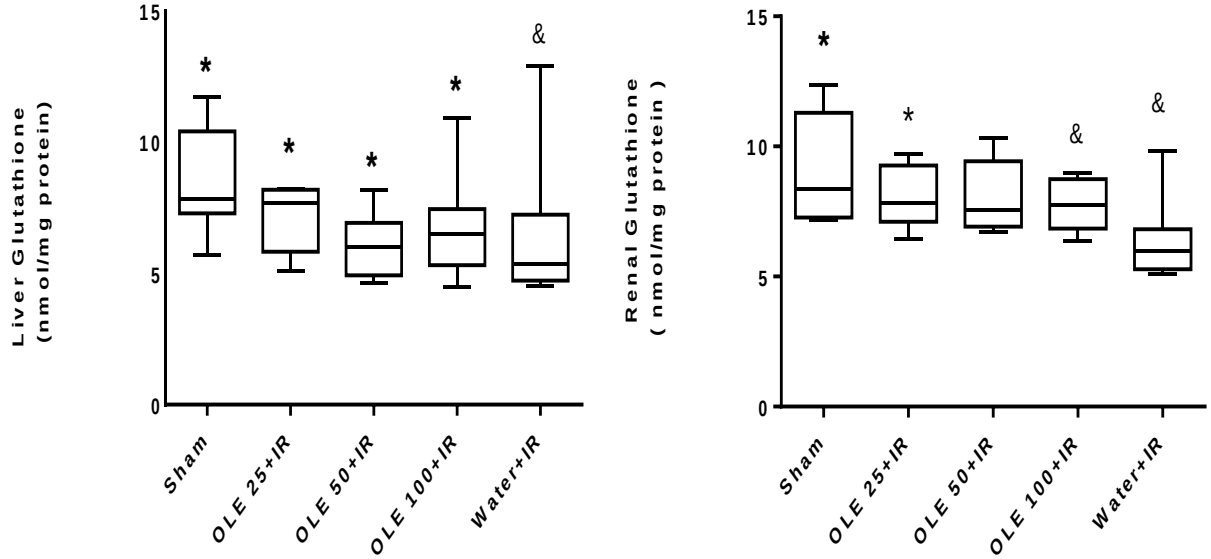

Fig. 4. Liver and Renal Glutathione levels in various experimental groups. Values are expressed as the Median (Min-Max).
P≤ 0.05 cpmpared to Sham group
* P≤ 0.05 compared to "Water+IR" (control) group.

cause oxidative injury of DNA, proteins, and lipids (4, 28). The lipid peroxidation could be estimated by

markers like MDA (27, 29). Dysfunction of antioxidant protective mechanisms in reperfusion period is considered as one of the reasons of IR injury (6, 31). Studies show that many antioxidant compounds such as mesna (17), vitamin C (18), EseroS-GS (30) can be effective in decreasing IR-induced renal injuries by inhibiting ROS (29, 31). On the other hand, OLE includes known antioxidants such as oleuropein, tyrosol, caffeic acid and hydroxy tyrosol. Especially Hydroxy tyrosol and oleuropein have powerful antioxidant characteristics and seems to be able to remove ROS and reinforce antioxidant system of the kidneys (32, 33). So it seems that antioxidants in olive leaf extract are at least partially responsible for diminishing renal injuries caused by IR.

Although many studies have reported the effect of various antioxidants on decreasing both serum BUN and Cr levels in renal ischemia-reperfusion models (6, 15, 16, 29, 30), our data showed significant amelioration of these two kidney function parameters after renal IR, only in OLE-treated rats with dose of 50 mg/kg. The more important indicator of renal function i.e. creatinine clearance was better in rats pretreated by all doses of OLE prior to IR.

It has been indicated previously that once daily gavage of 75mg/kg OLE for 14 days could partially protect the rat kidneys against cisplatin-induced nephrotoxicity (34). Also co administration of OLE (in 3 doses similar to present study for 12 days) with gentamycin had good protective effects on both renal functional and structural injuries caused by gentamicin (32). The results of our study in accordance with many other studies (29, 35-38) indicate that kidney IR, diminishes renal and liver antioxidant enzymes activities such as catalase and GPx and also decreases the level of glutathione which is likely due to dysfunction of protective antioxidant mechanisms in the time of reperfusion (6, 29). On the other hand there was a significant increase in amount of tissue glutathione, GPx and catalase in groups which received OLE prior to IR insult similar to many other studies about the effect of diverse antioxidants on renal IR injury (29, 39, 40).

Also OLE co-administration led to an increase in renal tissue activity level of antioxidant enzyme such as GPx, catalase and superoxide dismutas (SOD) in the rat model of gentamicin-induced nephrotoxicity

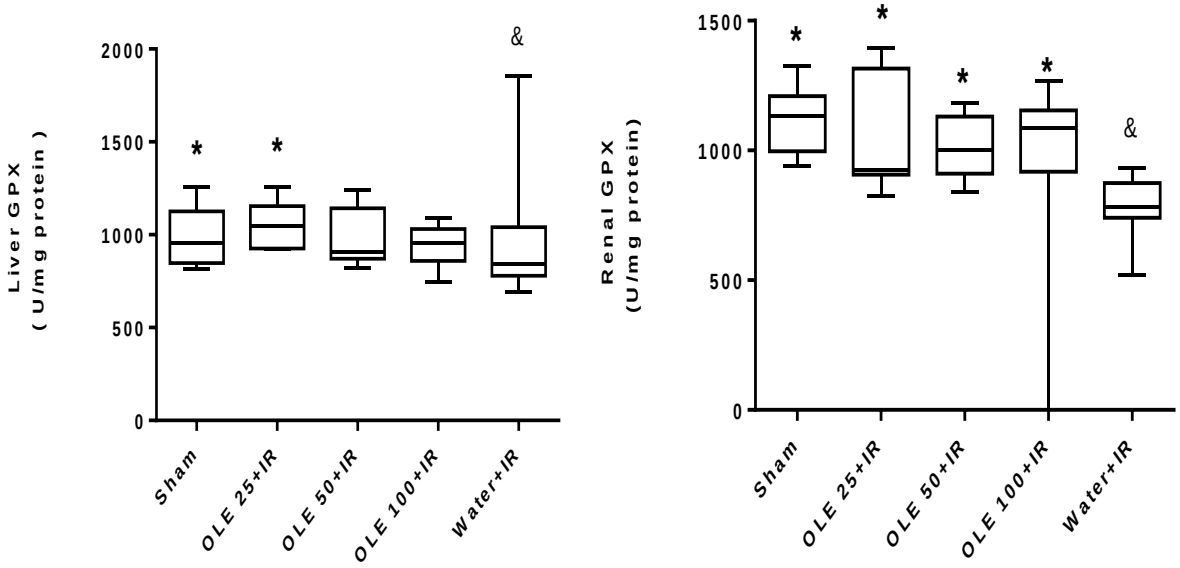

Fig. 5. Liver and Renal GPX levels in various experimental groups. Values are expressed as the Median (Min-Max).

P≤ 0.05 cpmpared to Sham group

* P≤ 0.05 compared to "Water+IR" (control) group.

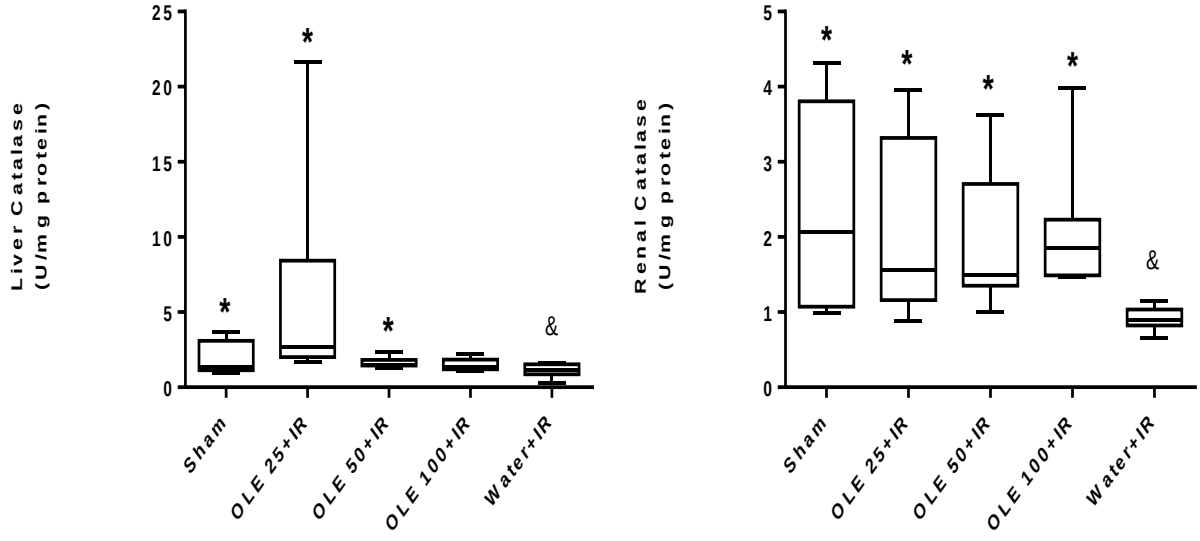

Fig. 6. Liver and Renal Catalase levels in various experimental groups. Values are expressed as the Median (Min-Max).
P≤ 0.05 cpmpared to Sham group
* P≤ 0.05 compared to "Water+IR" (control) group.

studied by Tavafi *et al* (32). Similar results i.e. increase in renal activity of GPx, catalase and SOD by OLE pretreatment has been reported in acute cisplatin-induced nephropathy model in rats (34). Both increases in amount of ROS production and diminishing of internal body antioxidant systems are partially responsible for various injuries caused by IR (35, 40) like IR-induced tubular necrosis in kidney tissue (29, 30, 35).

This study reveals that IR led to diminish volume density of the proximal convoluted tubules. The use of olive leaf extract was able to reduce structural changes of kidney tissue like volume density of the proximal convoluted tubules, probably not only due to its antioxidant characteristic but also due to reinforcement of body natural antioxidant systems.

It should be noted that OLE in doses applied in human studies is a safe herbal product with no determined toxicity (14). It has also been indicated that OLE or its chief constituent, oleuropein, has many possible preventive or therapeutic effects like protective effects on brain (18, 41, 42) and heart (43) ischemic injuries, blood pressure lowering effects

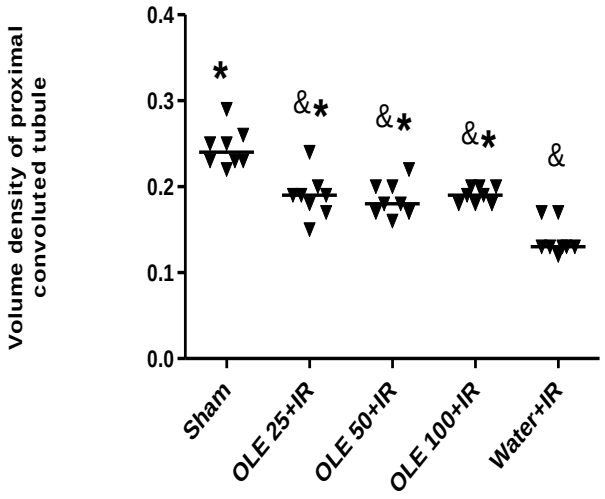

Fig. 7. Volume density of proximal convoluted tubules in various experimental groups. Values are expressed as the Median (Min-Max).
P≤ 0.05 cpmpared to Sham group
* P≤ 0.05 compared to "Water+IR" (control) group.

(14) decreasing blood glucose and lipids (15, 41) antinociceptive activity and potentiating morphine analgesic effects (44), antiarrhythmic effects (45), traumatic spinal cord injury lowering effects (46) and preventive effects on ethanol induced gastric ulcers (47).

Conclusion

In conclusion, the present study shows that once daily oral administration of olive leaf extract for 14 days, with three doses of 25, 50 and 100 mg/kg decreases renal injuries caused by IR in this rat model of bilateral renal ischemic injury. This protective effect may be somehow related to amelioration of IR-induced reduction in renal tissue antioxidant systems. Also OLE had similar significant protective effects on remote antioxidant system injuries of the liver tissue. Because of good antioxidant characteristic of OLE due to the presence of materials such as oleuropein, hydroxy tyrosol, tyrosol and so on, this beneficial effect on renal IR injury may be related to antioxidant characteristics of OLE. The effect of individual constituents of OLE is recommended to be considered in future researches on renal IR injury.

Acknowledgment

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Conflict of Interest

The authors declare that they have no conflict of interest.

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