

## Ultrastructural Study on the Effects of Green Tea Extract on Enrofloxacin - Induced Kidney Injury in Rats

Amal A.A. El-Daly

Department of Zoology, Faculty of Science, Benha University, Benha, Egypt

**Abstract:** The antimicrobial agents, enrofloxacin (EFX), established growing attention because its potential efficacy for the treatment of diseases in human as well as veterinary medicine. The present study was to recognize the protective effect of different doses of Green tea extract (1%, 1.5% and 3% of GTE) on enrofloxacin- induced kidneys ultrastructural abnormalities. Treating with 75 mg/kg body weight enrofloxacin daily dose for 10 days caused progresses nephrotoxic enhancement of glomeruli and acute tubular necrosis on both proximal and distal convoluted tubular cells. The results of EM studies displayed thickening of glomerular basement membrane GBM as electron-dense protein deposits along the sub-epithelial facet and mesangium revealed dense depositions. Also, EFX led to loss of foot processes (pediceles) of the podocytes. Moreover, proximal tubules epithelial cells evoked necrosis signs with loss of the microvilli, failure of basal membrane foldings, large vacuoles and an irregular basal distribution of the mitochondria in the cytoplasm. The distal convoluted tubule cells showed vesiculated mitochondria, abnormal nuclei and large dense bodies with loss of organelles. Co- administration of GTE improved alterations induced by EFX at altitude of the different doses. However, improvement was obvious after the consumption of higher doses attributed to the antioxidant properties of GTE constituents.

**Abbreviations:** Enrofloxacin (EFX), green tea extract (GTE)

**Key words:** Enrofloxacin • Kidneys • Green tea • Ultrastructure • Rat

### INTRODUCTION

Enrofloxacin (EFX) and its main metabolite ciprofloxacin (CFX) are fluoroquinolones commonly used in human as well as veterinary medicine. These compounds have high bioavailability in body fluids and organs by means of good absorption and tolerance [1]. Some studies showed an advanced antimicrobial potency of EFX [2, 3] in addition to its activity at low concentrations, compared with other classes of antimicrobial agents [4, 5]. EFX and CFX received growing attention because their potential efficacy for the treatment of numerous mammalian species as well as fish and birds [6-12]. Similarly, Colistin is effective primarily against gram-negative organisms give its action via binding with the anionic lipopolysaccharide molecules by displacing calcium and magnesium from the outer cell, leading to permeability changes in the cell envelope, leakage of cell contents and finally cell death [13].

Enrofloxacin possesses the bactericidal effects against gram-negative and gram-positive bacteria [14] through inhibition of DNA- gyrase [1] and it has approximately 4-folds greater microbiological activity than its parent drug [15]. The pharmacokinetic studies indicated that the drug has a large volume of distribution and low protein binding according to later author [15]. On the other hand, EFX showed hazardous effects in mammalian animals including bradycardia weakness and peripheral vasoconstriction [16]. Other authors, Christ [17] and Takayama [18] reported that all quinolones include toxic effects on the central nervous, cardiovascular, gastrointestinal systems, condrotoxicity, carcinogenicity and phototoxicity.

According to Gao [19] the potential toxicity of the drug result from the inhibition of CAT activity that is essential in the process of detoxification and plays a role in the cellular antioxidant mechanism. Furthermore, the nitrogen in ENR is responsible for the lipid solubility and

enhances the ability to penetrate tissues [20]. In a previous work Lykkeberg [21] identified that new ENR metabolite enrofloxacin N-oxide (ENR-N) produced in liver microsomes of pig.

GTE is an example of folkloric vegetation which is medically useful to health for the reason that richest source of normal antioxidant resources. Many studies projected out on GTE and its constituents revealed that it increases the activity of liver antioxidant enzymes glutathione peroxidase and improves total antioxidant status [22]. Feather studies showed that GTE contains polyphenols that has a protective effect on liver, serum and central nervous tissues [23]. Moreover, the compounds exhibit a protective effect against a variety of unpleasant oxidants, such as superoxide and peroxynitrite radicals [24].

Of the most popular use of GT is the assistance as chemopreventive and anticarcinogenic [25, 26]. Furthermore, Green tea is able to reduced hyperglycemia, prevented renal injury and autonomic dysfunction, reduced cardiovascular risk and target organ damage in diabetes [27]. As well, GT is amongst recent physicians medicinally useful in many diseases.

Consequently, kidneys are the main organ for elimination of the toxic substances and many drugs. The present work was undertaken to investigate the effect of EFX on rat kidneys and the possible protective effect of aqueous GTE.

## MATERIAL AND METHODS

**Material:** Male albino rats (*Rattus norvegicus*) were obtained from the animal colony of Dokki National Research Center (Dokki, Egypt). Enrofloxacin (10 %) was obtained from Sigma Chemical Co. (Egypt). Green tea (China green tea) was commercially purchased is packaged by the Egypt National Native Product sources.

**Preparation of Green Tea:** Green tea extract was freshly prepared everyday by brewing (25-30 gm) dried tealeaves in 500 ml of boiling water then cooled to room temperature. Tealeaves solution was filtered and reextracted with 500 ml of boiling water then filtered. The two filtrates were combined to obtain 3% green tea extract (3g tealeaves /100 ml water). 1.5 % and 1% GTE were prepared as previously described by Khan [28]. The resulting clear solution is similar to tea consumed by human. Each animal in the present study was orally given 1 ml of the final aqueous extract [29].

**Animals:** Adult male (*Rattus norvegicus*) weighing 100 -125 g were maintained under standard laboratory conditions at temperature ( $23 \pm 20^{\circ}\text{C}$ ) with a relative humidity and a photoperiod (12-h dark/light) were used for the experiment and housed in cages with the guide for the care and use of laboratory conditions [30]. The animals were acclimatized for one week before the initiation of treatment provided food and water *ad libitum*.

**Experimental Protocol:** Rats were divided into five groups (A-E), each group contains six rats. Group A kept as a control, group B was injected intraperitoneally with daily dose of 75 mg/kg body weight of enrofloxacin for 10 days. The animals of the residual groups (C-E) were administered daily with the above mentioned dose of EFX in one of the three concentrations, 1%, 1.5% and 3% respectively of fresh prepared GTE for 10 days. The experimental protocols were approved by the Institutional Animal Ethics Committee.

At the end of the experiment, the animals were anesthetized using ether, sacrificed and specimens from kidneys were collected and prepared for transmission Electron Microscope (TEM) analyses.

**Ultrastuctural Analyses:** Small pieces of kidneys from controls and treated animals were washed in 0.1 M cold phosphate buffer after fixation in 2% glutaraldehyde in 0.1M Na- cacodylate buffer, pH 7.2, (Karnovsky fixative) and post-fixed for 2 h in 1% osmium tetroxide in the same buffer at  $4^{\circ}\text{C}$ . After several washes in 0.1 M phosphate buffer, the specimens were dehydrated using graded acetone solutions, cleared and infiltrated in epoxy Resin.

Semithin sections were cut using Ultra microtome with glass knives and stained with toluidine blue in 1% borax. Specimens were then trimmed to select the area containing glomeruli. Ultrathin sectioning (80-100 nm) of the tissue blocks were carried out using an ultrathin microtome. The sections were stained in 2% alcohol uranyl acetate followed by Reynold's lead citrate stain and examined with a Siemens ELMISKOP I or Zeiss M-109 Turbo electron microscope.

## RESULTS

**Control Animals:** Semithin sections of the control rat kidneys showed normal cortical components including the glomeruli (G) with parietal layer of Bowman's capsule and observable capsular space (Fig. 1). The proximal (PCT) and distal tubules (DCT) with cubical epithelial lining cells as well as the interstitial were visible regular.

EM section of the control animal showed the wall of the glomerular capillaries (GC) is formed of inner fenestrated endothelial cells (EN), the middle continuous basement membrane and an outer layer of the pedicles of podocytes (P). Moreover, the podocytes give rise to numerous primary and secondary foot processes (Fo) interdigitated a delimit filtration slits. Besides, glomeruli have common mesangial cells (MC) with their normal matrix (MM) in between the capillaries (Fig. 2). Also, the proximal tubules cells exhibited numerous elongated mitochondria (M) at the base and parallel to the long axis. The brush border microvilli (MV) were generally present (Fig. 3). The distal convoluted tubules were usually appearance with small micovilli (Fig. 4).

**Animals Treated with Daily Dose of 75 ml/kg Enrofloxacin:** Semithin sections in rat kidneys treated with daily dose of 75 ml/kg EFX revealed evolution of glomerular damages, failure of capsular space and vacuolation were evident (Fig. 5). The glomerular capillaries were filled with erythrocytes by means of congested blood capillaries and the urinary spaces contained some hyaline material represent the trapped plasma proteins. The same shape denoted evolution of nephrotoxic necrosis of proximal and distal convoluted tubule cells and presence of hyaline casts in their lumina. On the other hand, various cells nuclei protruded (extruded) through the lumina.

EM section of the same treatment revealed various disorders at the altitude of the entire glomerular structures (Fig. 6). The changes represented by obliterated glomerular tuft and capillary lumen (CL) large erythrocytes as well as distinct lipid droplets create the capillary slim space owing to immune dysfunction. In addition, the lining endothelial cell nucleus (EN) revealed very dark chromatin satisfied nucleus. The glomerular basement membrane (GBM) showed electron-dense protein deposits along the subepithelial side therefore ill-distinct trilamellar structure become visible. Moreover, mesangial cells denoted karyolysed nucleus and densely matrix. Loss of foot processes (pediceles) of the podocytes was evident and the secondary ones were not well- precise. Moreover, abundant minute dense bodies in the urinary space gave it lessened.

Furthermore, the proximal convoluted tubule cells revealed necrosis of the nuclei with loss of the microvilli. The tubular basement membrane displayed failure of basal foldings. The cytoplasm contains numerous electron dense material, large vacuoles and sparse organelles. Mitochondria showed irregular distribution through the cytoplasm (Fig. 7). The distal convoluted tubule exhibited

abnormal necrotic indented nucleus and the cytoplasm have large dense bodies with loss of the organelles (Fig. 8). Damaged mitochondria were evident. Tubular basement membrane was ill definite.

**Animals Treated with Daily Dose of 75 ml/kg Enrofloxacin and 1% Green Tea Extract:** Examination of semithin sections in rat kidneys administered with EFX and 1%GTE exhibited a slight civilization of pathological characters compared to that of EFX alone. However, signs of damaged structures were still observable (Fig. 9). PCT and DCT with exhibited necrotic cells with few intact ones and their lumina contain hyaline casts. Glomerular leucocytic infiltrations were less detectable.

Ultrathin sections examination of the same treatment showed that trilaminar glomerular basement membrane was less density than the previous treatment (Fig. 10). The endothelial cells revealed vesicular nuclei. The glomerular capillaries occupied by large vacuoles of lipid droplets (V), many erythrocytes(er) and neutrophils. The monocytes appear engulfed highly dense material. The indented podocytes were highly proliferated with divided nucleus. Lucent materials occupied in the urinary space and fewer dense mesangial matrixes.

The proximal convoluted tubules cells still demonstrated nephrotoxic necrosis when nuclei with little chromatin materials accompanied by rupture of the membranes (tubulorrehesis) and occlusion of the tubular lumen by casts (Fig. 11). Mitochondria appear oval shape, slight basal striation, many of them discarded out cell membrane and others scattered in the cytoplasm. The cytoplasm denoted large electron dense material its content ruptured through the tubular lumen. Some necrotic cells away from home into the lumen leading to destruction of the brush border microvilli.

The distal convoluted tubule (Fig. 12) showed dark cells with vesicular nuclei and others were light stained. However, it showed healthier appearance than that of proximal ones. The DCT was still missing their typical microvilli. Peritubular capillaries showed prominent endothelial cells as well as interstitial nephritis (IS).

**Animals Treated with Daily Dose of 75 ml/kg Enrofloxacin and 1.5% Green Tea Extract:** Semithin sections of rat kidneys treated with EFX -1.5% GTE revealed relatively reduced renal cortex injury as compared to the EFX alone. Glomeruli denoted slim recovery existing. The proximal convoluted tubules showed intact cell membrane, euchromatic nuclei normal intact mitochondria and

## PLATE 1

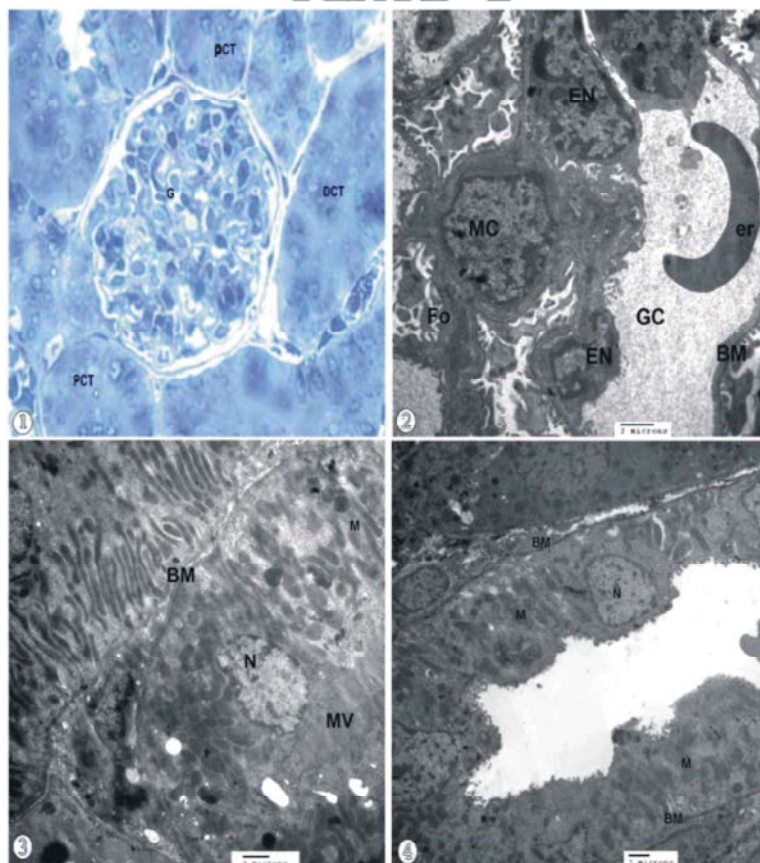


Plate 1: Represents photomicrograph in control group rat kidney of bothtoluidineblue stain and transmission electron microscope sections.

- Fig. 1: Displaying normal glomeruli (G), distal convoluted tubule (DCT), proximal convoluted tubule (DCT), Toluidine Blue stain, X400.
- Fig. 2: Showing ordinary glomerular capillary (GC) with its normal basement membrane (BM), endothelial cell (EN), mesangial cell (MC) and its matrix (Mm), foot process (Fo). (Bar = 2 $\mu$ )
- Fig. 3: Demonstrating common basal striation of proximal convoluted tubule with its normal basement membrane (BM), nucleus (N), elongated mitochondria (M), and normal microvilli (MV). (Bar = 2 $\mu$ )
- Fig. 4: Denoting regular distal convoluted tubule with normal tubular basement membrane (BM), elongated mitochondria (M) and small microvilli (Bar = 2 $\mu$ )

no longer detect the brush border (Fig. 13). In the other hand, little damaged or necrotic cells nuclei were detected into the tubular lumina.

Kidneys of the same treated group showed subtle ultrastructural changes in the glomerulus along with tubules of renal cortex. The glomerulus exhibited mild fluctuations thickness of the basement membrane with disconnected foot processes of the podocytes (Fig. 14). These improvements was also noticed in the filtration slits of the basement membrane, endothelial cells, both 1<sup>st</sup> and 2<sup>nd</sup> foot processes of the podocytes and the mesangial cells

slightly returned to come into view. In contrast, the urinary space contained electron-lucent materials. Glomerular capillary contained erythrocytes, electron lucent and dense materials.

Proximal convoluted tubules cells denoted better elongated mitochondria distributed parallel to the long axis of the cell, normal apical microvilli and loss of basal enfolding of the basement membrane. Little apical secretory granules were seen (Fig. 15). The nucleus appears slightly healthy with plentifully chromatin materials. Interstitial infiltration of inflammatory cells fairly observed.

## PLATE 2

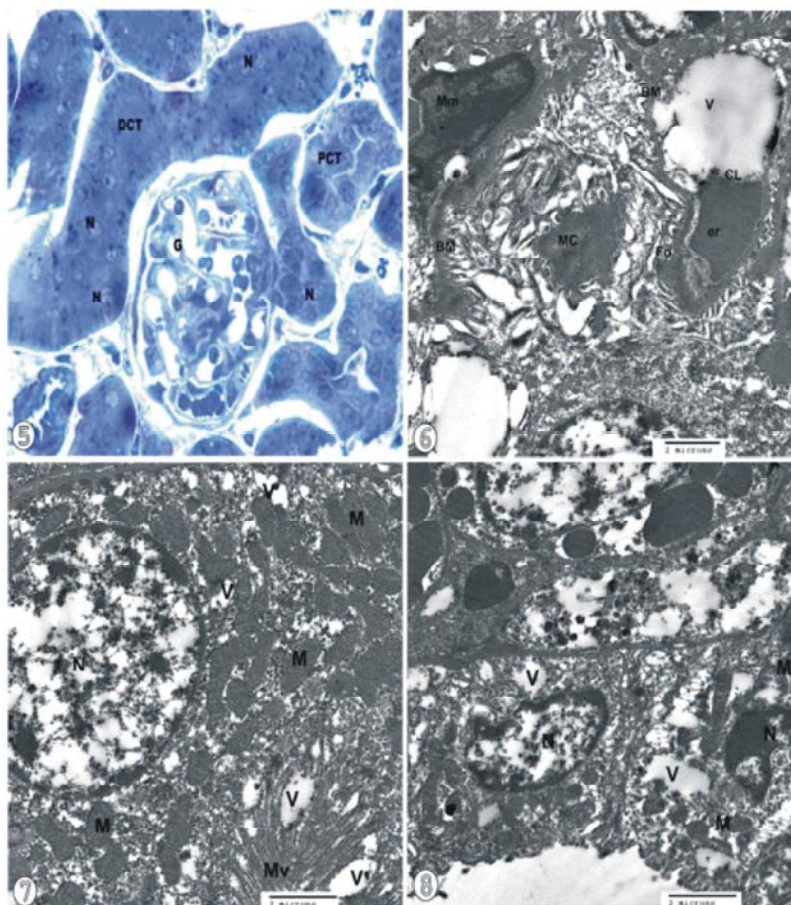


Plate 2: Represents photomicrograph in both toluidine blue stain and transmission electron microscope sections of rat kidneys treated with daily dose of enro-floxacin 10 % ( 75 mg/Kg B.W.)for10 days .

Fig. 5: Displaying injure of glomeruli ( G ) with numerous tubular cells necrosis in both proximal (PCT) and distal (DCT) tubules and some cells showed rupture necrotic nuclei (N), presence of hyaline casts in their lumina, Toluidine Blue stain, X400.

Fig. 6: Showing marked homogenous electron-dense deposits within the glomerular basement membrane (BM), capillary lumen (CL) with erythrocytes(er) as well as large lipid vacuole (V) due to some immune dysfunction, increase mesangial matrix (Mm), fusion of foot processes (Fo). (Bar = 2 $\mu$ )

Fig. 7: Showing marked proximal convoluted tubule epithelial cells necrosis with loss of the microvilli (MV), destructed cell membrane, burst down and scattered irregular distributed mitochondria, necrotic nuclei (N), vacuoles (V). (Bar = 2 $\mu$ )

Fig. 8: Showing necrotic distal convoluted tubule cells with irregular contoured nuclei (N), upper round mitochondria (M), large vacuoles (V). (Bar = 2 $\mu$ )

Distal convoluted tubule cells rest on thick basement membrane (basal lamina) and showed remarkable improvements. These including rounded vesiculated nuclei with irregular contour and prominent nucleolei, oval and elongated mitochondria with clearly cristae scattered in the cytoplasm and few vacuoles (Fig. 16).

**Animals Treated with Daily Dose of 75 ml/kg Enrofloxacin and 3 % Green Tea Extract:** Toluidine blue stained semithin sections of rat kidneys treated with EFX followed by 3% GTE for 10 days revealed considerable overturn the renal lesions that arise in a variety of the cortical structure. The sections demonstrated that most of the kidneys cortex to be normal glomerular structures,



## PLATE 3

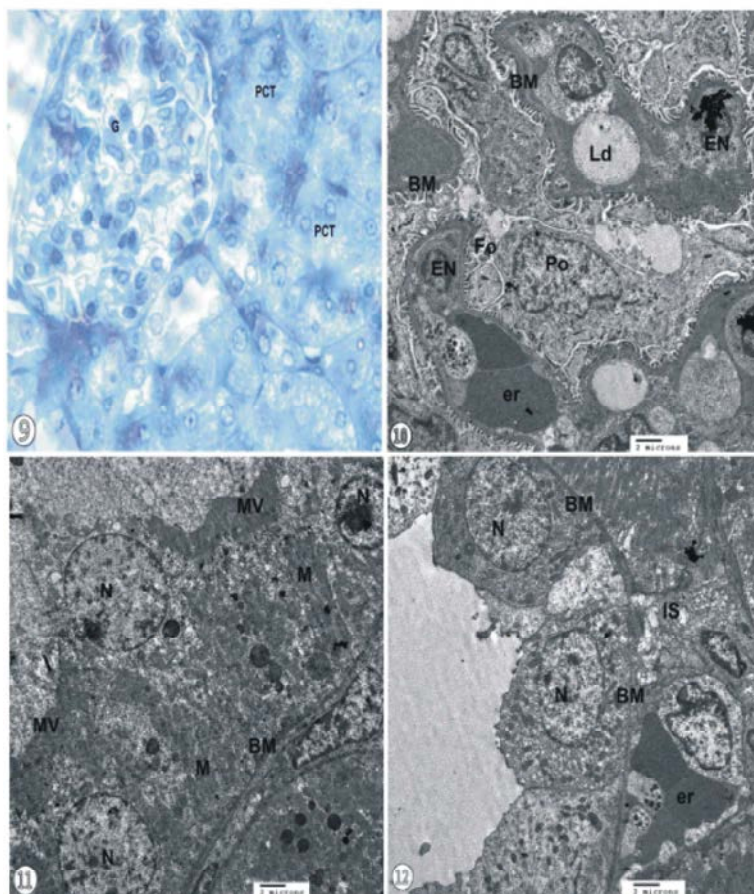


Plate 3: Represents photomicrographs for both toluidine blue stain and transmission electron microscope sections in rat kidney treated with daily dose of enro-floxacin 10 % ( 75 mg/Kg B.W.) as well as 1% GTE for 10 days:

Fig. 9: Displaying a congestion of the blood capillary, hyaline casts in the renal tubules with little normal intact PCT and DCT in addition to necrotic ones, less glomerular leucocytic infiltration (G). Toluidine Blue stain, X400.

Fig. 10: Showing slightly intact glomerular basement membrane (BM), capillaries lumen occupied by large vacuoles of lipid droplets (Ld), many erythrocytes(er) and neutrophil that engulfed fine dense materials. (Bar = 2 $\mu$ )

Fig. 11: An indistinctly intact microvilli (MV) of the proximal convoluted tubule, cell membrane that burst down discarded the mitochondria (M), large dense vacuoles, still loss of basal striation, interstitial nephritis (IS). (Bar = 2 $\mu$ )

Fig. 12: Fairly intact distal convoluted tubule, nucleus (N), rounded mitochondria (M) but still interstitial nephritis (IS). (Bar = 2 $\mu$ )

the proximal and distal convoluted tubules become healthy in the vicinity of the control with prominent fine developed microvilli, plentiful cytoplasm became more evident, healthier nuclei and normal nucleoli, no leucocytic infiltration manifest as compared to the lower doses and that of EFX alone given rats (Fig. 17). Interstitial widening was marked.

Ultrastructure sections examination of the same treatment showed remarkable improvements all over the renal cortex where the glomeruli gave the impression to be similar to the control. The glomerular capillaries contain different types of blood cells; leucocytes with clearly seen granules, lymphocytes and eosinophils (Fig. 18). Typical two podocytes extended to

## PLATE 4

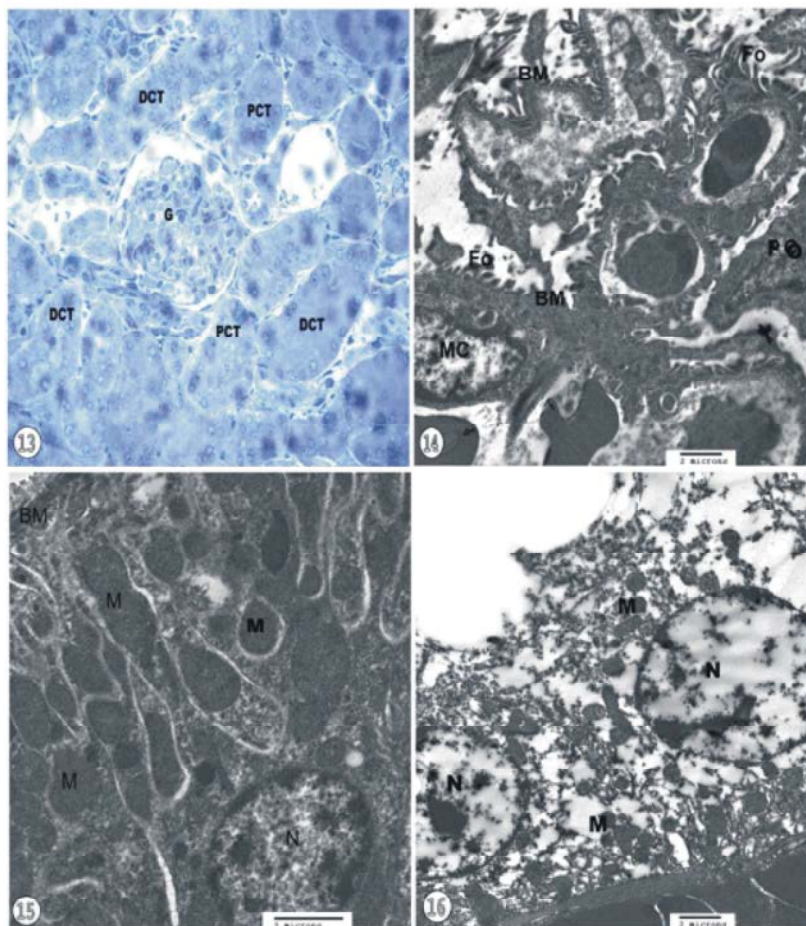


Plate 4: Represents photomicrographs for both toluidine blue stain and transmission electron microscope sections in rat kidney administered daily dose of enro-floxacin 10 % (75 mg/Kg B.W.) and 1.5%GTE for 10 days.

Fig. 13: Displaying a well intact of both proximal (PCT) and distal convoluted tubule (DCT) indicated an improve of their function, glomeruli (G), Toluidine Blue stain, X400.

Fig. 14: Distinct glomerular basement membrane (BM) with disconnected foot processes (Fo) of podocytes (Po), filtration slits of the endothelial cells, the mesangial cells (MC) slightly return into view, the urinary space was still containing lucent material. Glomerular capillary was still containing erythrocytes and electron - lucent and dense materials. (Bar = 2 $\mu$ )

Fig. 15: Observing improved proximal convoluted tubule, large dense vacuoles, elongated mitochondria (M) little loss of basal striation discarded from the burst down cell membrane, slightly intact microvilli (MV) indicated, (Bar = 2 $\mu$ )

Fig. 16: A bit abnormal configuration of distal convoluted tubule indicated worse reabsorption, nucleus (N) with obvious nucleolus, rounded mitochondria (M) but still interstitial nephritis (IS) with spot oozing red blood corpuscles (R). (Bar = 2 $\mu$ )

give primary foot process and in-betweens forming secondary foot ones forming what is called filtration slits. The basement membrane become more obvious and consists of a dense central lamina bounded on both sides by alight-staining lamina rara indicated the trilaminar structure.

Moreover, EM in the proximal convoluted tubule cells exhibited normal appearance nucleus with prominent clear nucleolus, well developed brush border microvilli (MV) not different from that control, based rounded mitochondria (M), numerous dense lysosomes (Fig. 19).

## PLATE 5

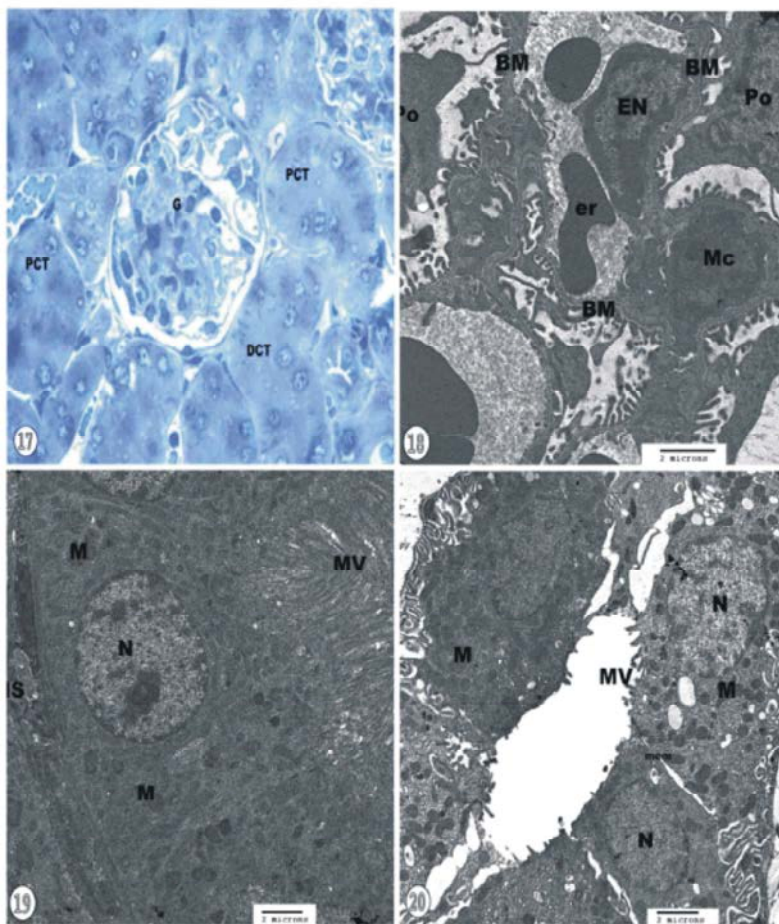


Plate 5: Represents photomicrographs for both toluidine blue stain and transmission electron microscope sections of rat kidney treated with daily dose of enro-floxacin 10 % ( 75 mg/Kg B.W.) and 3% GTE for 10 days.

Fig. 17: Viewing to some extent unbroken distal convoluted tubule with enhanced nucleus (N), slight interstitial oedema with leucocytes infiltration, glomeruli (G) denote appreciable better constituents, Toluidine Blue stain, X400.

Fig. 18: Denoting development of tri- laminar basement membrane (BM) through filtration slits of the endothelial cells (EN) and typical foot processes (Fo) extending from the podocytes (Po), the mesangial cells (MC) turn into control. The urinary space was still containing lucent material. Clear glomerular capillary contained erythrocytes. (Bar = 2 $\mu$ )

Fig. 19: Displayed slightly normal configuration of proximal convoluted tubule indicated by healthy nucleus (N) with prominent nucleolus, developed brush border microvilli (MV), rounded mitochondria (M), and dense lysosomes (Ly), interstitial (IS). (Bar = 2micron).

Fig. 20: Showing healthy distal convoluted tubule similar to control, nucleus (N), vigorous cell membrane (mem) with apical small microvilli (MV) and basal enfoldings, and the junction between the cell and its neighbor cell (Bar = 2 $\mu$ ).

The distal convoluted tubule cell showed normal ultrastructural features with prominent nucleolus and typically normal basement membrane invaginations, abundant rounded scattered mitochondria, junction appearance between the epithelial cells and small

microvilli (Fig. 20). Some cells appear dark while others noted light with few vacuoles. The same figure showed dilated intercellular spaces filled by inters digitations membrane leaflets, a sign of active water transport or sodium chloride.



## DISCUSSION

Enrofloxacin (EFX) is a fluoroquinolones commonly used in human as well as veterinary medicine associated with side effects, like bradycardia weakness and peripheral vasoconstriction [16]. Interestingly, it was previously found that GTE have a good antioxidant mechanism against intoxication in the liver and kidneys [31, 28]. Here we assessed the oxidative status of the kidneys during EFX co-exposed with green tea extract compared to separate intoxication of EFX alone.

In the present work, toluidene blue stained sections of rat kidneys showed deterioration effects subsequent to 75 mg/Kg B.W. EFX intoxication. It caused severe damage of all the cortical components as evolution of glomerular damages and vacuolation were manifest. The results agree with a wide range of studies on the effects of EFX which was unfavorable. The treatment with the drug associated with histological lesions and abnormal development of immature cartilage, urinary and gastrointestinal tracts and the central nervous system [22, 23]. Other studies denoted EFX toxicity as evaluation of genotoxicity in animals as well as toxic effects on the growth rate and inhibition of catalase activity in *Eisenia fetida* [32, 33, 34, 19, 35]. These adverse effects may be contributed to the production of free radicals and improve the generation of free oxygen species which induces apoptotic cell death causing a reduced glomerular ultrafiltration [36, 21].

In the current study ultrastructure deformation was recorded. The growing thickness in the glomerular basement membrane with loss of trilaminar structure and nephrotoxic tubular necrotic cells was apparent. Hotta [37] considered that this effect may be attributed to the increased deposition of glycoproteins. Furthermore, the fusion of foot process of the podocytes was observed after EFX treatment. It might be explained proteinuria associated through the indistinct basement membrane of the glomerular damage [38].

The present work showed a well distinct lipid droplet in the glomerular capillary after EFX treatment. It possibly due to reactive oxygen species (ROS) produced by EFX oxidative stress in cells and tissues causing lipid peroxidation (LPO). Moreover, the highly lipophilic characteristic was previously explained by the amphoteric properties of the drug which attributes to its toxicity [39].

Consequently, some necrotic cells extruded to the lumen leading to destruction of the microvilli of the brush borders and accompanied by rupture of the

membranes in phenomena of tubulorrehesis, electron dense materials and vacuolations was present in the current results. However, Cotran [36] showed that free radicals cause depletion of adenosine triphosphate and accumulation of intracellular calcium which results in cell detachment.

The present work showed that GTE give retardations of various abnormalities in renal tissues reported due to EFX exposure in rat kidneys. The GTE protection could be resulted as a scavenger of the free radicals produced by EFX leading to preventing lipid peroxidation and protein failure.

Conversely, consumption of a lower dose of GTE showed a slight improvement in the kidney cortex compared to the EFX alone. The results exhibited some obvious necrotic appearance at the level of the glomeruli, PCT and DCT lining epithelial cells. Moreover, congestion of blood vessels was still present within histological and ultrastructural examinations.

Kang [40] reported that GT (*Camellia sinensis* var. *sinensis*) has beneficial preventive tendencies towards diabetic nephropathy. The author added that it is associated with beneficial preventive effects in oxalate-induced cytotoxicity plus production of lipid peroxidation in addition to acting by preventing glomerular hyperfiltration, hypertrophic changes and subsequent protein loss in the urine.

Previous studies confirmed that GTE exhibits antioxidant defense mechanism through a number of antioxidant enzymes such as superoxide dismutase (SOD) and catalase enzyme. Kumar [41] added that SOD is responsible for the dismutation of the highly reactive and potentially toxic superoxide radicals ( $O_2^-$ ) to  $H_2O_2$ . On the other hand, catalase is responsible for the detoxification of reactive oxygen species.

In a remarkable study, Khan [28] and Abdel-Raheem [42] investigated the GT protection against gentamicin nephropathy or oxidative damage. The authors investigated the GT protection either by SOD-mediated or catalase mediated mechanism or both mechanisms simultaneously in rat renal cortex. They suggested that these mechanisms led to decrease in lipid peroxidation (LPO). So that the effects of GTE in the present results can antagonize the controversy the unfavorable outcome of EFX since it was found EFX could inhibit catalase [19].

Moreover, it was reported that GT contains polyphenols, mainly catechins and their derivatives which possess antioxidant, antimutagenic and

anticarcinogenic effects that reduce the risk of various forms of cancer, cardiovascular and renal disorders against certain environmental agents [43, 44, 45].

Administration of 1% GTE showed slight improved rat kidney tissues induced by EFX. Glomerular basement membrane still showed some protein precipitations indicated by the presence of electron dense materials. In addition, the proximal convoluted tubules still demonstrating nephrotoxic tubular necrotic cells. Thus, a low dose of GTE could not completely eradicate the dynamic toxicity of EFX. The presence of the necrotic cells in the proximal convoluted tubular cells might be due to an increase in potassium secretion due to the toxic agent. Increased potassium secretion in the proximal convoluted tubules will be compromised by an increase in potassium absorption in the distal tubules. This will eventually occur in case of cell apoptosis [46].

More interestingly, higher doses of GTE showed a remarkable improvement in glomerular endothelial cells. This improvement reached its peak after the treatment with 3% GTE where the vast majority of the EFX adverse effects were eliminated. This was noticed in the glomerular machinery, the proximal and the distal convoluted tubules. In the case of the PCT, well developed brush border microvilli were observed to be similar to the control. The ameliorations effects of GTE were dose-dependent. These findings are consistent with our previous study on the protective role of GTE against EFX in rat livers [31]. Moreover, GTE polyphenol are stimulators of mitochondrial biogenesis which are suppressed in the kidney after chronic cyclosporine A nephrotoxicity in rats as well as significant antiproteinuric effects during its antioxidative activity [42, 47, 48].

Furthermore, GTE was known to increase blood pressure in hypertensive rats. This effect is attributed to its antioxidant component (EGCG) properties [49] that inhibit lipid peroxidation and leakage of enzymes into the blood [50]. Moreover, other GTE components are effective free radical-scavengers [51] and also effective metal chelators [52]. Consequently GTE keeps the normal homeostasis of the body, protects phospholipids from enhanced peroxidation and prevents biochemical and morphological changes. Also, it increases catalase which is responsible for detoxification of reactive oxygen species [28].

## CONCLUSION

It is concluded from the present study that intoxication with EFX induced serious damage to the

kidney. This damage is suggested to be a consequence of the free radicals produced by EFX. Protective effect of GTE against kidneys damage induced by EFX may be attributed to its antioxidant activity which is more obvious with intake of higher dose (3%).

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