Ameliorating Effect of Olive Leaf Extract on Cyclosporine-induced Nephrotoxicity in Rats

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Introduction. Olive leaves are traditionally used in the Mediterranean basin in many medical conditions for its potent antioxidant activity. Cyclosporine A, a well-known immunosuppressant, can induce nephrotoxicity through oxidative stress. This study investigated the effect of olive leaf extract (OLE) on cyclosporine-induced nephrotoxicity in rats.

Materials and Methods. Thirty Wistar rats (180 g to 200 g) were classified into 5 groups, each containing 6 rats. The first group received normal saline and served as control. The second group was treated with cyclosporine, 25 mg/kg for 21 days for nephrotoxicity induction. Groups 3 to 5 were treated with cyclosporine, 25 mg/kg in addition to different doses of OLE (40 mg/kg, 80 mg/kg, and 120 mg/kg), respectively, for 21 days. After 21 days, the rats' body weights were recorded and the rats were sacrificed. Blood samples were collected and the animals were necropsied. Both kidneys were removed, one for histopathological and one for antioxidant activity evaluations.

Results. Cyclosporine significantly reduced body weight and kidney weight; serum total protein, albumin, and sodium levels; and renal glutathione peroxidase, catalase, and superoxide dismutase. It also increased serum urea, creatinine, and calcium levels as compared to the control group. Groups 4 and 5 showed a significantly greater body weight and kidney weight; higher serum sodium, total protein, and albumin levels; greater glutathione peroxidase, catalase, and superoxide dismutase; and lower serum urea, creatinine, and calcium levels as compared to group 2.

Conclusions. Treatment with OLE can alleviate cyclosporine-induced nephrotoxicity when used in a proper dose.

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INTRODUCTION

Cyclosporine A is a powerful immunosuppressive medication that is widely used to prevent organ rejection and to treat certain autoimmune diseases.[1] However, the clinical utility of cyclosporine is often limited by the occurrence of nephrotoxicity.[2] Several mechanisms have been proposed in cyclosporine-induced nephrotoxicity, namely, the activation of the renin-angiotensin system and enhanced sympathetic tone,[3] increased synthesis of endothelin,[4] inductions of cytochrome P450 enzymes in renal microsomes,[5] and renal vasoconstriction attributed to an imbalance in releasing of the vasoactive substances, including reduction of vasodilator factors in particular nitric oxide.[6] Other studies have clearly demonstrated

that cyclosporine-induced oxidative stress plays a pivotal role in producing structural and functional impairment of the kidney.[2]

The olive tree (*Olea europaea L* [*Oleaceae* family]) has been cultivated in the Mediterranean area for more than 1000 years. Not only the olive oil, but also the leaves have been used for medical purposes and were introduced into the European Pharmacopoeia.[5] Olive leaf contains the active iridoid constituent oleuropein (chief constituent). Other secoiridoids include 11-demethyloleuropein, 7,11-dimethyl ester of oleoside, ligustroside, oleuroside, and unconjugated secoiridoid aldehydes. Triterpenes and flavonoids, including luteolin, apigenin, rutin, and diosmetin, are also present. Oleasterol, leine, and glycoside oleoside have also been found in the leaves.[7] Olive leaf extract (OLE), a phenolic compound derived from olive leaves, is known to have anti-oxidative, antimicrobial, antiviral and anti-inflammatory properties. Moreover, OLE can protect low-density lipoprotein from oxidation and has shown a hypotensive activity in experimental animals.[8-10]

Based on the involvement of oxidative stress and formation of oxygen free radical in the mechanism of cyclosporine-induced nephrotoxicity and antioxidant activity of the OLE, the present study was designed to investigate the effect of OLE against cyclosporine-induced nephrotoxicity.

MATERIALS AND METHODS

Chemicals

Cyclosporine was purchased as Sandimmune capsules (Novartis International AG, Basel, Switzerland).

Preparation of Ethanolic Olive Leaf Extract

Fresh olive leaves (*Olea europaea*) were collected from the Olive orchid of Misurata, Libya, in August 2013, and were identified by a specialist of the Department of Pharmacognosy, College of Pharmacy, Misurata University. The collected olive leaves were air-dried and grinded into fine powder. The extract was prepared by Soxhlet extraction process using 70% ethanol (1/10, w/v) as solvents. The extract was then centrifuged and filtered using a 0.45-μm filter (Millipore, Japan), and the filtrate was freeze-dried. For use, a portion of the extract was weighed and dissolved in distilled water in a ratio of 8:1.

Animals

Male Wistar rats (body weight, 180 g to 200g) were obtained from the Animal house, Misurata Technical Institute, Misurata, Libya. The rats were kept under constant environmental conditions (12 hours of dark-light cycle, 25 ± 2°C, and humidity of 50 ± 10%) and were supplied with laboratory chow and water *ad libitum*. All experimental procedures were conducted according to the ethics standards approved by the Institutional Animal Ethics Committee guidelines for animal care and use, Misurata University.

Experimental procedures

The animals were randomly divided into 5 groups, 6 animals each, as follows: group 1 (control group) received only normal oral saline; groups 2 to 5 received oral cyclosporine, 25 mg/kg/d, for 21days[11]; groups 3 to 5 also received OLE, 40 mg/kg/d, 80 mg/kg/d, and 120 mg/kg/d, respectively, for 21 days. At the end of the experiment, the rats were sacrificed and blood samples were collected and centrifuged to obtain clear sera. A midline abdominal incision was made and both kidneys were retrieved. The left kidney was deep frozen till enzymatic analysis, whereas the right kidney was sectioned and fixed in 10% formalin for the histopathological evaluation.

Biochemical Analysis

Biochemical studies were performed using commercially available kits, and serum levels of creatinine (Diamond Diagnostics, Egypt) as well as serum levels of urea, uric acid, total protein, albumin, sodium, potassium, and calcium (Biodiagnostic, Egypt) were quantified according to the manufacturer's instructions.

Histopathological Examination

Formalin-fixed right kidney was routinely processed, paraffin embedded, sectioned at 5 µm and routinely stained with hematoxylin-eosin.[12] Slides were examined under light microscope.

Assessment of Renal Antioxidant Enzymes

The kidney homogenate was used to assay glutathione peroxidase, superoxide dismutase (SOD), and catalase activity as described below:

Reduced glutathione. Kidney glutathione peroxidase was assayed by the method reported

by Jollow and colleagues.[13] In brief, the postmitochondrial supernatant was precipitated with 4% sulphosalicylic acid, kept at 4°C, and then centrifuged. The yellow color developed was read immediately at 412 nm on a spectrophotometer and was expressed as mol/mg protein $\times 10^{-4}$.

Catalase. Catalase activity was assayed using the reported method of Claiborne.[14] Briefly, the assay mixture consisted of 1.95 mL of phosphate buffer (0.05 M; pH, 7.0), 1.0 mL of hydrogen peroxide (0.019 M), and 0.05 mL of postmitochondrial supernatant (10%) in a final volume of 3.0 mL. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated and expressed as k/min.

Superoxide dismutase. Superoxide dismutase activity was assayed by the reported method of Kono.[15] The assay system consisted of ethylenediaminetetraacetic acid, 0.1 mM; sodium carbonate, 50 mM; and nitro blue tetrazolium, 96 mM. Two milliliter of above mixture, 0.05 mL of hydroxylamine, and 0.05 mL of postmitochondrial supernatant were taken in the cuvette, and the auto-oxidation of hydroxylamine was observed by measuring the absorbance at 560 nm and expressed as units/mg protein.

Statistical Analysis

Results were expressed as mean ± standard error of mean and were analysed for statistically significant differences using the 1-way analysis of variance followed by the Turkey-Kramer postanalysis test to compare the means from different groups. A *P* value less than .05 was considered significant. The SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA) was used for statistical calculations.

RESULTS

Body and Kidney Weight

Following the 21-day interventions, body weight and kidney weight were significantly lower in group 2 (cyclosporine) as compared to the control group. Compared to group 2, these parameters were significantly greater in groups 4 and 5 that received 80 mg/kg and 120 mg/kg of OLE, respectively (Figures 1 and 2).

Blood Chemistry

After the 21 days, serum creatinine and urea levels were significantly higher in group 2 (cyclosporine) as compared to the control group (Figures 3 and 4). Moreover, the nephrotoxicity effect of cyclosporine in group 2 was confirmed by the significantly lower levels of total protein and albumin (Figure 5) as compared to those in the control group, as well as significantly lower serum sodium levels and higher calcium levels in this group (Table 1). No significant changes were found regarding uric acid levels between the studied groups (Table 1).

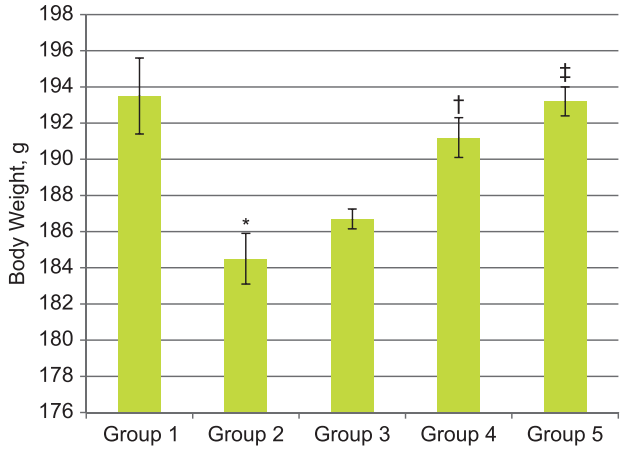

Figure 1. Mean ± standard error of mean for body weight in the rats. Group 1 is the control; group 2, cyclosporine; and groups 3 to 5, cyclosporine and olive leaf extract at a dose of 40 mg/kg, 80 mg/kg, and 120 mg/kg, respectively.
*P < .01 compared to group 1
†P = .01 compared to group 2
‡P < .001 compared to group 2

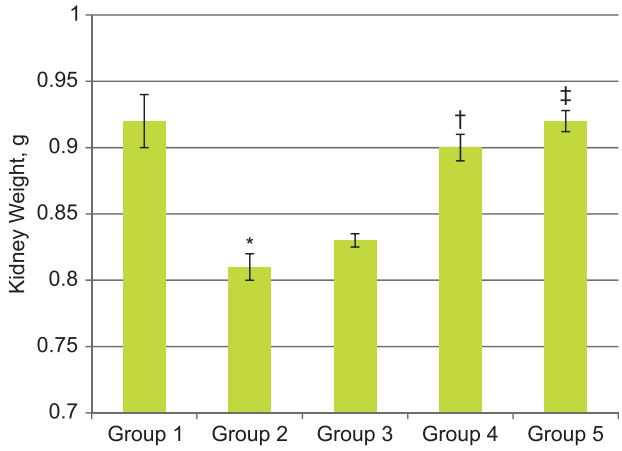

Figure 2. Mean ± standard error of mean for kidney weight in the rats. Group 1 is the control; group 2, cyclosporine; and groups 3 to 5, cyclosporine and olive leaf extract at a dose of 40 mg/kg, 80 mg/kg, and 120 mg/kg, respectively.
*P < .001 compared to group 1
†P < .001 compared to group 2
‡P < .001 compared to group 2

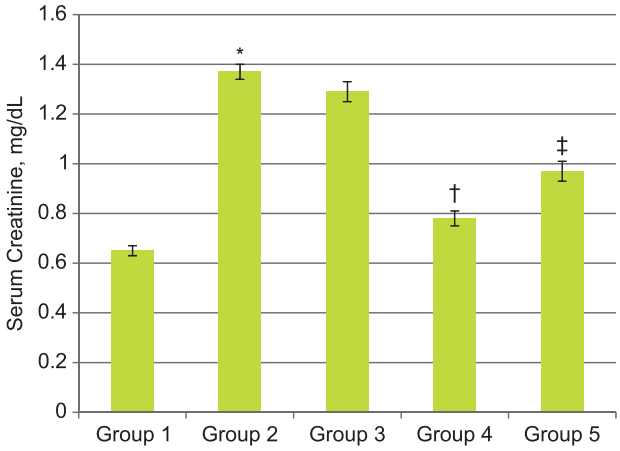

Figure 3. Mean ± standard error of mean for serum creatinine level in the rats. Group 1 is the control; group 2, cyclosporine; and groups 3 to 5, cyclosporine and olive leaf extract at a dose of 40 mg/kg, 80 mg/kg, and 120 mg/kg, respectively.
*P < .001 compared to group 1
†P < .001 compared to group 2
‡P < .001 compared to group 2

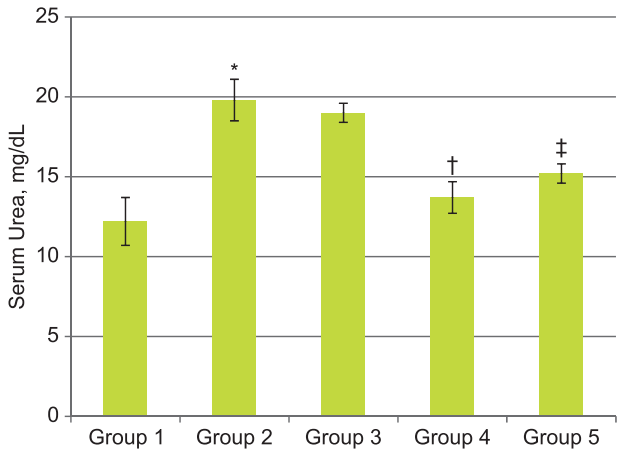

Figure 4. Mean ± standard error of mean for serum urea level in the rats. Group 1 is the control; group 2, cyclosporine; and groups 3 to 5, cyclosporine and olive leaf extract at a dose of 40 mg/kg, 80 mg/kg, and 120 mg/kg, respectively.
*P < .001 compared to group 1
†P < .001 compared to group 2
‡P = .002 compared to group 2

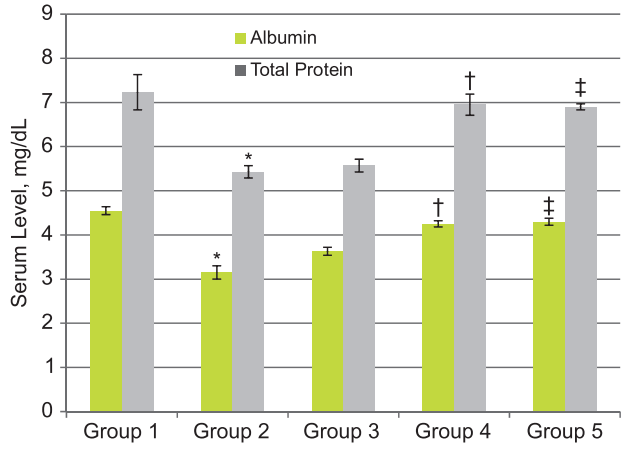

Figure 5. Mean ± standard error of mean for serum levels of total protein and albumin in the rats. Group 1 is the control; group 2, cyclosporine; and groups 3 to 5, cyclosporine and olive leaf extract at a dose of 40 mg/kg, 80 mg/kg, and 120 mg/kg, respectively.
*P < .001 compared to group 1
†P < .001 compared to group 2
‡P < .001 compared to group 2

Groups 4 and 5 showed significantly better kidney function test as compared to group 2 (Figures 3 and 4). Serum creatinine level was significantly lower in group 5 than group 4 (P = .02); however, both mean values were in reference range (Figure 3). Impairments in serum levels of total protein, albumin, sodium, and calcium were significantly less in groups 4 and 5 as compared to group 2 (Figure 5 and Table 1).

Renal Antioxidant Activities

Administration of cyclosporine in group 2 significantly decreased renal glutathione peroxidase, catalase, and SOD levels as compared to those parameters in group 1 (Table 2), while administration of OLE in groups 4 and 5 resulted in significantly higher levels of renal glutathione peroxidase, catalase, and SOD as compared to those in group 2 (Table 2).

Table 1. Mean Serum Levels of Uric Acid, Sodium, and Calcium in Rats*

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Uric acid, mg/dL	1.60 ± 0.12	1.60 ± 0.05	1.58 ± 0.05	1.53 ± 0.05	1.55 ± 0.04
Sodium, mEq/L	136.2 ± 1.0	130.0 ± 1.5†	131.0 ± 0.6	139.5 ± 1.8§	140.2 ± 1.0§
Calcium, mg/dL	9.55 ± 0.15	11.16 ± 0.19‡	10.67 ± 0.07	9.71 ± 0.39§	9.67 ± 0.07§

*Group 1 is the control; group 2, cyclosporine; and groups 3 to 5, cyclosporine and olive leaf extract at a dose of 40 mg/kg, 80 mg/kg, and 120 mg/kg, respectively.
†P = .02 compared to group 1
‡P < .001 compared to group 1
§P < 0.001 compared to group 2

Table 2. Mean Levels of Renal Antioxidants in Rats*

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Reduced glutathione, mol/mg protein $\times 10^{-4}$	18.80 ± 0.31	11.20 ± 0.33†	11.60 ± 0.12	18.30 ± 0.11‡	18.60 ± 0.11‡
Catalase, k/min	0.390 ± 0.006	0.250 ± 0.004†	0.260 ± 0.008	0.380 ± 0.003‡	0.390 ± 0.003‡
Super oxide dismutase, U/mg protein	1.52 ± 0.02	0.56 ± 0.07†	0.61 ± 0.01	1.45 ± 0.091‡	1.53 ± 0.02‡

*Group 1 is the control; group 2, cyclosporine; and groups 3 to 5, cyclosporine and olive leaf extract at a dose of 40 mg/kg, 80 mg/kg, and 120 mg/kg, respectively.
†$P < .001$ compared to group 1
‡$P < .001$ compared to group 2

Histopathological Examination

The nephrotoxicity effects of cyclosporine (group 2) were found to be prominent in histopathological examination with marked vacuolization of the cortical distal convoluted tubular epithelium, hyaline arteriolosclerosis, and cystic dilatation of the Bowman capsule as compared to the unaffected tissue (Figures 6A to 6C). Moreover, interstitial nephritis and shrunken glomerular tufts were observed. Similar results were seen in the rats of group 3, where vacuolization of the renal tubular epithelium and shrunken glomerular tufts were recorded (Figure 6D).

The examined sections from the rats of group 4 showed few shrunken glomerular tufts with mild interstitial nephritis represented by few fibroblasts and mononuclear cell infiltrations (Figure 6E), as compared to the control group. The rats of group 5 showed mild dilatation of the Bowman capsule with restoration of the normal histological features of the renal tissue (Figure 6F).

DISCUSSION

In the present study, we assessed the protective and antioxidant activities of OLE against cyclosporine-induced nephrotoxicity. Cyclosporine, in a dose of 25 mg/kg/d for 21 days, significantly reduces rats' body and kidney weight. Such a reduction is attributed to the increased metabolic rate and catabolism consequent to cyclosporine administration.[16] Our rats that were treated with OLE did not experience the adverse effect of cyclosporine on both body and kidney weight as evident by significantly greater weights for the rats of the groups with OLE at a dose of 80 mg/kg and 120 mg/kg. Similar results were recorded by Park and colleagues[17] who described a protective effect of OLE against body and organ weight loss in sreptozotocin-induced diabetic mice.

The cyclosporine-induced deterioration of kidney function was evidenced by the elevated serum creatinine and urea levels. Such results were previously reported[18-20]; in addition, elevation of serum calcium and decrease in serum total protein, albumin, and sodium levels reported in the present study confirm the cyclosporine nephrotoxic effect. Renal lesions associated with cyclosporine treatment, mainly tubular vacuolization, arteriole haylinosis, and interstitial nephritis, were confirmed in the rats and explained the elevated renal enzymes. These were in agreement with the previously reported lesions of the cyclosporine-induced nephrotoxicity.[21]

Administration of OLE counteracted the kidney dysfunction and morphological abnormalities induced by cyclosporine at doses of 80 mg/kg and 120 mg/kg, as evident by significant decrease in urea, creatinine, and calcium levels. Also, significant increases in sodium, total protein, and albumin levels compared to the cyclosporine-treated group were documented. Such a protective effect is evidenced by the less severity of the microscopic lesions recorded and the preserved normal renal architecture of these groups. Interestingly, the OLE-treated groups showed less inflammatory reaction in the renal tissues that might be attributed to OLE's anti-inflammatory effects.[10]

The pathogenesis of the cyclosporine-induced nephrotoxicity is not fully understood, but it is thought to be as result from the low-grade hypoxic injury to renal tubular cells and reactive oxygen species inducing apoptosis.[2,22] The development of cyclosporine nephrotoxicity in the present study was associated with a significant decrease in renal glutathione peroxidase, catalase, and SOD activity, indicating that oxidative stress plays a crucial role in the pathogenesis of cyclosporine-induced nephrotoxicity. In the present study, cotreatment with OLE prevented the depletion of renal glutathione peroxidase, catalase, and SOD associated with cyclosporine administration. The protective characteristics of the OLE are

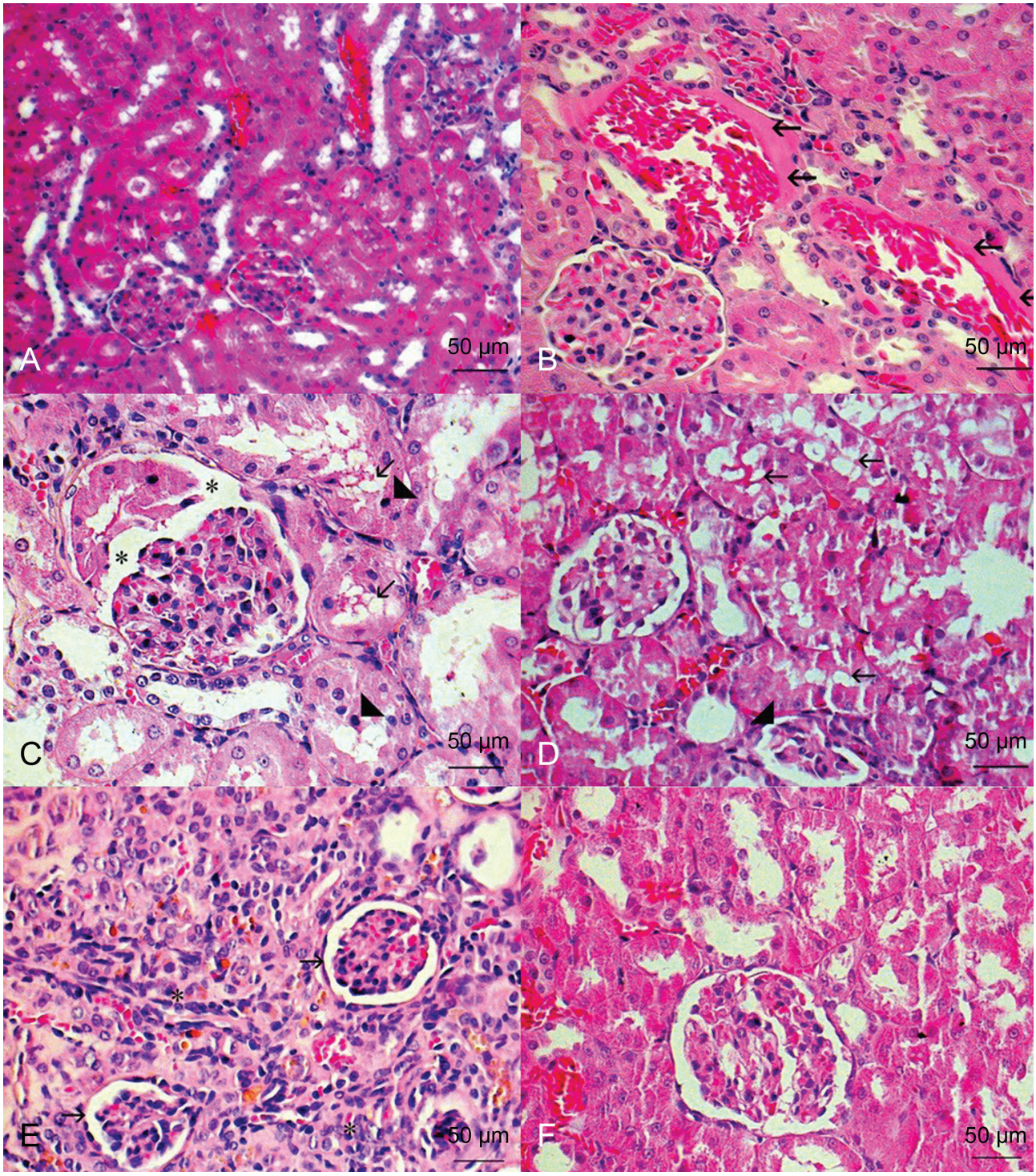

Figure 6. Histopathological assessment (hematoxylin-eosin). A, Normal renal tissues of group 1. B, Homogenous hyaline thickening of the renal blood vessels wall (arrows) in group 2 (cyclosporine). C, Cystic dilatation of the Bowman capsule (asterisks), moderate vacuolation of the distal convoluted renal tubules (arrows), and few pyknotic nuclei of renal epithelium (arrowheads) in group 2 (cyclosporine). D, Moderate vacuolation of renal tubules (arrows) and few pyknotic nuclei of renal epithelium (arrowheads) in group 3 (cyclosporine and olive leaf extract, 40 mg/kg). E, Shrunken glomerular tufts (arrows) with mild interstitial nephritis represented by few fibroblasts and mononuclear cell infiltrations (asterisks) in group 4 (cyclosporine and olive leaf extract, 80 mg/kg). F, Mild dilatation of the Bowman capsule with restoration of the normal histological features of the renal tissue in group 5 (cyclosporine and olive leaf extract, 120 mg/kg).

probably due to its rich content from oleuropein, hydroxytyrosol, and tyrosol. These compounds have strong free-radical scavenging capacity and show a synergistic behavior when combined.[23]

Such results were obtained as well by Tavafi and coworkers, as they reported the OLE's protective effect against gentamicin-induced nephrotoxicity in rats due to its antioxidant activity.[24] The high oleuropein content and the important antioxidant activities of OLE could be useful sources for industrial extraction and pharmacological application in the promotion of health and prevention of damages caused by radicals.[25] In the present study, the small dose of OLE (40 mg/kg/d) was not associated with protection of cyclosporine-induced nephrotoxicity. However, Tavafi and coworkers[24] showed that OLE could attenuate nephrotoxicity induced by gentamicin when used in a dose as small as 25 mg/kg/d, probably due to the differences of the induction of nephrotoxicity by gentamicin.

CONCLUSIONS

On the basis of the obtained results, it can be concluded that with the antioxidant activities of OLE, in an appropriate dose, cyclosporine-induced impairment of kidney function can be markedly ameliorated and antioxidant activities can be maintained. In addition, OLE diminished the severity of the related renal lesions.

CONFLICT OF INTEREST

None declared.

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