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RESEARCH ARTICLE

PROTECTIVE EFFECT OF ELLAGIC ACID AGAINST CYCLOSPORINE A-INDUCED RENAL HISTOPATHOLOGICAL AND ULTRASTRUCTURAL CHANGES, AND OXIDATIVE STRESS IN MALE ALBINO RATS

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ABSTRACT

Cyclosporine A (CsA) is an effective immunosuppressor agent, which is used in preventing graft rejection and in treating autoimmune diseases. The present study aimed to investigate the protective effect of ellagic acid (EA) against CsA-induced renal toxicity in male albino rats. Animals were divided into six groups, each of seven rats: the 1st group was used as a control, the 2nd group received a subcutaneous injection of slightly alkaline solution, the 3rd group received orally olive oil, the 4th group was injected subcutaneously with EA (10 mg/kg body weight, dissolved in slightly alkaline solution), the 5th group was orally treated with CsA (15 mg/kg body weight, dissolved in olive oil), and the 6th group was treated simultaneously with CsA and EA, daily for 30 days. The results indicated that CsA induced a significant increase in renal lipid peroxidation and a significant decrease in renal catalase and peroxidase activities, and reduced glutathione concentration. Histopathological changes of the renal cortex region of CsA-administrated rats revealed vacuolated cytoplasm of the epithelial cells and fibrosis, while the renal medullary region showed tubular calcification or casts, and damaged collecting tubules. Ultrastructural changes included marked degeneration of glomeruli, multiple cytoplasmic vacuoles of the cuboidal epithelial cells, thickened basal lamina, and sparse short microvilli of the proximal tubules. Treatment with EA simultaneously with CsA resulted in a significant alleviation of the renal histopathological and ultrastructural changes, and oxidative stress. Thus, it is recommended to use EA to alleviate CsA-induced renal toxicity due to its antioxidant effects.

INTRODUCTION

Cyclosporine A (CsA), a neutral lipophilic cyclic undecapeptide ($C_6H_{11}N_{11}O_{12}$), was isolated from the fungus *Tolypocladium inflatum* Gams[1]. Although it was firstly identified in 1976 as a novel antibiotic agent, it was subsequently discovered to be a powerful immunosuppressive agent. The potent immunosuppressive effect of CsA is due to its specific inhibiting effect on the lymphokines generation and differentiation, and signal transduction pathways of T-cell

receptor that affects immune responses[1]. In addition, the direct cytotoxic effect of CsA activates the pro-oxidant pathway and increases the synthesis of reactive oxygen species (ROS) and lipid per-oxidation products[2]. Despite its therapeutic importance, it has been reported that CsA causes serious toxicity in humans and experimental animals[3,4]. Moreover, several experiments reported that clinical use of CsA is limited by its side effects of which the most important are nephrotoxicity[3], hepatotoxicity[5], vascular dysfunction[6], testicular toxicity[7], and ovarian damage[8]. The exact mechanism of CsA-induced nephrotoxicity remains obscure, but several studies suggested that a defect in intra-cellular calcium handling[9], magnesium deficiency[10], oxidative stress[11], and nitric oxide system are involved[12]. Acute renal failure due to CsA is widely attributed to the generation of ROS by CsA[11]. A previous study on rats showed that exposure to CsA resulted in a significant increase in serum creatinine and urea[13]. Also, glomerulo-sclerosis, atrophy in the renal tubule cells, vacuolation, stripped interstitial fibrosis, mononuclear inflammatory reaction, and vascular changes were shown in CsA-treated rats[13].

A combination of the CsA delivery together with a potent and safe natural antioxidant may be the appropriate approach to ameliorate CsA-induced nephrotoxicity. Among natural polyphenols, dilactone of hexahydroxydiphenic acid (ellagic acid, EA) is found, as both free and bound forms, in numerous fruits and vegetables, especially in raspberries, strawberries, nuts and in the pomegranate. EA has been previously shown to possess antioxidant and anti-carcinogenic properties both *in vitro* and *in vivo* models[14-17]. The potential chemo-preventive effects of EA have been attributed to various mechanisms including growth-inhibition and apoptosis-promoting activities in several cancer cell lines[15-16]. In addition, Pari and Sivasankari[14] reported that EA contains four hydroxyl groups and two lactone groups in which the hydroxyl groups are known to increase its antioxidant activity, and protect cells from lipid per-oxidation and oxidative damage. Previously, Yüce *et al*.[18] reported that EA has the ability to alleviate CsA-induced renal damage associated with oxidative stress. The study of Yüce *et al*.[18] did not investigate the alterations in kidney ultrastructural and functions. Therefore, the current study was designed to scrutinize the protective effect of EA against CsA-induced renal oxidative stress and histopathological/ultrastructural alterations, as well as kidney dysfunction in male albino rats.

MATERIAL AND METHODS

Chemicals

CsA was purchased from Novartis (Istanbul, Turkey) in a form of Sandimmune capsule (each capsule contains 25 mg CsA). EA and other laboratory chemicals were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Experimental animals

The experimental animals used in this work were random bred adult males of laboratory albino rats (*Rattus norvegicus*), each weighing 130-190 g. The animals were obtained from Ophthalmology Research Institute, Giza, Egypt. The experiments were performed parallel to the ethical standards and according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Institute of Laboratory Animal Resources, 1996). All animals were housed in plastic cages with wired covers and kept under normal laboratory conditions. The animals were not treated with antibiotics, vitamins, or insecticides and were fed on a standard commercial diet (ATMID Company, Giza, Egypt) and tap water *ad libitum*.

Experimental design

Forty two male albino rats were randomly organized as six groups, each of seven animals. All applied treatments in the present study were maintained daily for 30 days.

- Group 1 (G1), normal control group: It did not receive any treatments.
- Group 2 (G2), slightly alkaline solution group: The rats received subcutaneous injection of 0.5 mL slightly alkaline solution/kg body weight.
- Group 3 (G3), olive oil group: The rats received orally 1.0 mL olive oil/kg body weight through gastric intubation.
- Group 4 (G4), EA group: The rats received subcutaneous injection of EA (10 mg/kg body weight). EA is hardly dissolved under natural condition. Therefore, it was dissolved in alkaline solution (0.01 N NaOH; approximately pH = 12), where the pH of the final solution after the addition of EA was approximately 8[18].
- Groups 5 (G5), CsA group: The rats were administered orally CsA (15 mg/kg body weight, dissolved in 1.0 mL olive oil) by a gastric tube[18].
- Group 6 (G6): EA/CsA group: The rats were injected subcutaneously with EA (as in G4) parallel to orally administration of CsA (as in G5).

Blood and tissues sampling

Blood samples were individually collected from rats of the different groups at the time of killing (30 days of the experiment), left to coagulate at room temperature, and then centrifuged to separate serum. The clear non-haemolysed serum was quickly removed and kept at −20ºC till used for estimation of serum urea and creatinine levels. At the end of the experiment, the rats in each group were killed after mild diethyl ether anaesthesia. About 0.5 g of kidney tissues were removed quickly, ice-cooled, and homogenised in 5 mL of 0.9% NaCl (1% weight/volume) using Teflon homogenizer (Glas-Col, Terr Haute, IN, USA). The homogenate was centrifuged at 3000 g for 15 min at 4°C. The supernatant was collected and preserved at −20°C till used for determination of lipid per-oxidation and antioxidant defence system including reduced glutathione (GSH) content, and catalase (CAT) and peroxidase (Px) activities. Other pieces of kidney tissues were fixed for histopathological and ultra-structural studies.

Determination of lipid peroxidation and antioxidant-capacity in kidney tissues

In kidney homogenates, lipid peroxidation was colorimetrically determined based on the determination of malondialdehyde (MDA), an end product of lipid per-oxidation, which can react with thio-barbituric acid to yield a pink coloured thiobarbituric acid reacting substance (TBARS) using a Biodiagnostic kit (Giza, Egypt) and following the manufacture's instruction. The level of GSH was measured based upon the reduction of 5,5`-dithiobis-2-nitrobenzoic acid by thiol group that is present in GSH to form 5-thio-2-nitrobenzoic acid, which can be measured colorimetrically[19]. CAT activity was analysed according to the method of Cohen *et al*.[20] and Sinha[21] by monitoring the enzyme-catalysed decomposition of hydrogen peroxide using potassium permanganate and following the first order kinetics. Px activity was determined according to Kar and Mishra[22], and the activity was expressed as unit absorbance of the amount of purpurogallin formed from oxidation of pyrogallol in the presence of hydrogen peroxide.

Kidney function tests

Urea concentration was estimated in serum as described previously[23]. Briefly, urea in the sample is hydrolysed enzymatically into ammonia and carbon dioxide. The formed ammonia ions react with sodium hypochlorite in presence of the catalyst nitroprusside to form a green indophenol; the intensity of the colour formed is proportional to the urea concentration in the sample. The assay of serum creatinine is based on the reaction of creatinine with alkaline picrate forming a red complex[23]. The time interval chosen for measurements should avoid interferences from other serum constituents. The intensity of the red colour is proportional to the creatinine concentration in the sample.

Paraffin sections preparation for histological assay

Tissue samples of the kidneys were fixed in 10% neutral buffered formalin (pH 6.8) for 24 hours. After dehydration, tissue samples were embedded in paraffin wax, sectioned at 4 to 5 µm and stained with haematoxylin and eosin[24] for histo-pathological examination.

Ultrastructure sections preparation

Thin sections for transmission electron microscopy were prepared according to the method described by Bozzola and Russell[25]. Briefly, 1-3 mm segments of kidney of control, CsA and treated rats were immediately fixed in 3% glutar-aldehyde (freshly prepared) buffer at pH 7.4 for 4 hours at 4°C, washed in phosphate buffer (pH 7.4) and post-fixed in 1% osmium tetroxide for one hour at 4°C. The samples were then processed and embedded in propylene oxide-resin mixture at the ratio of 3:1 for one hour, then in a propylene oxide-resin mixture (1:1) for one hour, followed by propylene oxide-resin mixture (1:3) overnight. The samples were embedded in fresh pure resin at room temperature overnight. The specimens were then transferred to capsules containing fresh resin and placed in the oven at 60°C for 24 hours. Semi-thin sections (1.0 mm) of kidney tissue were cut with glass knives using an ultramicrotome, stained with toluidine blue, and examined using light microscope. Ultrathin sections (70–90 nm) were prepared and picked up on grids, and then stained with uranyl acetate and lead citrate. Stained grids were then examined on a transmission electron microscope (model CX 100, JEOL, Japan) operated at 60 kV.

Statistical analysis

The biochemical results were statistically analysed by one way analysis of variance ANOVA[26] and post hoc LSD test using the SPSS/PC program (Version 20.0; Chicago, IL). $P < 0.05$ was considered significant.

RESULTS

Effect of ellagic acid on defects in renal antioxidant defence system and kidney functions of cyclosporine A-treated rats

A significant increase in the lipid peroxidation and a significant decrease in the GSH content and the activities of CAT and Px were noticed in the kidneys of CsA-treated group when compared with the control, slightly alkaline solution, olive oil, and EA groups (Table 1). EA-treated group showed almost similar results in MDA and GSH levels along with Px and CAT activities in comparison with the control, olive oil, and slightly alkaline solution groups. In addition, simultaneous administration of EA along with CsA revealed a significant reduction ($P < 0.05$) in MDA level versus CsA-treated group. Moreover, administration of EA to CsA-treated rats induced a significant amelioration in the level of GSH and the activities of CAT and Px (Table 1).

The CsA-intoxicated rats exhibited a significant increase in serum urea and creatinine levels when compared with the control, slightly alkaline solution, olive oil, and EA groups (Table 2). However, administration of EA in concomitant with CsA produced observable amelioration in kidney functions when compared with the CsA-treated group (Table 2). On the other hand, there was no significant difference in serum urea and creatinine concentrations among the control, slightly alkaline solution, olive oil, and EA groups (Table 2).

Effect of ellagic acid on histopathological changes in kidneys of cyclosporine A-treated rats

The microscopic examination of the kidney sections of the untreated control showed normal histological structure of the renal corpuscles and renal tubules. The renal corpuscle includes the glomerulus that is a small tuft of fenestrated capillaries covered with thin diaphragm and Bowman′s capsule. The renal tubules consist of proximal convoluted tubules, distal convoluted tubules, and collecting tubules. The proximal

Table 1: Protective effect of ellagic acid against cyclosporine A-induced changes in renal lipid peroxidation (malondialdehyde level), catalase and peroxidase activities, and reduced glutathione concentration.

	MDA (nmol/g tissue)	GSH (nmol/g tissue)	CAT activity (K × 10^2)	Px activity (U/g tissue)
G1 (negative control)	$4.36 \pm 0.08^{\mathbf{b}}$	$2.84 \pm 0.01^{\mathbf{b}}$	$66.60 \pm 0.60^{\mathbf{b}}$	$46.40 \pm 0.17^{\mathbf{b}}$
G2 (slightly alkaline solution)	$4.35 \pm 0.10^{\mathbf{b}}$	2.83 ± 0.02^{b}	66.16 ± 0.73^{b}	45.78 ± 0.83^{b}
G3 (olive oil)	4.37 ± 0.10^{b}	2.76 ± 0.03^{b}	66.16 ± 0.80^{b}	47.64 ± 0.13^{b}
G4 (EA)	4.89 ± 0.14^{b}	2.79 ± 0.05^{b}	63.22 ± 0.97^{b}	48.48 ± 0.15^{b}
G5 (CsA)	8.98 ± 0.17^{a}	1.91 ± 0.12^{a}	44.98 ± 0.63^{a}	30.63 ± 0.13^{a}
G6 (CsA + EA)	5.87 ± 0.31^{c}	2.30 ± 0.10^{c}	61.27 ± 0.91^{c}	43.95 ± 1.7^{c}
One way ANOVA (*P* value)	$F_{5,36} = 110.668$ ($P < 0.001$)	$F_{5,36} = 25.019$ ($P < 0.001$)	$F_{5,36} = 110.518$ ($P < 0.001$)	$F_{5,36} = 71.678$ ($P < 0.001$)

Data are expressed as mean ± standard error. EA: ellagic acid, CsA: cyclosporine A, MDA: malondialdehyde, GSH: reduced glutathione, CAT: catalase, Px: peroxidase, K: the first-order reaction rate constant. Values with different letters in the same column were significantly different ($P < 0.05$).

Table 2: Protective effect of ellagic acid against cyclosporine A-induced changes in serum urea and creatinine levels.

	Urea (mg/dL)	Creatinine (mg/dL)
G1 (negative control)	$29.33 \pm 0.77^{\mathbf{b}}$	$0.51 \pm 0.05^{\mathbf{b}}$
G2 (slightly alkaline solution)	$27.18 \pm 0.25^{\mathbf{b}}$	$0.56 \pm 0.08^{\mathbf{b}}$
G3 (olive oil)	$28.53 \pm 0.20^{\mathbf{b}}$	$0.50 \pm 0.07^{\mathbf{b}}$
G4 (EA)	$29.75 \pm 0.70^{\mathbf{b}}$	$0.82 \pm 0.04^{\mathbf{b}}$
G5 (CsA)	48.90 ± 0.98^{a}	1.20 ± 0.04^{a}
G6 (CsA + EA)	35.80 ± 0.63^{c}	$0.74 \pm 0.06^{\mathbf{b}}$
One way ANOVA (*P* value)	$F_{5,36} = 155.970$ ($P < 0.001$)	$F_{5,36} = 19.187$ ($P < 0.001$)

Data are expressed as mean ± standard error. EA: ellagic acid, CsA: cyclosporine A. Values with different letters in the same column were significantly different ($P < 0.05$).

convoluted tubules are lined with simple cuboidal epithelium, having prominent brush borders and acidophilic cytoplasm. The distal convoluted tubules are identified on account of simple cuboidal epithelium, clearly defined and wider lumina than those of the proximal convoluted tubules, and closely packed nuclei (Figures 1a and 2a). In control EA-treated rats, the microscopic examination of the kidney sections showed

normal histological structure of the renal corpuscles and renal tubules at 10 mg/kg body weight (Figures 1b and 2b). Cortex region of CsA-administrated rats showed atrophy of the renal corpuscles, widening of the Bowman′s spaces, dissolution in some areas, and periglomerular infiltration of inflammatory cells. Some cells in the renal tubules showed vacuoles, others exhibited pyknotic nuclei, and also haemorrhage was observed (Figures 1c and d). In addition, the cortex region of CsA-administrated rats had fibrotic tissues (indicated hydropic degeneration) and inflammatory cells infiltration (Figure 1e). The tubules of the medullary region showed calcifications (or casts appear in the lumina of the tubules), infiltration of inflammatory cells, and damaged collecting tubules (Figure 2c). The rats treated with EA simultaneously with CsA showed almost normal structure of the glomeruli, Bowman's spaces, proximal tubules, distal tubules, loops of Henle, and collecting tubules (Figures 1f and 2d) similar to those of the control groups.

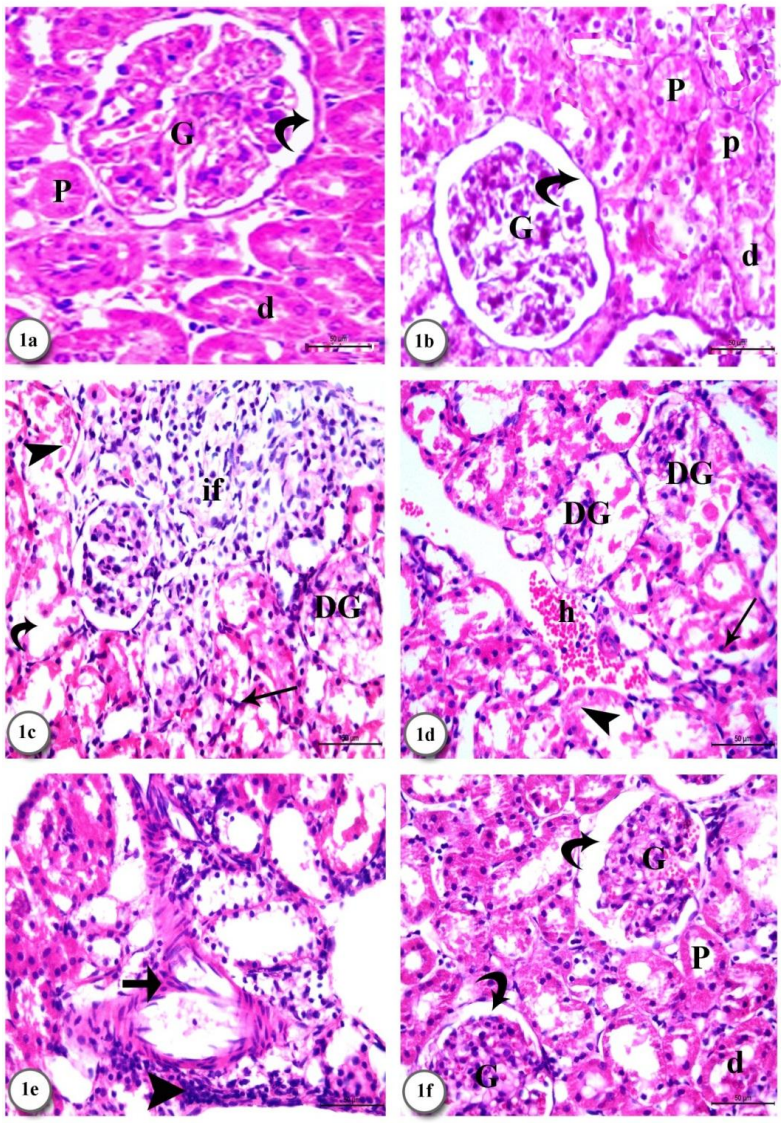

Figure 1: Photomicrographs of the renal cortex (scale bar = 50 µm; haematoxylin and eosin). **(a)** Control kidney showing renal corpuscles, glomerulus (G), Bowman's capsule (curved arrow), proximal tubules (P) and distal tubules (d). **(b)** The kidney of ellagic acid group showing cortex region with glomerulus (G), Bowman's capsule (curved arrow), proximal tubules (P) and distal tubules (d), which are nearly similar to those of the control group. **(c** and **d)** The kidney of cyclosporine A group showing cortex region with dissolution in tubules (curved arrow), atrophied glomerular tuft (DG), severe infiltration of inflammatory cells (if); some cells appear with vacuolated cytoplasm (arrowhead) and others with pyknotic nuclei (arrows) and haemorrhage was appeared in the cortex (h). **(e)** The kidney of cyclosporine A group showing cortex region with inflammatory cells infiltration (arrowhead) and fibrotic tissues (arrow), indicating hydropic de-generation. **(f)** The kidney of cyclosporine A plus ellagic acid group showing cortex region with normal glomeruli (G), Bowman's capsules (curved arrows), proximal tubules (P) and distal tubules (d) similar to those of the control group.

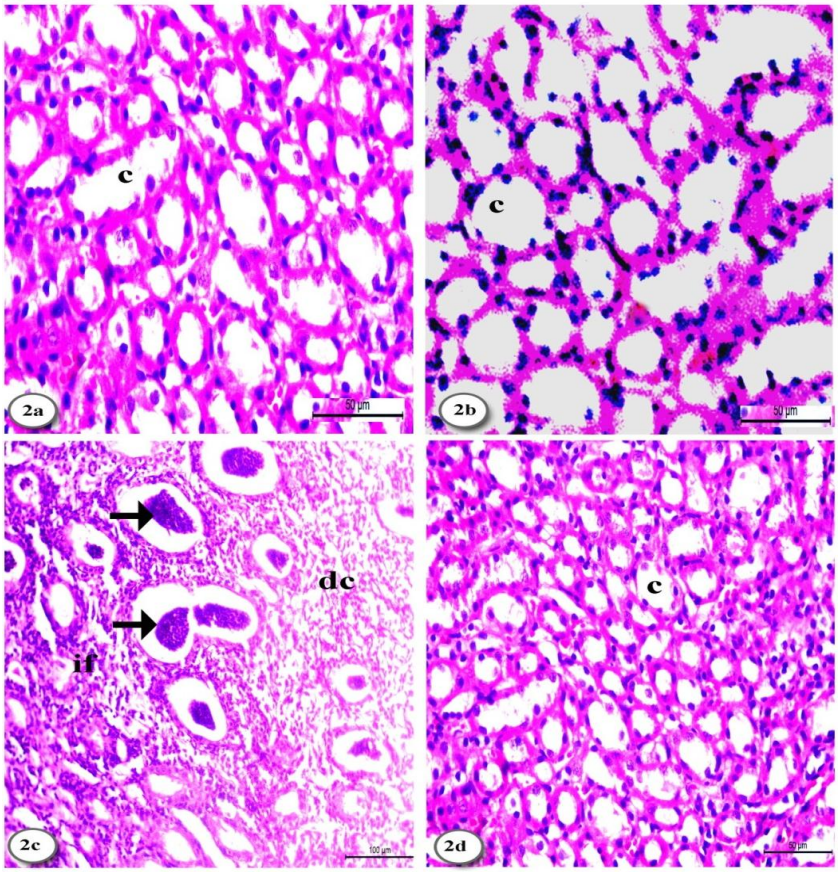

Figure 2: Photomicrographs of the renal medulla (scale bar = 50 µm; haematoxylin and eosin). **(a)** The kidney of control group showing renal collecting tubules (c) and loop of Henle. **(b)** The kidney of ellagic acid group showing medulla region with collecting tubules (c), which are almost similar to those of control group. **(c)** The kidney of cyclosporine A group showing medulla region with calcifications of the tubules or casts appear in the lumina of the tubules (arrows), and infiltration of the inflammatory cells (if), as well as damaged collecting tubules (dc). **(d)** The kidney of cyclosporine A plus ellagic acid group showing medulla region with normal collecting tubules (c) similar to those of the control group.

Effect of ellagic acid on ultrastructural alterations in kidneys of cyclosporine A-treated rats

The renal corpuscles of the control rats appeared as dense spherical tufts of capillaries. The outer layer of Bowman´s capsule is the parietal layer and the inner visceral layer applies closely to the glomerular capillaries. Each capillary loop is lined with endothelial cells. The podocytes give rise to primary processes which in turn give numerous secondary foot processes or pedicels that rest on a thin basal lamina. The mesangium is comprised of two components, mesangial cells and mesangial substance; it provides support for several capillary loops. The mesangial cell is characterized by large heterochromatin nucleus. The mesangial matrix largely encloses the mesangial cells (Figures 3a and b). In the rats given CsA, the renal corpuscles showed marked degeneration of almost all the structures of the glomeruli including the podocytes with electron dense nuclei, effacement of the foot processes, damaged fenestrated endothelium of the capillary and focal thickening of the glomerular basement membranes (Figure 3c). After treatment with EA, the renal corpuscles showed marked amelioration of almost all structures including the podocytes, which appeared with irregular nuclei, primary processes, secondary processes, and uniform thickness of the glomerular basement membrane (GBM). Fenestrated blood capillaries were also noticed (Figures 3d and e).

The cells of the normal proximal tubules have a brush border of numerous microvilli. Their nuclei are spherical in shape. Numerous mitochondria present in the cytoplasm, the basal lamina is thin, and the basal infolding runs upwards among the mitochondria (Figure 4a). The proximal

tubules of rats given CsA showed distorted and irregular basal mitochondria. Multiple cytoplasmic vacuoles, electron-dense bodies (phagosomes), and areas of rarified cytoplasm were noticed. Also, sparse short microvilli were observed. In addition, irregular and thickened basal lamina was seen (Figure 4c). After treatment with EA in concomitant with CsA, marked reduction of basal lamina thickness was noticed. The apical cell membrane, microvilli, and mitochondria between basal infoldings appeared normal. In addition, the nuclei displayed normal appearance (Figure 4d).

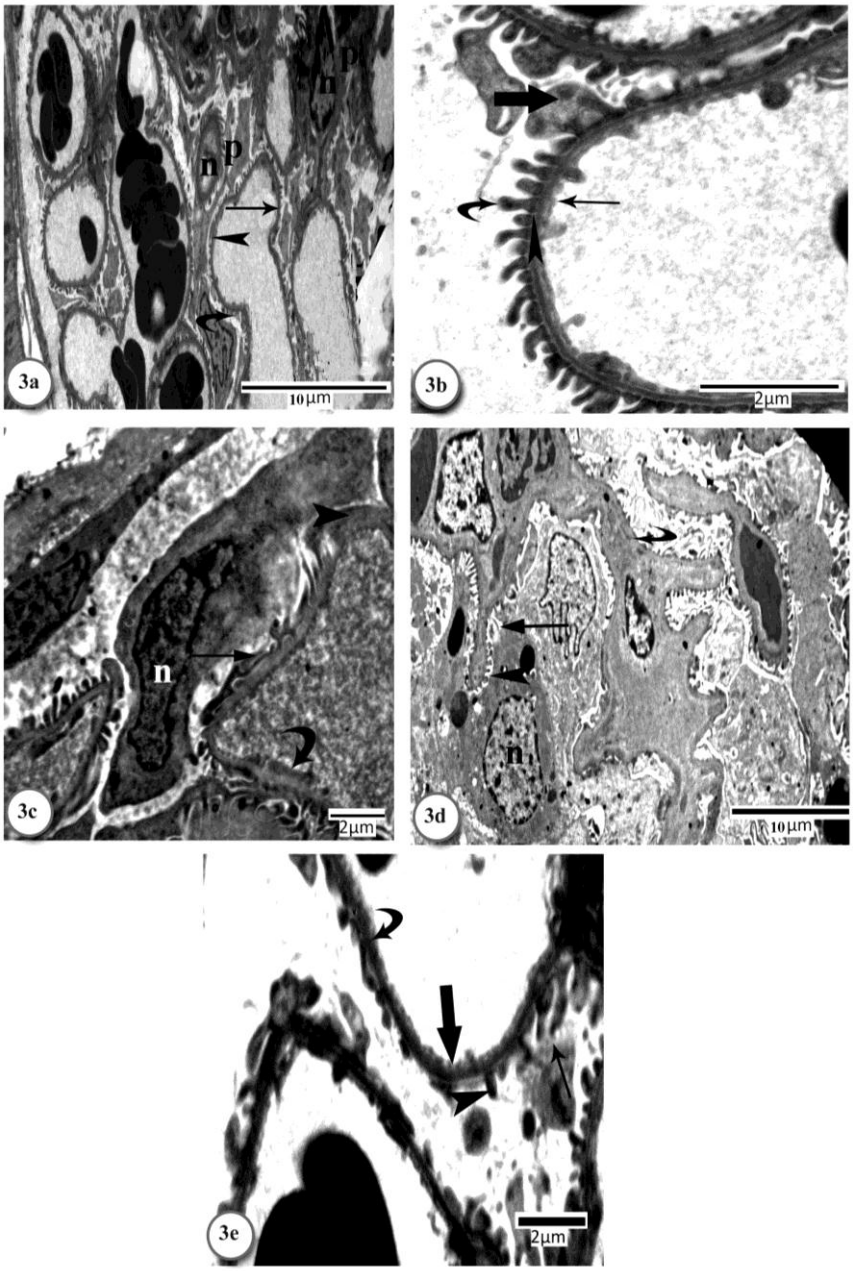

Figure 3: (a and **b)** The control group shows podocytes (P) and processes. The irregular euchromatic nucleus (n) of the podocyte and the fenestrated endothelium of the capillary (arrow) are observed. The primary process (thick arrow) and interdigitating secondary process (curved arrow) are noticed. The glomerular basement membrane (arrowhead) is formed of a central electron dense lamina densa, and lamina rara on either side. **(a)** Scale bar = 10 µm. **(b)** Scale bar = 2 µm. **(c)** The cyclosporine A-treated group showing podocytes with electron-dense nuclei (n) and effacement of the foot processes (arrow), damaged fenestrated endothelium of the capillary (indicated by curved arrow), and focal thickening of the glomerular basement membrane (arrowhead), scale bar = 2 µm. **(d** and **e)** Cyclosporine A plus ellagic acid-treated group showing podocytes with the irregular electron-lucent nuclei (n). The primary processes (indicated by arrow), and secondary processes (arrowhead), as well as the uniform thickness of glomerular basement membrane (curved arrow) are also detected. The fenestrated blood capillary (thick arrow) is also noticed. **(d)** Scale bar = 10 µm. **(e)** Scale bar = 2 µm.

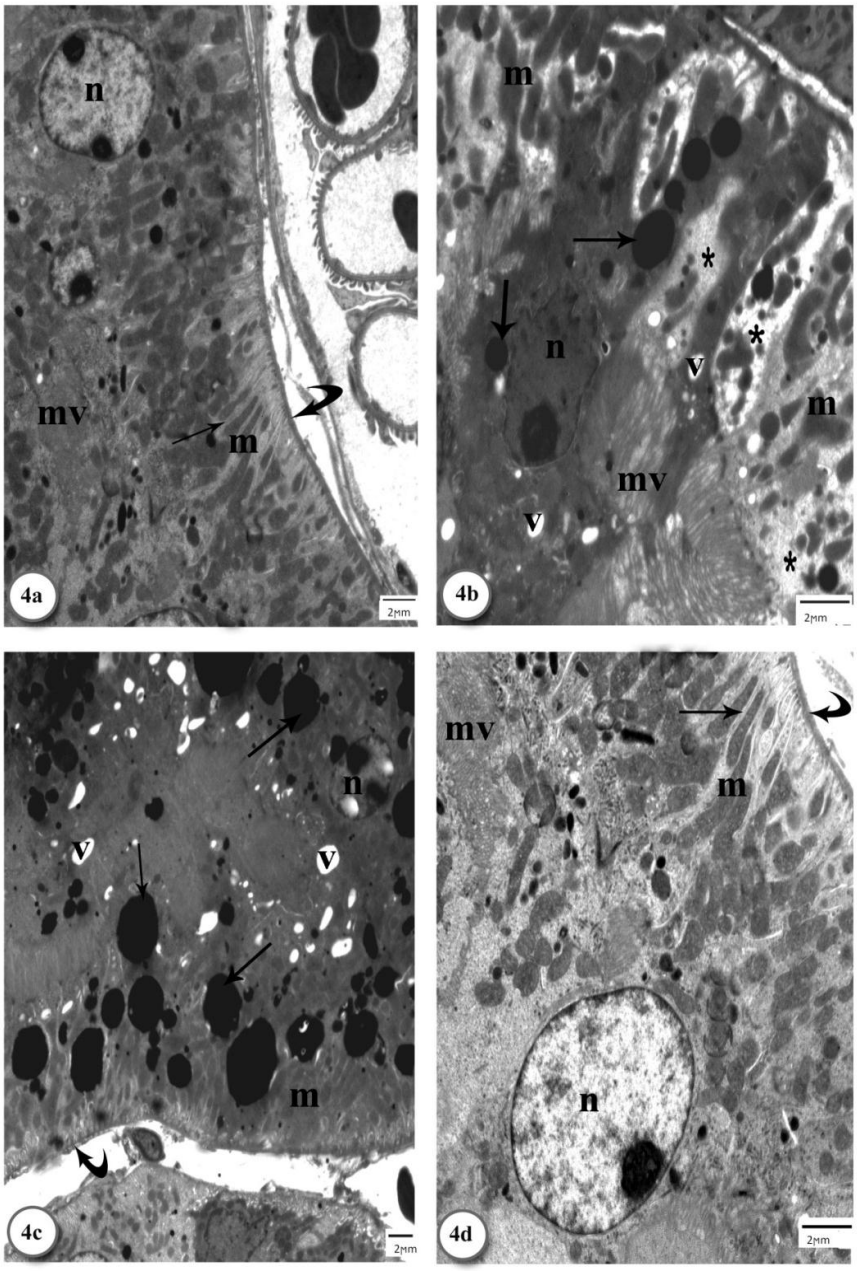

Figure 4: Electron micrographs of a proximal convoluted tubule cells (scale bar = 2 µm). **(a)** The control group shows epithelial cells of proximal convoluted tubule lining with large euchromatic nuclei (n) and microvilli (mv). Many of elongated basal mitochondria (m) are seen in between the extensive basal infoldings (arrow). All cells rest on basal lamina (curved arrow). **(b** and **c)** The cyclosporine A-treated group demonstrating distorted and irregular basal mitochondria (m). The multiple cytoplasmic vacuoles (v), electron-dense bodies (phagosomes) (arrows) and areas of rarified cytoplasm (*****) are noticed. The damaged nuclei (n) and sparse short microvilli (mv) are also detected. Irregular basal lamina (curved arrow) is seen. **(d)** The cyclosporine A plus ellagic acid-treated group showing normal nuclei (n) of epithelial cells, and mitochondria (m) are also seen in between extensive basal infoldings (arrow). Apical long microvilli (mv) are noticed. Regular basal lamina is also noticed (curved arrow).

DISCUSSION

Over the last 20 years, the immunosuppressive drug CsA has revolutionized organ transplantation by preventing graft rejection, and has been successfully used in the treatment of the autoimmune diseases[27]. Unfortunately, the extensive usage of CsA is questionable due to its severe adverse effects[3-8]. Hence, a strategy that diminishes the side effects of CsA, but preserves its therapeutic efficacy, is necessary.

The results of the present investigation revealed that administration of CsA-induced histopathological, ultrastructural, and biochemical alterations including the antioxidant defence system. Previous studies showed that ROS-induced oxidative stress and lipid peroxidation may be involved in

the pathophysiology of CsA renal toxicity[11,18]. The data of the present study showed that the renal MDA levels, the by-products of lipid peroxidation, were significantly higher in the CsA-treated group when compared with the control group. Rezzani[1] indicated that CsA generates ROS, such as superoxide anion and hydroxyl radicals; hence stimulates tissue lipid peroxidation. It has also been reported that CsA administration causes an increase in lipid peroxidation and formation of free radicals in kidney tissues, leading to oxidative damage of cell components (e.g. proteins, lipids and nucleic acids)[28]. Therefore, the increases in lipid peroxidation of the studied tissues, observed in the current study, may be attributed to the excessive production of free radicals caused by CsA. GSH is one of the essential compounds for maintaining cell integrity because of its reducing properties and participating in the cell metabolism. Alterations in the ratio of GSH and the oxidized glutathione have been used as indicators of oxidative stress and/or diseases in humans and laboratory animals[29]. In the cases of oxidative stress, GSH is converted into oxidized form, and its depletion leads to lipid peroxidation. Therefore, the role of GSH as a reasonable marker for the evaluation of oxidative stress is important[29]. So, to prevent lipid per-oxidation, it is very important to maintain the level of GSH. In the present study, CsA treatment caused significant decreases in GSH level, and Px and CAT activities of kidney tissues. When ROS begin to accumulate, the living cells exhibit a defensive mechanism using various antioxidant enzymes. The main detoxifying systems for peroxides are CAT and GSH. CAT is an antioxidant enzyme, which destroys H_2O_2 into a highly reactive hydroxyl radical in the presence of iron as a catalyst. By participating in the glutathione redox cycle, GSH together with Px convert H_2O_2 and lipid peroxides to non-toxic products[29]. In the present work, significant increases in the levels of serum urea and creatinine were observed in CsA-treated group indicating impairment in kidney functions. On the other hand, CsA plus EA-treated group showed a significant improvement in renal antioxidant defence system and functions in comparison with the CsA-treated group. Also, EA was able to improve the renal antioxidant defence system and kidney functions in rats treated with cisplatin (alkylating agent) and triclosan (broad spectrum bactericide)[30-32].

The present study revealed histo-pathological changes in CsA-treated rats, which go hand in hand with the biochemical changes. These changes were detected in both renal corpuscles and kidney tubules. The observed prominent signs of devastation were glomerulosclerosis, tubules' cells atrophy, vacuolation, stripped interstitial fibrosis, mononuclear inflammatory reaction, and vascular changes such as damaged fenestrated endothelium of the capillary. Such histopathological changes may be attributed to the chronic ischemia resulting from CsA-associated arteriolopathy, and this ischaemia might lead to apoptosis which further resulted in fibrosis[13,33]. Also, several mechanisms have been involved in CsA nephrotoxicity; these include increased production of ROS[34]. Nicolli *et al.*[35] reported that CsA produced an excess of ROS *via* alteration of mitochondrial respiratory chain and impairment of antioxidant defence system. In addition, Young *et al.*[33] demonstrated that the renal vasculopathy and glomerulosclerosis induced by CsA could be due to endothelial dysfunction and activation of smooth muscle cell proliferation leading to vascular wall thickening.

Ultrastructural examination of the current material showed marked degeneration of almost all the structures of the glomeruli including podocytes with electron dense nuclei, effacement of the foot processes and damaged fenestrated endothelium of the capillary. Tipping[36] postulated that the podocytes are generally regarded as targets of ROS by stimulating podocyte production of the granulocyte-macrophage colony stimulating factor, the pro-

inflammatory cytokine that induces apoptosis in podocytes and directly injures the GBM. Thickened GBM with distorted foot processes of podocytes was noticed in the current study in CsA-treated rats. Rateb and Abdel-Hafez[37] suggested that this thickening may develop to compensate for the increased glomerular permeability and proteinuria. In addition, kidney tubules were also affected by CsA where some of their cells appeared with vacuolated cytoplasm and others with pyknotic nuclei. Haemorrhage, inflammation and hydrobic degeneration were shown in between. Similar results were described by Thliveris *et al*.[38]. The increase in the size of the tubules resulted in increased proximal reabsorption, which in turn caused glomerular hyperfiltration *via* hyper-absorption[39]. The hyperfiltration caused increased glomerular capillary pressure; the glomerular hypertension caused endothelial mesangial and podocyte injuries with increased filtration of proteins to the lumina of the tubules[40]. Medulla region of the kidney of CsA-treated rats appeared with tubular calcification or casts, and damaged collecting tubules. These results were in agreement with Kim and Suh[41].

In the current study, the proximal tubules of rats given CsA showed distorted and irregular basal mitochondria. Multiple cytoplasmic vacuoles, electron-dense bodies (suggestive of early micro-calcification), and areas of rarified cytoplasm were noticed. Apoptotic nuclei and sparse short microvilli were also observed. In addition, irregular and thickened basal lamina was shown in CsA-treated rats. The inefficiency of renal function and structure is attributed to mitochondrial destruction; mitochondrial dysfunction reduces active transport of the renal tubules[42]. The appearance of acute renal failure and the morphological evidence of proximal tubule pathology following administration of CsA to patients suggested that the drug might be proximal tubules-toxin. Toxicity of the tubules may be seen when CsA levels were elevated above 1500-2000 ng/mL in whole blood, where the proximal tubules may show giant mitochondria and isometric vacuolization (i.e. the cytoplasm of the epithelial cell contains many clear vacuoles)[43]. CsA treated patients gave decreased lithium clearance, suggesting increased proximal tubules solute reabsorption[3]. In the current study, EA showed a protective effect against oxidative injury, as well as histopathological and ultrastructural changes induced by CsA in kidneys, possibly due to its antioxidant activity. Similarly, Yüce *et al*.[44] reported that giving EA to cisplatin-treated animals decreased the MDA levels. Also, Al-Kharusi *et al*.[31] proved that EA ameliorates oxidative stress, as well as decreases the tubules' necrosis and apoptotic cells in the renal cortex of rats treated with cisplatin. In addition, Ateşşahín *et al*.[30] reported that EA protected the kidney tubules from degeneration, desquamation and dilatation in cisplatin-treated rats.

In conclusion, CsA leads to alterations in the renal biochemical and oxidative stress. In addition, it caused tissue damage, and ultrastructural changes of kidney tissues. However, treatment with EA showed marked amelioration in the biochemical, histological and ultrastructural configurations; this confirms the efficiency of its antioxidant properties to counteract the toxic effect of CsA. So, EA can be used as a protective agent against CsA nephrotoxicity due to its antioxidant effect.

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التأثير الوقائي لحمض الإلجيك من التغيرات الهستوباثولوجية والتركيبية الدقيقة والإجهاد التأكسدي التى يُحدثها السيكلوسبورين فى كُلى ذكور الجِرذان المُهْق

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تهدف هذه الدراسة إلى التحقق من الدور الوقائي لحمض الإلجيك فى مواجهة التأثيرات السُمية الكُلوية لعقار السيكلوسبورين الذي يستخدم على نطاق واسع لمنع رفض الأعضاء المزروعة وعلاج أمراض المناعة الذاتية. وقد استخدم في هذه الدراسة إثنان وأربعين من ذكور الجِرذان المُهْق والتي قسمت بالتساوي على ست مجاميع على النحو التالى: المجموعة الأولى بمثابة المجموعه الضابطة، المجموعة الثانيه حقنت تحت الجلد بمحلول قاعدي قليلا، المجموعة الثالثه تم تجريعها عن طريق الفم بزيت الزيتون، المجموعة الرابعة حقنت تحت الجلد بحمض الإلجيك (10 مليجرام/كيلو جرام من وزن الجسم مذاب في محلول قاعدي قليلا)، المجموعة الخامسة تم تجريعها عن طريق الفم بعقار سيكلوسبورين (15 مليجرام/كيلو جرام من وزن الجسم مذاب في زيت الزيتون)، المجموعة السادسة تم تجريعها عن طريق الفم بعقار سيكلوسبورين بالتزامن مع الحقن تحت الجلد بحمض الإلجيك. وكانت جميع الجرعات في المجموعات المختلفة مرة واحدة يوميا ولمدة ثلاثين يوما. وقد أظهرت النتائج التأثيرات السُمية الكُلوية لعقار السيكلوسبورين من خلال بعض التغيرات البيوكيمائية والنسيجية. وقد أدى ذلك إلى خلل فى وظائف الكُلى، وأكسده الدهون والنظام المضاد للأكسدة في النسيج الكُلوي. وقد بينت الدراسة الهستوباثولوجية لقشرة النسيج الكُلوى بعد المعاملة بالسيكلوسبورين ظهور فجوات سيتوبلازمية في بعض الخلايا، بالإضافة إلى ألياف عضلية والتى تبرهن على وجود تلف شديد. بينما شوهد ظهور تكلس فى أنيبيبات منطقة النخاع، وارتشاح فى الخلايا الالتهابية، وتلف فى الأنيبيبات الجامعة. وأظهرت أيضا الدراسات الميكروسكوبية الدقيقة تحلل في الكثير من الكبات، وتلف في خلايا الإندوثيليم للأوعية الدموية، وقصر فى زُغَيْبات الأنيبيبات القريبة. بينما أدت المعاملة بحمض الإلجيك إلي خفض الضرر الكُلوي الناجم عن عقار السيكلوسبورين في الجِرذان وذلك يرجع لتأثيراته المضادة للأكسدة.