Does Olive Oil Attenuate Ciprofloxacin-Induced Renal Cortical Toxicity? Light and Electron Microscopic Study

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Original Article

ABSTRACT

Introduction: Ciprofloxacin (CPFX) is a broad spectrum antibiotic used for treatment of many infections. Olive oil is rich in compounds that have strong antioxidant activity.

Aim: To assess CPFX-induced renal cortical structural changes in albino rats and to evaluate whether or not olive oil could attenuate such changes.

Materials and Methods: Thirty two male albino rats were used and divided into 4 groups. Control group received distilled water, OO group received olive oil (5ml/kg/day), CPFX group received CPFX (20 mg/kg/day) and CPFX+OO group received both CPFX and olive oil as the previous groups. All medications were continued for 14 days. Renal cortex specimens were processed for light and electron microscopy. Morphometric study and statistical analysis for the results were also performed.

Results: CPFX group revealed irregularity and shrinkage of renal corpuscles with loss of vascular component and capsular space obliteration. The mesangial cells were significantly increased. The renal tubules showed dilatation with significant increase in proximal convoluted tubules diameter, marked degeneration, significant increase in the darkly stained nuclei of their lining cells and intraluminal casts. Congested glomerular capillaries and peritubular blood vessels were also observed. Ultrastructurally, there were thickening of glomerular basement membrane, fusion of glomerular capillary endothelium and podocyte pedicles. Cytoplasmic empty areas and vacuolations, swollen mitochondria, nuclear changes were also found in the tubules. CPFX + OO group showed less structural changes.

Conclusion: Olive oil has ameliorating effect on ciprofloxacin-induced renal cortical toxicity.

Keywords: Ciprofloxacin, olive oil, rat, renal cortex

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INTRODUCTION

Ciprofloxacin (CPFX) belongs to the fluoroquinolones. The fluoroquinolones are a set of antibacterial agents that have been used since 1980 as a routine treatment for skin, gastrointestinal, respiratory, urinary, bone and joint infections. They are also used for treatment of sexually transmitted diseases [1, 2].

CPFX is characterized by a high and rapid bactericidal activity[2]. It acts through the suppression of the bacterial topoisomerase/DNA gyrase enzyme which in turn leads to inhibition of DNA synthesis in the bacterial cells[3].

Unfavorable effects have been reported in patients with the use of fluoroquinolones including gastrointestinal (nausea, vomiting, abdominal pain and diarrhea), CNS (insomnia, headache, depression and tremors) and skin effects. These effects are reversible after withdrawal of the drug and are not dose dependent[4]. Studies in experimental animals showed various side effects after CPFX such as chondrotoxicity [5, 6] and damage in testicular structure and function[7, 8].

Renal side effects as crystal nephropathy were reported in some clinical cases after high CPFX dose. The incidence of the renal side effects increased with chronic kidney disease[9].

Olive oil (OO) is obtained from the first pressing of olives that belong to Oleaceae family [10]. It has been used in several nutritional and medical concerns and it has many health benefits [11]. Olive oil is rich in monounsaturated fatty acids[12]. It is composed of triglyceride esters of oleic acid, palmitic acid, and other fatty acids in a mixture. It contains also some antioxidants as oleocanthal, oleuropein, vitamin E, and carotenoids.

Olive oil has been reported to exert antiplatelet[13], antithrombotic, anti-inflammatory and antihypertensive effects. Also, it causes vasodilation on both animals and humans[14]. It could be used as a prophylactic mean for

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gastric ulcer or cancer [15]. In addition, it aids in cancer prevention via reduction of oxidative damage to DNA and RNA [16] and helps the nerves to act properly and increases serotonin [17]. Besides, the oleuropein content of olive oil can block low density lipoprotein oxidation [18].

Unfortunately, experimental studies concerning the effect of CPFX on the kidney especially from the structural point of view are relatively rare. Also, the available studies revealed a controversy about the effect of CPFX on the kidney. Moreover, literatures concerning the concomitant use of olive oil, as a possible protective agent with CPFX are also rare.

AIM OF THE WORK

So, this work was done to assess ciprofloxacin-induced structural changes in the renal cortex of albino rats and to evaluate whether olive oil could attenuate such changes or not.

MATERIALS AND METHODS

Drugs:

Ciprofloxacin packed tablets (250 mg ciprofloxacin HCL, each) with a trade name "Ciprobay" (European Egyptian Pharm. Ind. Company Alexandria-Egypt) were used. The Olive oil (DARIA) was purchased from the local market in the form of glass bottles (750 ml) that contain virgin olive oil.

Animals and experimental protocol:

Thirty two adult male albino rats (180 – 200 g) were selected for this 14-days experiment. The experimental protocol was approved by Institutional Research Board (IRB); the ethics committee of Mansoura Faculty of Medicine.

The animals were housed in clean properly ventilated cages at 25 ± 2 ˚C with 1212/ h light/dark photoperiod with free access to standard laboratory diet and water. All experimental procedures were done at the Department of Histology and Cell Biology, Mansoura Faculty of Medicine and Tanta University.

Animal groups:

The animals were randomly divided into 4 groups (8 animals each):

1- Control group: The rats received distilled water.

2- OO group: The rats received olive oil (5ml/kg body weight) daily by orogastric tube [19].

3- CPFX group: The rats received ciprofloxacin (20 mg/ kg body weight) dissolved in distilled water daily by orogastric tube (20)

4- CPFX + OO group: The rats received both ciprofloxacin and olive oil as the previously mentioned doses in CPFX and OO groups.

All rats were sacrificed the day after the last dose by decapitation. Thereafter, intra-cardiac perfusion was performed with 2.5% glutaraldehyde with 0.1 M phosphate buffer at pH 7.4 for partial fixation of specimens. The two kidneys were dissected out and processed for histological study.

Histological study:

1- Light microscopy:

Small specimens from the cortex of the right kidney were fixed in 10% neutral buffered formalin overnight, dehydrated, cleared, and embedded in paraffin wax. Serial sections (5 um thick) were cut using a rotatory microtome and stained with Hematoxylin and Eosin (H&E) stain[21].

2- Electron microscopy:

For transmission electron microscopy, small fragments from the cortex of the left kidney were fixed in 2.5% buffered glutaraldehyde dehydrated and embedded in resin. Semithin sections (1µm) were stained with toluidine blue and used for selection of fields examined by transmission electron microscope (TEM). Ultrathin sections (6080- nm) were stained with uranyl acetate and lead citrate [22] for examination by JEOL-JEM-100 SX TEM in the electron microscopy unit at the Faculty of Medicine, Tanta University, Egypt.

Morphometric study:

The slides were photographed by Olympus digital camera (E420, China) installed on Olympus microscope with photo adaptor (0.5x), using objective lens X 40 (TX 31, Philippines). The images were then analyzed by Intel Core 13 based computer using VideoTesT Morphology software (VideoTesT, Russia, Saint-Petersburg) with a specific built-in routine for calibrated distance and object counting.

The number of mesangial cells, the diameter of proximal convoluted tubules and the number of condensed darkly stained nuclei in the renal tubules were quantified from 10 randomly selected images obtained from H&E stained renal cortical sections per each animal of all groups using objective lens X 40.

Statistical analysis:

Data obtained from the morphometric study was analyzed using SPSS 17.0 (SPSS Inc., Chicago). A Kolmogorov -Smirnov test was used to verify the normality of data. For statistical comparison between different groups; the significance of difference was done by using the following tests:

-**Anova test (analysis of variance):** used to compare between more than two groups of numerical (parametric) data followed by post-hoc tukey.

-Kruskal Wallis test: used to compare between more than two groups of numerical (non-parametric) data followed by mann-whitney test for multiple comparisons.

Data were represented in the form of mean ±SD or median and range (minimum–maximum). P values <0.05 were considered statistically significant.

RESULTS

Light microscopic results:

Examination of H&E stained renal cortical sections of the control and OO groups revealed that the cortex contained renal corpuscles, proximal and distal convoluted tubules (PCTs & DCTs) and collecting tubules. The corpuscles were formed of glomerulus surrounded by Bowman's capsule. Two layers were lining the Bowman's capsule; an outer parietal layer formed of simple squamous epithelium and an inner visceral layer formed of podocytes. A capsular space was found between the two layers. The mesangial cells with dark nuclei were located in the mesangium between the glomerular capillaries. The convoluted tubules were lined with cubical cells that had rounded vesicular nuclei. The DCTs had less acidophilic cytoplasm and wider lumen than PCTs (Fig. 1).

The CPFX group demonstrated marked structural changes in the corpuscles and tubules. Some corpuscles showed obliteration of the capsular space or irregular outline of the glomerulus with widening of some areas of the capsular space. Severe damage was observed in other corpuscles in the form of irregularity and shrinkage with loss of their vascular component. The mesangial cells were apparently more frequently encountered between the glomerular capillaries. The PCTs revealed degeneration with marked cytoplasmic vacuolations of the lining cells. The lumen of some PCTs contained remnants of degenerated epithelial cells. Their nuclei showed dark staining, different degrees of degeneration (karyorrhexis, karyolysis or loss). The PCTs also showed obliteration of the lumen. Moreover, dilatation of the renal tubules and presence of intraluminal casts were found. Congestion of glomerular capillaries, dilatation of peritubular blood vessels and congestion were also observed (Fig. 2).

The CPFX + OO group revealed less noticeable histological changes. The renal corpuscles and the tubules appeared more or less intact with a relatively preserved structure. However; dilatation of some PCTs and DCTs and some darkly stained nuclei were found in the tubular cells (Fig. 3).

Electron microscopic results:

Electron microscopic examination of the renal cortex of the control and OO groups showed similar ultrastructure. The corpuscle contained glomerular capillaries that were lined with fenestrated endothelial cells and covered by the cell bodies of podocytes. The podocytes had primary processes that gave rise to many secondary processes or pedicles that end on the capillary basement membrane. Filtration slits were found between the pedicles of podocytes (Fig. 4). The PCTs & DCTs were lined with cubical cells that had rounded euchromatic nuclei. Numerous longitudinally oriented mitochondria were found at the basal part of the cells between the basal cell membrane infoldings. Some lysosomes were also found in the cytoplasm of the tubules. Numerous long microvilli were seen projecting from the luminal surface of PCTs while few or no microvilli were seen in DCTs (Fig. 5).

The CPFX group showed prominent ultrastructural changes. There were thickening in the glomerular capillary basement membrane, thickening and fusion of the capillary endothelial cells and fusion of some podocytes's pedicles (Fig. 6). The renal tubules revealed also changes. The PCTs showed areas of partial loss of microvilli, Cytoplasmic empty areas, vacuoles, swollen mitochondria and loss of normal mitochondrial arrangement between the basal membrane infoldings. Some nuclei appeared shrunken, heterochromatic and others appeared with few chromatin (Fig.7 A, B, C). The DCTs revealed marked disruption or loss of the cell membrane of tubular cells, empty cytoplasmic areas, few degenerated mitochondria with loss of their normal arrangement between the basal infoldings and irregular disrupted nuclear membrane (Fig 7 D).

On the other hand, the CPFX + OO group demonstrated a well maintained ultrastructure. The glomerular basement membrane, the lining endothelium of capillaries and the podocytes's pedicle appeared intact to some extent (Fig. 8). The PCTs displayed intact microvilli, mitochondria and nuclei (Fig. 9 A and B). The DCTs also had a conserved ultrastructure (Fig. 9 C and D).

Histomorphometric and statistical results:

Anova test showed a significant difference in the number of mesangial cells and the diameter of proximal convoluted tubules among the groups. By post-hoc tukey test; the two parameters showed significant increase in CPFX group compared to control group and a significant decrease in CPFX+OO group compared to CPFX group (Tables 1 and 2).

Kruskal-Wallis test revealed a significant difference in the number of darkly stained nuclei among all groups. By mann-whitney test a significant increase in the number of darkly stained nuclei in CPFX group compared to control group and a significant decrease in CPFX+OO group compared to CPFX group was observed (Table 3).

Table 1: The number of mesangial cells

		Groups				
		Control group	OO group	CPFX group	CPFX+OO group	P
Number of mesangial cells	Mean	16.40	17.00	32.40	20.00	
	±SD	1.26	1.63	3.47	2.26	
	P1		0.9	<0.0001	0.007	<0.0001
	P2			<0.0001	0.03	
	P3				<0.0001	

SD: standard deviation P: probability

Test used: ANOVA followed by post-hoc tukey

P1: significance relative to control group

P2: significance relative to OO group

P3: significance relative to CPFX group

Table 2: The diameter of proximal convoluted tubules

		Groups				
		Control group	OO group	CPFX group	CPFX+OO group	P
Diameter of PCTs	Mean	32.32	32.46	46.45	39.31	
	±SD	2.19	2.33	5.62	2.69	
	P1		1.00	<0.0001	<0.0001	<0.0001
	P2			<0.0001	0.004	
	P3				<0.0001	

SD: standard deviation P: probability

Test used: ANOVA followed by post-hoc tukey

P1: significance relative to control group

P2: significance relative to OO group

P3: significance relative to CPFX group

Table 3: The number of condensed darkly stained nuclei in the renal tubules

		Groups				
		Control group	OO group	CPFX group	CPFX+OO group	P
Number of condensed darkly stained nuclei	Median	1.00	1.00	16.50	4.00	
	Range	.00-2.00	.00-2.00	12.00-24.00	3.00-7.00	<0.0001
	P1		0.79	<0.0001	<0.0001	
	P2			<0.0001	<0.0001	
	P3				<0.0001	

P: Probability

Test used: kruskal wallis followed by mann-whitney for multiple comparison.

P1: significance relative to control group

P2: significance relative to OO group

P3: significance relative to CPFX group

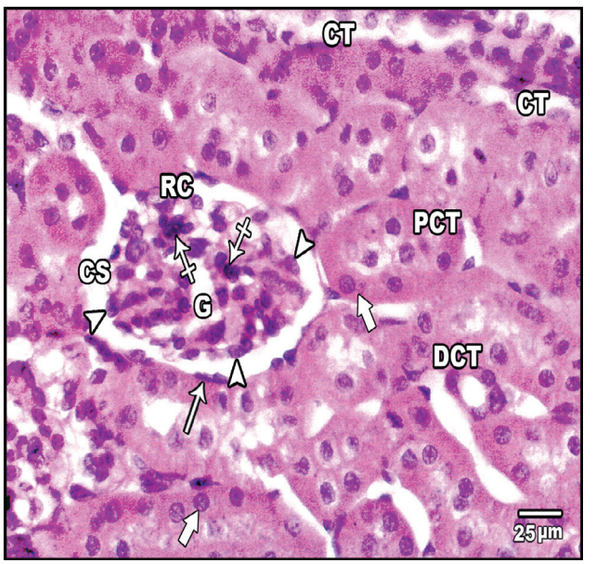

Fig. 1: Representative photomicrograph of the control group renal cortex showing the renal corpuscle (RC), proximal (PCT) and distal (DCT) convoluted tubules and collecting tubules (CT). The renal corpuscle consists of glomerulus (G) surrounded by Bowman's capsule which has two layers; outer parietal simple squamous epithelial layer (arrow) and inner visceral layer formed of podocytes (arrow heads). A capsular space (CS) is present between the two layers. Mesangial cells (crossed arrows) with dark nuclei are present in the mesangium between the glomerular capillaries. The lining cells of the convoluted tubules have rounded vesicular nuclei (thick arrows). The DCTs have wider lumen and less acidophilic cytoplasm than PCTs (H&E X400).

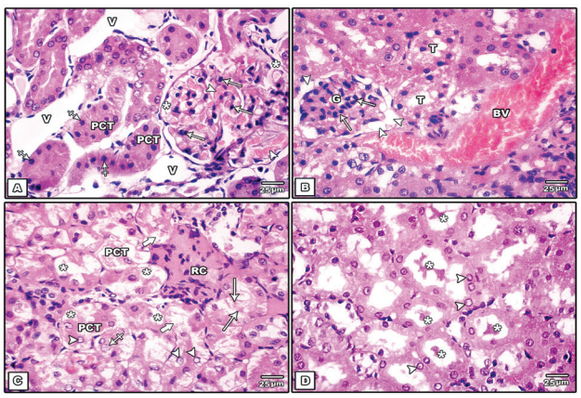

Fig. 2: Representative renal cortex photomicrographs of CPFX group. A: showing obliteration of most of the capsular space (asterisks) with congestion of glomerular capillaries (arrow heads). The mesangial cells are apparently more encountered (arrows). Dilatation of peritubular blood vessels (V), obliteration of some PCTs lumen (PCT) and darkly stained nuclei (crossed arrows) of their lining cells are also observed. B: showing irregular outline of the glomerulus (G) with widening of some areas of the capsular space (arrow heads) and apparent increase in the mesangial cells (arrows). Congested peritubular blood vessels (BV) and degenerated tubules (T) are also noticed. C: revealed irregular shrunken renal corpuscle (RC) with loss of its capsular space and vascular component. Degenerated PCTs (PCT) that are lined with cells having markedly vacuolated cytoplasm (asterisks) are also seen. The lumen of some PCTs contained remnants of degenerated epithelial cells (arrows). The nuclei of the tubular cells showing various degrees of degeneration; karyorrhexis (arrow heads), Karyolysis (crossed arrows) or even loss (thick arrows) D: showing dilated renal tubules with intraluminal casts (asterisks). Many nuclei with karyorrhexis (arrow heads) are also observed (H&E X400).

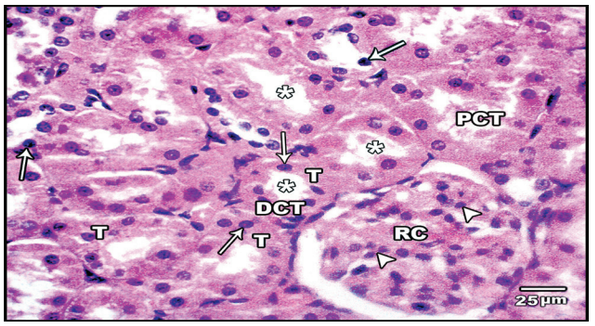

Fig. 3: Representative photomicrograph of the renal cortex of CPFX + OO group. The renal corpuscle (RC) and the tubules (T) are more or less intact with preservation of their histological structure. However, there is dilatation of the PCTs (PCT) and DCTs (DCT). In addition condensed darkly stained nuclei in the lining cells of the tubules (arrows) are also observed. Note mesangial cels are found between the glomerular capillaries (arrow heads) (H&E X 400).

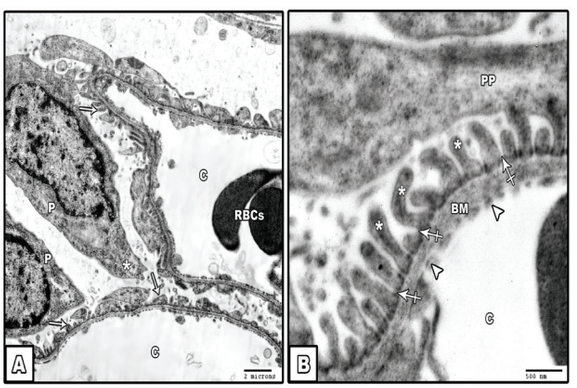

Fig. 4: Electron micrographs of a part of the glomerulus of control group. A: The cell bodies of the podocytes (P) cover the glomerular capillaries (C). The podocytes have primary processes (asterisk) arise from the cell body and many pedicles cover the capillary surfaces (arrows). Note the red blood cells (RBCs) in the capillary lumen. B: the glomerular capillary (C) is lined with fenestrated endothelium (arrow heads). The primary process (PP) of the podocyte gives rise to many pedicles (asterisks) separated by filtration slits (crossed arrows) and end on the capillary basement membrane(BM) (TEM; A: X1500 & B: X 8000).

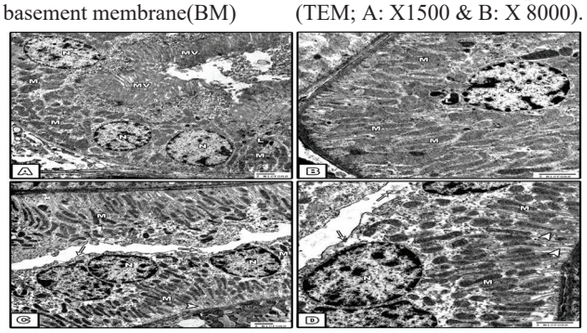

Fig.5: Electron micrographs of the control group renal tubules. A&B: the PCT is lined with cubical cells with rounded euchromatic nuclei (N). Numerous long microvilli (MV) are projecting from the luminal surface. Numerous long mitochondria (M) are found in the basal part of cells. Some lysosomes are also seen (L). C&D (D: a higher magnification of C): the DCTs are lined with cubical cells with euchromatic nuclei (N). Few or no microvilli (arrows) are projecting into the lumen. Numerous longitudinally oriented mitochondria (M) are found between basal membrane infoldings (arrow heads) (TEM; A, C X 1000, B, DX 2000).

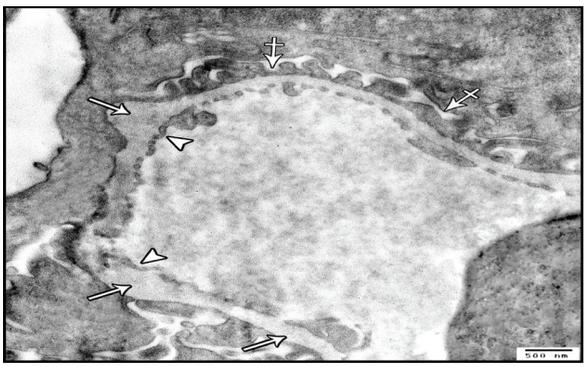

Fig. 6: Electron micrograph of a part of the glomerulus of CPFX group showing thickening of the glomerular basement membrane (arrows), thickening and fusion of the capillary endothelium (arrow heads) and fusion of podocytes pedicles (crossed arrows) (TEM X 5000).

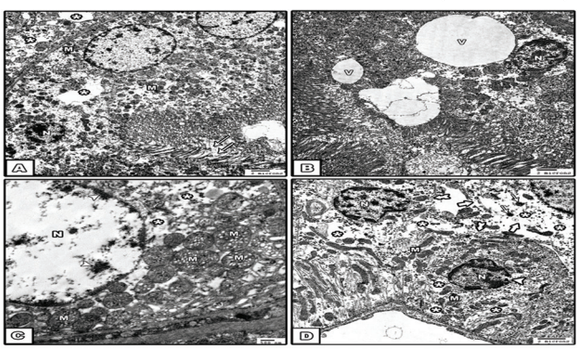

Fig. 7: Electron micrographs of the renal tubules of CPFX group. Obvious changes are observed in the PCTs (A,B&C). A&B: showing areas of partial loss of microvilli (arrows). Empty cytoplasmic areas (asterisks), vacuoles (V), shrunken heterochromatic nucleus (N) and Loss of mitochondrial arrangement (M) between the basal infoldings are noticed in tubular cells. C: showing empty areas of cytoplasm (asterisks) and swollen mitochondria (M). The nucleus (N) has few chromatin (arrow heads). D: illustrated changes in DCTs. Disruptions of the tubular cell membrane (tailed arrows), empty cytoplasmic areas (asterisks) and few mitochondria (crossed arrows) with loss of their arrangement between the basal infoldings are observed. The nucleus (N) has irregular nuclear membrane with area of disruption (arrow head). A completely degenerated cell is seen with complete loss of the cell membrane (thick arrows) and a large empty cytoplasmic area (asterisks) contained few degenerated darkly stained mitochondria (arrows). (TEM; A,B X1000, C X 3000, D, 1500).

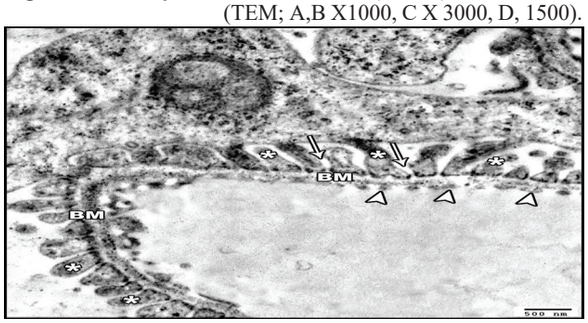

Fig. 8: Electron micrograph of a part of the glomerulus of CPFX + OO group. The glomerular basement membrane (BM) and the endothelium (arrow heads) appear more or less intact. The pedicles of podocytes (asterisks) are nearly intact and are separated by filtration slits (arrows) (TEM, X5000).

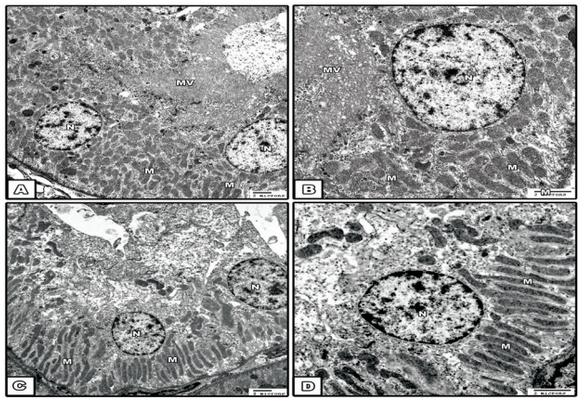

Fig. 9: Electron micrographs of the renal tubules of CPFX + OO group. A&B (B: higher magnification of A): showing more or less intact structure of PCTs with intact microvilli (MV), nuclei (N) and mitochondria (M). C&D (D: higher magnification of C): showing preserved structure of DCTs with normal arrangement of mitochondria (M) between the basal infoldings and intact nuclei (N) (TEM A, C X 1000, B, D X 2000).

DISCUSSION

Despite the undesirable effects of CPFX, it is widely used as a treatment for various infections [23]. CPFX-induced renal toxicity is a matter of argument. Some authors reported that the renal toxicity caused only by CPFX is uncommon and various factors appear to predispose to this toxicity including old age, concurrent intake of other nephrotoxic drugs (such as aminoglycosides, vancomycin and cisplatin) and the hydration status of the patients [24, 25].

Acute renal failure was reported in patients receiving CPFX without a previous history of renal problem. The duration of ciprofloxacin therapy ranged from several days to several weeks [26]. CPFX-induced allergic interstitial nephritis was also reported [27]. Moreover, some reports declared the development of crystal nephropathy either with the use of CPFX alone [5] or with NSAIDs [28]. The latter added that the simultaneous administration of CPFX with NSAIDs may cause acute renal failure. Loh and Cohen [29] stated that chemical agents and medications that cause formation of crystalline deposits can induce kidney damage by causing tubular obstruction and injury in addition to interstitial inflammation.

Experimental studies on the effect of CPFX on the kidney are relatively rare especially on its histological structure. So, this study aimed to reveal the renal cortical histological changes caused by CPFX. Also, to study the effect of olive oil, as a possible ameliorating agent for such changes was evaluated.

The present study revealed that CPFX induced structural changes in the renal cortex of CPFX group. These changes involved both the renal corpuscles and the tubules. The corpuscles showed irregularity and shrinkage with loss of the vascular component and obliteration of the capsular space. Congestion of glomerular capillaries was also observed. These findings were in agreement with Elbe *et al.* [23] who found similar structural changes in renal corpuscles after intraperitoneal administration of 20 mg/kg CPFX twice daily in albino rats for 10 days.

Our results revealed that the number of mesangial cells was significantly increased in CPFX group compared to the control. Mesangial cell proliferation is a prominent feature of some glomerular diseases such as membranoproliferative glomerulonephritis, IgA nephropathy, lupus nephritis or diabetic nephropathy [30]. Mesangial proliferative glomerulonephritis is considered the most common type of glomerulonephritis and is characterized by abnormal proliferation of mesangial cells, increase release of inflammatory mediators and mesangial matrix which often cause glomerulosclerosis that may be irreversible and lead to end stage renal disease [31].

Various structural changes were detected in the renal tubules particularly the PCTs. The tubules showed dilatation, obliteration of the lumen and severe degeneration with marked cytoplasmic vacuolations of their lining cells and various degrees of nuclear degeneration. Also congestion of peritubular blood vessels was noticed. In accordance with our results Elbe *et al.* [23] observed renal tubular dilatation, atrophy and necrosis and interstitial hemorrhagic areas following injection of CPFX for 10 days in rats. In addition, Al-Shawi [32] demonstrated swelling and necrosis of renal tubular cells in juvenile rats treated intraperitoneally with a dose of 25 mg/kg CPFX, and tubular cell necrosis in animals that were receiving 50 mg/kg of CPFX i.p for one week. In addition, aggravation of gentamicin induced nephrotoxicity by ciprofloxacin was also reported [33].

The statistical results of the present work showed a significant increase in the diameter of PCTs in CPFX group compared to the control which might be explained by the tubular dilatation that was observed by light microscopy. The renal cortical tubular dilatation is often observed in different renal conditions. For instance, it was detected by Amer and Hussien [34] after the use of gibberellic acids (GA3), and by Mahmoud *et al.* [35] after using ketoprofen.

Condensed darkly stained nuclei were detected in the renal tubules mainly PCTs of CPFX group by light microscopic examination. This was confirmed statistically by a significant increase in the number of condensed darkly stained nuclei in the tubular cells compared to control, suggesting a possible apoptotic effect caused by CPFX. The mechanism by which CPFX induces apoptosis remains a point of research. Some researchers studied the possible apoptotic effect of CPFX on variable tumor cells. Aranha and colleagues [36, 37] found that CPFX causes apoptosis in bladder cancer cells and suggested some probable mechanisms of apoptosis

induction. Those mechanisms included Bax upregulation and its redistribution to the mitochondrial membrane, mitochondrial swelling in addition to disruption of calcium homeostasis. Besides, Herold *et al*. [38] mentioned that colon carcinoma cell apoptosis that induced by CPFX was associated with an increased activity of caspases 3, 8 and 9 and Bax upregulation. Moreover, they recommended that CPFX as adjuvant agent for colorectal cancer should be assessed. Zobeiri *et al.* [39] showed that CPFX may cause toxicity of sperm cells by apoptosis induction through the mitochondrial pathway.

Intraluminal casts were seen in some renal tubules in this study. These luminal casts were composed of cellular fragments, sloughed epithelial cells and proteinaceous substance [29]. Fuller [40] added that the luminal casts can obstruct the tubules and later an inflammatory cascade is activated that leads to more kidney damage.

On the contrary, Baykal *et al*. [41] studied the effect of CPFX (400mg/kg) for 4 and 7 days on rat kidneys and found that CPFX did not induce nephrotoxicity. Also, Bourgeois *et al*. [42] investigated the influence of subcutaneous administration of different doses of CPFX (10, 30 and 100mg/kg once per day) in newly born mice and observed that the kidney was normal in its histological structure.

Ultrastructural studies of CPFX-induced renal changes are rare. In this study, electron microscopy revealed a variety of changes in the renal cortex of CPFX group. Glomerular changes were in the form of thickened glomerular capillary basement membrane, thickening and fusion of the capillary endothelium in addition to fusion of podocyte pedicles. The renal tubular epithelial cells revealed disruption and loss of cell membrane, cytoplasmic vacuolations, empty cytoplasmic areas, swollen mitochondria, partial loss of apical microvilli in PCTs together with nuclear changes. Some nuclei appeared shrunken and heterochromatic while others had few chromatin.

Marked cytoplasmic vacuolations and mitochondrial affection with CPFX were also detected in the submandibular gland by electron microscopy [43]. The authors suggested that cytoplasmic vacuoles may represent degenerated mitochondria. It was previously reported that the mitochondria were very vulnerable to harmful agents and when they were damaged, cellular metabolism failed with further damaging effects to the cells [44].

A variety of experimental studies revealed that CPFX caused structural degenerative changes in different organs including submandibular gland [43], articular cartilage [20] and liver of juvenile rats [32]. Ibrahim *et al.* found that the CPFX-induced changes were reversible after the drug withdrawal [43], however, Halawa found them irreversible [20]. Also, the use of CPFX caused abnormalities in the sperm structure, function and count [2, 45].

The kidney is a favored target for many environmental toxins and therapeutic agents such as antibiotics, which interact with it [33]. A possible cause of CPFX toxicity was stated by Elbe *et al.* [23] who attributed the renal histopathological changes of CPFX to the oxidative stress confirmed by the biochemical findings. They found an increase in malondialdehyde level, a decrease in superoxide dismutase, catalase activities and glutathione level in kidney tissue of rats receiving CPFX.

This study revealed that intake of olive oil attenuated the CPFX-induced renal cortical structural changes that was observed by light and electron microscopy. Moreover it improved the changes in the measured histomorphometric parameters. These effects could be attributed in part to its antioxidant effect. Olive oil is a source of many phenolic compounds as oleuropein, hydroxyl tyrosol and tyrosol which are free radical scavengers and strong antioxidants [46, 47]. Phenolic compounds have strong antioxidant activity against DNA, lipids and low density lipoprotein oxidation [48]. This activity is greater than that of other known antioxidants such as vitamins C and E [49]. Moreover, the phenolic compounds exert strong anti-inflammatory actions. The newly discovered phenolic compound oleocanthal was reported to have similar anti-inflammatory properties as ibuprofen [50].

Olive oil is rich in omega 3 and omega 6 fatty acids [51]. Olive oil also produces a balance between omega-3 fats and omega-6 fatty acids [52]. It has been reported that omega-3 fatty acid, docosahexaenoic acid, has an important role during stress [53]. High levels of omega-3 fatty acid protect from stress by decreasing the pro-inflammatory cytokines [54].

Our research revealed a significant decrease in the number of condensed darkly stained nuclei in CPFX+OO group compared to CPFX group. This might be due to an antiapoptotic effect of olive oil. In context, Haider *et al.* [54] stated that olive oil possesses an antiapoptotic effect. On the other hand, some authors mentioned that olive oil phenolic compounds as oleuropein and hydroxytyrosl induce apoptosis in many cancer cells as breast cancer cells [56].

CONCLUSION

Since, CPFX can induce renal cortical structural changes. Subsequently, we recommend monitoring of the renal functions in patients receiving it. Besides, olive oil can be used as adjuvant in CPFX therapy as it has a proven ameliorating effect against CPFX-induced renal cortical toxicity.

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربى

هل زيت الزيتون يخفف تسمم القشرة الكلوية الناتج عن السيبروفلوكساسين؟
دراسة بالميكرسكوب الضوئي و الالكتروني

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المقدمة:سيبروفلوكساسين من المضادات الحيوية واسعة المدي التي تستخدم في علاج كثير من العدوي .زيت الزيتون غني بالمركبات التي لها نشاط قوي مضاد للاكسدة.

الهدف من البحث:تقييم التغيرات التركيبية في القشرة الكلوية الناجمة عن السيبروفلوكساسين في الفئران البيضاء و ايضا تقييم ما اذا كان زيت الزيتون يمكنه ان يخفف هذه التغيرات ام لا .

مواد وطرق البحث: استخدام اثنان و ثلاثون من ذكور الفئران البيضاء و قسمت إلى اربع مجموعات. المجموعة الضابطة تلقت مياه مقطرة ، مجموعة زيت الزيتون تلقت زيت الزيتون (٥ مل/كجم يوميا) ، مجموعة سيبروفلوكساسين تلقت سيبروفلوكساسين (٢٠ مل/كجم يوميا) و مجموعة سيبروفلوكساسين+زيت الزيتون و التي تلقت كل من سيبروفلوكساسين و زيت الزيتون كما في المجموعات السابقة. كل العلاج استمر لمدة ١٤ يوما. و قد عولجت عينات من القشرة الكلوية للميكروسكوب الضوئي و الإلكتروني. و أيضا تم عمل دراسة تركيبية قياسية و تحليل احصائي للنتائج.

النتائج: أوضحت مجموعة سيبروفلوكساسين تعرج وانكماش في الكريات الكلوية مع فقد في المكون الدموي منها و طمس في فراغ المحفظة. و زادت خلايا مسراق زيادة هامة .و أوضحت الانابيب الكلوية اتساعا مع زيادة هامة في قطر الانابيب الملتوية القريبة واعتلال ملحوظ وزيادة في عدد الانوية الداكنة الصبغة لخلاياها المبطنة و قوالب داخل التجويف. و لوحظ ايضا احتقان في الشعيرات الدموية الكبيبية و الاوعية الدموية حول الانابيب. و علي المستوي التركيبي الدقيق كان هناك زيادة في سمك الغشاء الكبيبي القاعدي ، و التحام في الخلايا المبطنة للشعيرات الدموية الكبيبية وعنيقات الخلايا الرجلاء. و وجد ايضا في الانابيب مناطق فارغة وتجاويف في السيتوبلازم و تورم في الميتوكندريا و تغيرات في الانوية. و قد اوضحت مجموعة سيبروفلوكساسين+زيت الزيتون تغيرات تركيبية اقل..

الخلاصة: زيت الزيتون له تأثير محسن على تسمم القشرة الكلوية الناتج عن السيبروفلوكساسين.