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RESEARCH ARTICLE

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HISTOLOGICAL AND HISTOCHEMICAL EFFECTS OF GREEN TEA EXTRACT ON ENROFLOXACIN-INDUCED KIDNEY INJURY IN ALBINO RATS

ABSTRACT:

Enrofloxacin (EFX) is an antibacterial drug associated with inhibition of many enzymes and many other effects. The aim of the present work is to investigate the protective effect of green tea extract (GTE) on EFX-induced kidneys abnormalities in albino rats. The rats were divided into five groups (A–E); each group contains six rats. Group A was kept as control, whereas rats of group B were injected intraperitoneally with a daily dose (75 mg/kg body weight) of enrofloxacin for 10 days. In the remaining groups (C–E), the animals were treated daily with one of the following three concentrations i.e. 1%, 1.5%, and 3% of freshly prepared green tea extract (GTE) daily the above mentioned dose of EFX for 10 days. At the end of the experiment, small pieces of kidney tissue were taken and processed for histological and histochemical examinations. The histological results revealed progresses nephrotoxic effects on the glomeruli and acute necrobiotic changes in both the cells of the proximal and distal convoluted tubules after treating the animals with EFX. Moreover, presence of hyaline casts in the tubules lumina with loss of the microvilli, disturbance of basal membranes enfolding and large vacuoles were recorded. The histochemical results showed depletion of the polysaccharides as well as the total proteins in glomeruli and lining epithelial cells. The wall of the tubules indicated marked decreases in many enzymatic activities. Besides, EFX-co administration of aqueous extract with GTE produces cytoprotective effects. In addition, marked improvements in the levels of glycogen and total proteins were recorded. Accordingly, it was concluded that aqueous extract of GTE improves the histological and the histochemical alterations induced by EFX at altitude of the different doses. More improvement was observed after the consumption of higher dose (3%).This may be attributed to the antioxidant properties of GTE constituents.

KEY WORDS:

Green tea, Enrofloxacin, Rat, Kidneys, Histology, Histochemistry, Ultrastructure

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INTRODUCTION:

Drugs are very important in the field of medicine both for human and animals. On the other hand, they may cause hazardous effects on different tissues. Enrofloxacin (EFX) is a fluoroquinolone antimicrobial drug that inhibits bacterial DNA gyrase (topoisomerase II) and topoisomerase IV (Lecomte *et al.,* 1998; Sissia *et al.,* 1998; Elmas *et al.,* 2001; Michiels *et al.,* 2002; Noble and Maxwell, 2002; Xu *et al.,* 2006). Besides, quinolones were found to induce singlet oxygen and superoxide anion (Abd-Allah *et al.,* 2000a) and this was accompanied by inhibition of protein synthesis (Scholar and Pratt, 2000). Other hazardous effects of these compounds are their sufficient lipid-solubility that able them to penetrate tissues and show low binding to plasma protein (Abo El-Sooud, 2003). At the level of the histological lesion, Coetzee *et al.* (2009) found that a dose of 4.0 mg/ml of EFX leads to condensation and vacuolization of nuclear material with signs of advanced degeneration in *A. marginale* organs. Moreover, Sweet *et al.* (1999) reported that EFX decreases the activity of the antioxidant enzyme CAT that might be inhibited possibly due to cellular dysfunction or apoptosis. Furthermore, EFX strongly decreased all the hepatic P450 dependent monooxygenases (Vaccaro *et al.,* 2003).

Medicinal foliage has an important role in pharmacology and medicine today. It is projected that about 80% of the world population relies on botanical preparations as medicine to meet their health needs (Ogbera *et al.,* 2010). Many investigations showed that green tea has different pharmacological protection for many diseases including antioxidant properties (Sung *et al.,* 2000; Nakagawa and Yokozawa, 2002), hypocholesterolemic (Lin *et al.,* 1998;

Riemersma *et al.,* 2001), antihyperglycemic (Tsuneki *et al.,* 2004), hepatoprotective (Hasegawa *et al.,* 1995; Sai *et al.,* 1998), chemopreventive (Chung *et al.,* 2003; Fujiki *et al.,* 2005), anticarcinogenic (Wang *et al.,* 1992; Lou *et al.,* 1999; Hayakawa *et al.,* 2001). The present study was undertaken to investigate the effects of EFX on the histological and histochemical structure of kidney of rats and the possible protective effect of GTE aqueous extract.

MATERIAL AND METHODS:

Male albino rats were obtained from the animal colony of the Dokki National Institute (Dokki, Eygpt). Enrofloxacin (10%) was obtained from Sigma Chemical Co., Egypt. Green tea (China green tea) commercially purchased and packaged by the Egypt National Native Product sources. Twenty five, 3-4 months old, male albino rats were housed in individual cages. All the animals received food and water *ad libitum.* The rats were kept in standard environmental conditions (temperature 25 ± 20^{o}C and a 12 h light/dark cycle). The animals were assigned randomly to one of five groups (n= 5/group) and kept in separate cages. The protocols employed were approved by the local committee for animal experiments.

Preparation of green tea:

Green tea extract (GTE) was used and based on the method of Khan *et al.*, (2009). Briefly, GTE was freshly prepared everyday by adding 25-30 g of dried tealeaves in 500 ml of boiling water, and then cooled to room temperature. Tea leaves solution was filtered and re-extracted with 500 ml of boiling water then filtered. The two filtrates were mixed and concentration of the extract is 3% green tea extract (3 g tea leaves /100 ml water). 1.5% and 1% green tea extracts were prepared as previously described. The resulting clear solution is similar to tea consumed by humans. The animal in the present study was orally given 1 ml of the final aqueous extract (Kamtchouing *et al*., 2002).

Experimental protocol:

The animals were divided into five groups (A–E); each group contained six rats. Group A was kept as a control, group B was injected intraperitoneally with a daily dose of 75 mg/kg body weight of enrofloxacin for 10 days. The animals of the remaining groups (C–E) were treated daily with the above mentioned dose of EFX and one of the following three concentrations, i.e. 1%, 1.5%, and 3% of freshly prepared GTE for 10 days. The experimental protocols were typically by the Institutional Animal Ethics Committee (National Res. Council, 1985).

At the end of the experiment, the animals were killed. Small pieces of kidneys were fixed in neutral buffer formalin, routinely processed and embedded in paraffin. Paraffin sections were cut at 5µm thickness. For histological examination the sections were stained with haematoxylin and eosin (H&E). Other sections were stained according to periodic acid-Schiff (PAS) method for demonstration of polysaccharides (Bancroft *et al.,* 1990) and were counterstained with haematoxylin. Total proteins were detected using the mercury bromophenol blue method (Pearse, 1972). All the slides were then examined with light microscope.

RESULTS:

Histological observations:

Figure 1a shows the histological picture of kidney cortex of the control rat with normal cortical components. The glomeruli (G), the proximal convoluted tubules (PCT) and the distal convoluted tubules (DCT) that are lined with cubical epithelial cells appear normal. Normal brush border was noticed in the proximal tubules.

The animals treated with daily dose of 75 ml/kg enrofloxacin for 10 days exhibited distinct histological alterations compared with the control. The glomeruli showed hypercellularity and failure of capsular space (Fig. 1b). The glomerular capillaries were obliterated by means of congested blood and the urinary spaces contained some hyaline material. Both proximal and distal convoluted tubules lining epithelium showed signs of progression nephrotoxic necrosis, ruptured membranes and presence of hyaline casts. Proximal tubules cells showed degenerated cytoplasm and dark necrotic nuclei. Some nuclei protruded in the lumina of the tubules. Destruction of the brush border was observed.

These histopathological alterations were less obvious in animals treated with EFX and 1% GTE. However, damaged structures were observed (Fig. 1c). Eosinophilic hyaline casts in the renal tubules and few normal intact PCT and DCT, less glomerular leucocytic infiltration and congestion of the blood were observed. The renal tubules showed few normal intact cells; others still demonstrated necrotic cells with scanty nuclear chromatin materials. Rupture of the membranes (tubulorrehexis) was present.

Treating rats with EFX and 1.5% green tea extract induced slight pathological signs compared to those given EFX alone and the control. Some proximal convoluted tubules cells displayed intact cell membranes and nuclei (Fig. 1d); but little showed necrotic cells and pyknotic nuclei that were protruded into the tubules lumina.

Examination of kidneys of animals treated with EFX and 3% GTE revealed less prominent histopathological changes when

compared with the animals treated with EFX only. Haematoxylin -stained sections showed normal glomeruli with no hypercellularity. Most of the epithelium of the proximal and distal convoluted tubules restored their normal structure and appeared almost normal (Fig. 1e). The cytoplasm became more eosinophilic with prominant nuclei and no leucocytic infiltration was manifested as compared to rats given EFX only and those of the lower dose.

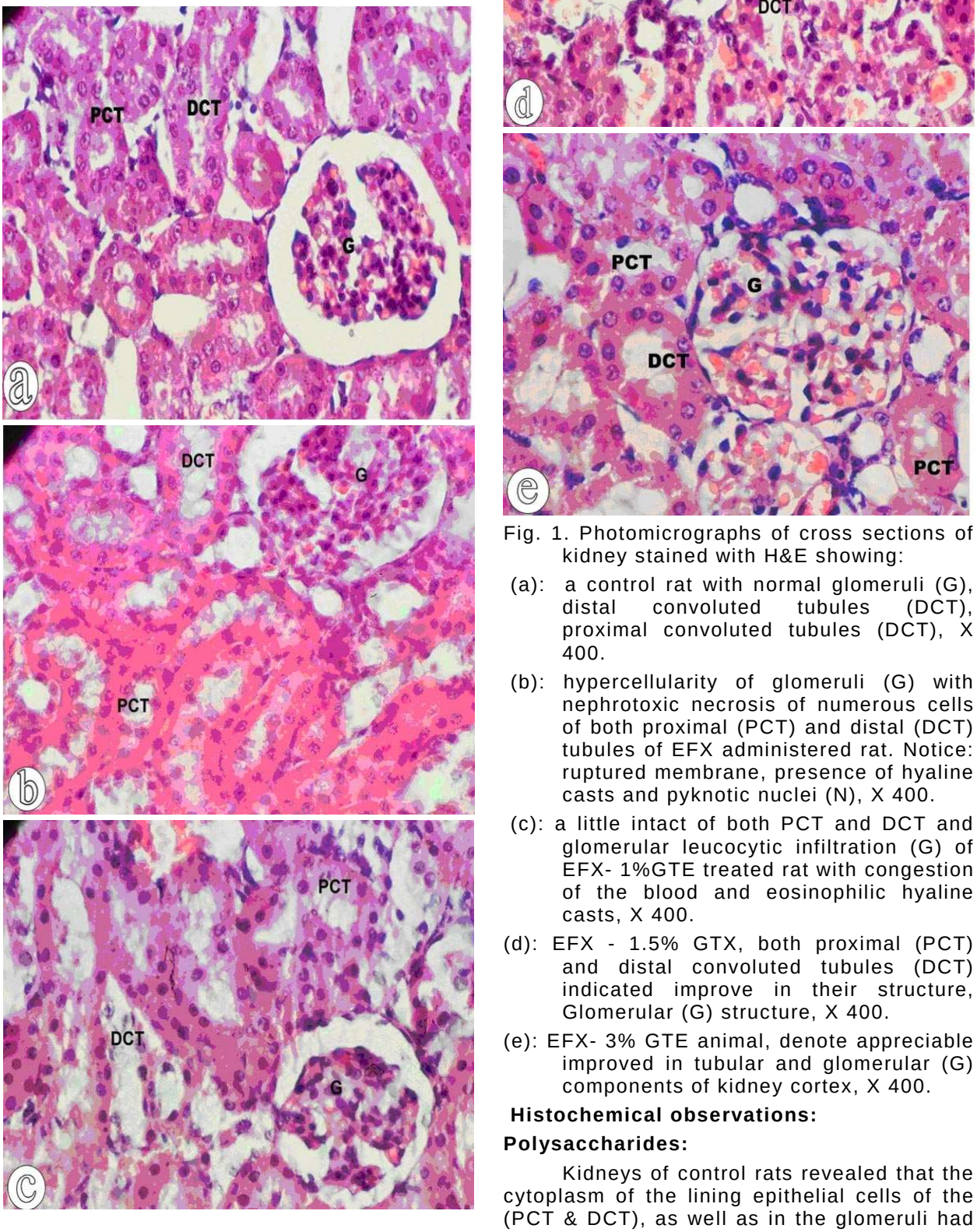


Fig. 1. Photomicrographs of cross sections of kidney stained with H&E showing:

(a): a control rat with normal glomeruli (G), distal convoluted tubules (DCT), proximal convoluted tubules (DCT), X 400.

(b): hypercellularity of glomeruli (G) with nephrotoxic necrosis of numerous cells of both proximal (PCT) and distal (DCT) tubules of EFX administered rat. Notice: ruptured membrane, presence of hyaline casts and pyknotic nuclei (N), X 400.

(c): a little intact of both PCT and DCT and glomerular leucocytic infiltration (G) of EFX- 1%GTE treated rat with congestion of the blood and eosinophilic hyaline casts, X 400.

(d): EFX - 1.5% GTX, both proximal (PCT) and distal convoluted tubules (DCT) indicated improve in their structure, Glomerular (G) structure, X 400.

(e): EFX- 3% GTE animal, denote appreciable improved in tubular and glomerular (G) components of kidney cortex, X 400.

Histochemical observations:

Polysaccharides:

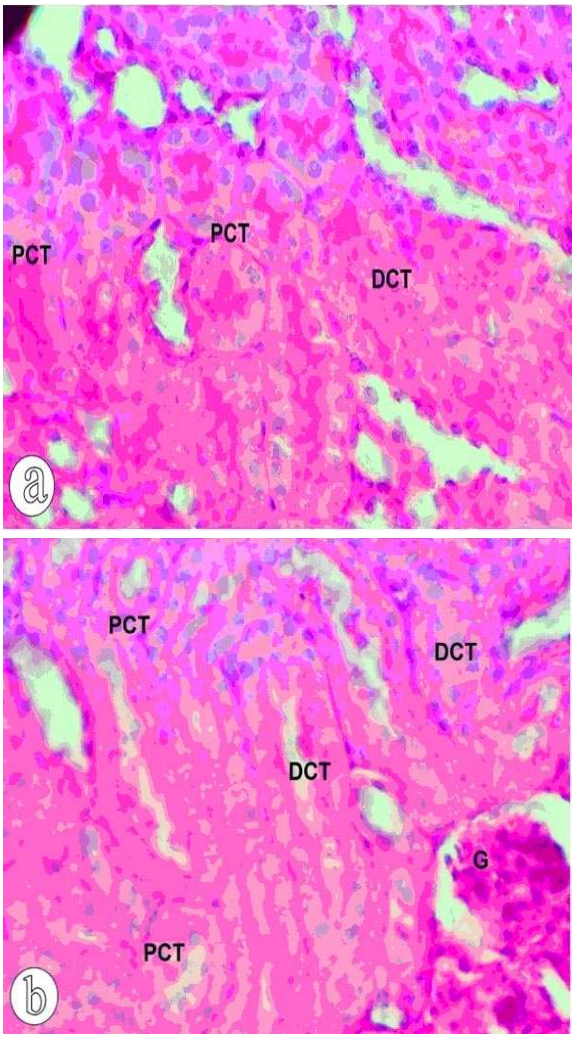
Kidneys of control rats revealed that the cytoplasm of the lining epithelial cells of the (PCT & DCT), as well as in the glomeruli had intense PAS-positive materials while the

nuclei were counterstained with H&E stain. The brush borders of the epithelial cells appeared with strong PAS reaction (Fig. 2a). Examination of kidneys of rats treated with EFX followed by 3% green tea extract showed an increase of the polysaccharide content as control (Fig. 2e).

The kidneys of rats treated with EFX displayed of PAS-positive materials. This weak reaction was noticed in both glomeruli and the renal tubular epithelial cells (PCT & DCT) as shown in figure 2b.

The kidneys of rats treated with EFX and GTE revealed gradual increase of PAS-positive material and reached their maximum in case of 3% GTE. This increase started to develop after treatment with 1% green tea extract (Fig. 2c) as it displayed moderate polysaccharides content in cell of both proximal (PCT) and distal (DCT) tubules cells and glomeruli (G) after PAS reaction. Animals treated with EFX and 1. 5% GTE more or less polysaccharides contents were illustrated (Fig. 2d).

The moderate reaction was noticed in cell cytoplasm of both proximal (PCT) and distal (DCT) tubules cells, more intense in the basement membrane of the tubules, in brush border and glomeruli (Fig. 2d).



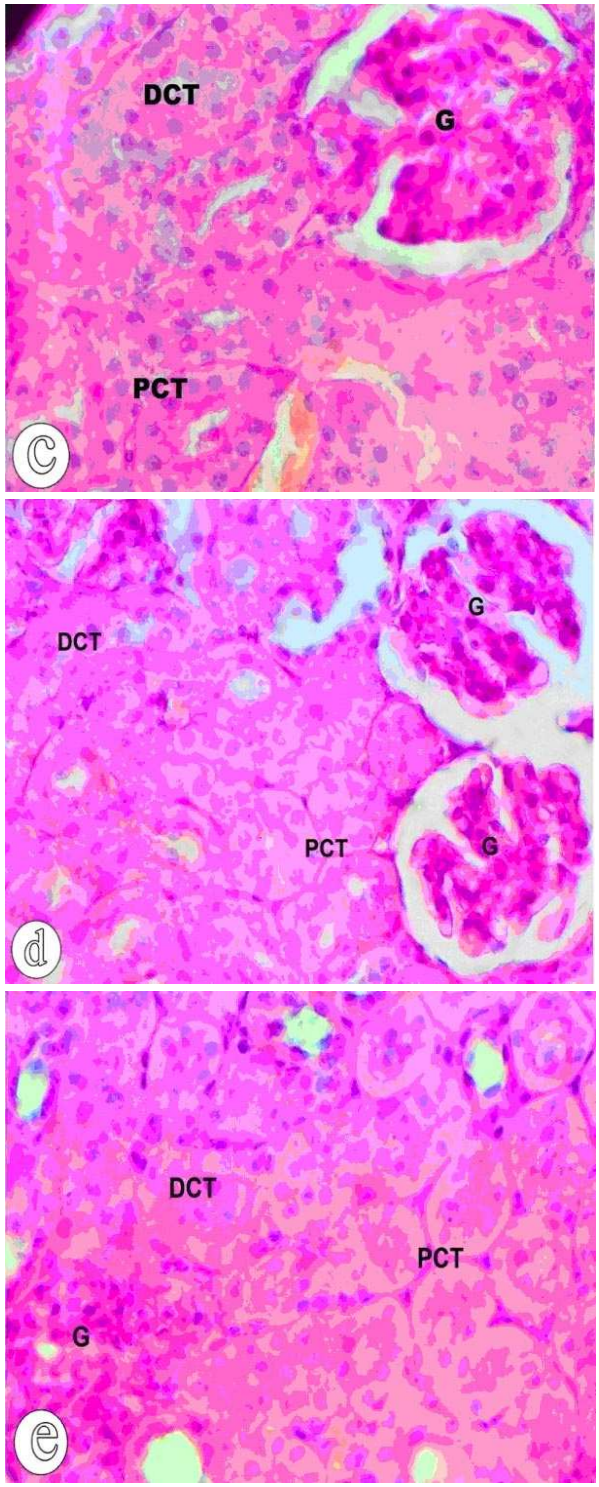


Fig. 2. Photomicrographs of cross sections of rat kidney (PAS- reaction) showing:

(a): a control rat displays an intense PAS +ve reaction in the cytoplasm of tubules epithelium and in the glomerular components, X 400.

(b): EFX-treated rat for 10 days illustrates weak polysaccharides content in cells of both proximal (PCT) and distal (DCT) tubules and glomeruli (G), X 400.

(c): EFX-1%GTE treated animal for the same period displaying moderate polysaccharide content in proximal (PCT) and distal (DCT) tubules cells, as

well as glomeruli (G), PAS reaction, X 400.

(d): a moderate of PAS-positive materials in kidney of EFX- co-exposed with 1.5% GTE treated rat, polysaccharide content in the cells of both proximal (PCT) and distal (DCT) tubular cells but an intense PAS reaction was observed in glomeruli (G), basement membrane of the tubules and brush border, PAS reaction, X 400.

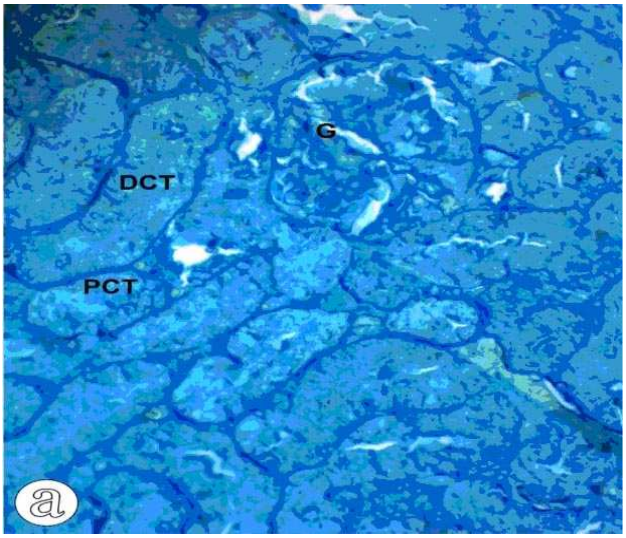
(e): EFX-3 %GTE treated rat displays an intense polysaccharides content in proximal (PCT) epithelial cells brush border and glomeruli (G), as in the control kidney, X 400

Total proteins:

Using mercury bromophenol blue method, the total proteins appeared in the kidney cortex tissues of control rats as deeply stained granules inside the nuclei and the cytoplasm of the lining epithelial cells of the convoluted tubules as well as glomeruli (Fig. 3a). The Animal treated with EFX for 10 days showed obvious decrease in the protein content (Fig. 3b). It is in the form of sparsely stained blue faint granules spread in the cytoplasm. The glomeruli have faint affinity for the dye after EFX treatment.

After treatment with EFX and 1% GTE the cortex components restored some of their protein content but these still had a diffused form (Fig. 3c). The same section show moderate protein distributions in the glomeruli (G), basement membrane of distal (DCT) and proximal convoluted tubules (DCT). No obvious changes in the total proteins were observed after treatment with 1.5 % GTE than in 1% GTE (Fig. 3d).

The intensity of the total proteins content was similar to that of the control after treatment with 3% GTE (Fig. 3e). It was observed that the total proteins increase in the cytoplasm of the tubular epithelium and basement membrane.



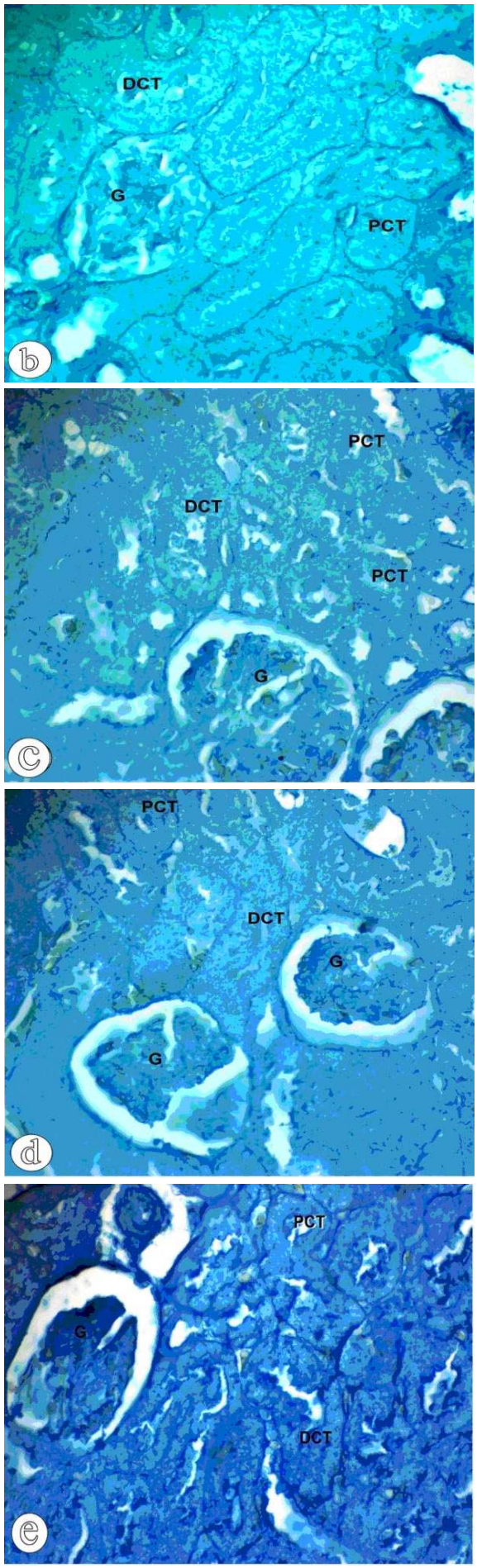


Fig. 3. Photomicrographs of cross sections of

rat kidney stained with Bromophenol Blue:

(a): a control section illustrates normal or deeply stained protein distributions in the glomeruli (G), distal convoluted tubule (DCT) and proximal convoluted tubule (DCT), X 400.

(b): decreased protein distributions in the glomeruli (G), distal convoluted tubules (DCT), and proximal convoluted tubules (DCT) of rat kidney treated with EFX for 10 days, X 400.

(c): EFX- 1%GTE treated show moderate protein distributions in the glomeruli (G), basement membrane of distal convoluted tubule (DCT), proximal convoluted tubules (DCT), X 400.

(d): Specimen obtained from an animal treated with EFX- 1.5 %GTE for 10 days showing moderate protein particles in kidney tissue, X400.

(e): a rat treated with EFX- 3% GTE showing improvement of protein content in the glomeruli (G), distal convoluted tubules (DCT), proximal convoluted tubule nearly similar to the control (DCT), X 400.

DISCUSSION:

Although EFX is important in the treatment of mammalian diseases, biochemicals and morphological alterations in treated animal have been reported. On the other hand, it exhibits cytotoxicity because it induces free radicals (Brown, 1996; Abd-Allah *et al.,* 2000a). The present work conducted histological and histochemical investigations on kidney cortex to evaluate the protective role of green tea against EFX treatment, and to determine and recognize the metabolism and machinery by which the epithelial changes are affected. Giving green tea extract simultaneously with EFX to rat leads to normalization of the metabolic process of its toxicity as previously obtained in the rats liver (Elasrag, 2010; El- Daly, 2011). This may be due to some of GTE constituents which exhibit scavenging characteristics.

The present study demonstrated that rats treated with EFX assessed severe histological changes in the kidney cortex, especially in glomeruli and proximal and distal tubules. The changes characterized by ruptured of the tubular epithelium membrane and presence of hyaline casts and necrotic nuclei of the proximal and distal tubules, destruction of the brush border, vacuolation and the necrobiotic glomerular cells. These deformations may be caused by the free radical release from EFX metabolism which induces toxic oxidative stress and cause kidneys injury. Parola and Robino (2001) reported that the free radical is the common factor for many diseases. Moreover, Ostrowska *et al.* (2004) concluded that the active hydroxide radicals initiate lipid peroxidation. Therefore, it is suggested that the created reactive oxygen species should react with most cellular organelles as well as organic molecules mainly membrane phospholipids. Additionally, the EFX-induced toxicity detected in this work was in accordance with several previous studies (Abd-Allah *et al.,* 2000 a&b; Aral *et al.*, 2008; Coetzee *et al.,* 2009; Elasrag, 2010; El-Daly, 2011).

Histochemical detection of total proteins of rat kidney cortex in the present work illustrated marked decrease in various degrees after EFX administration. The observed depletion of protein could be attributed to disturbances in the chromatin materials of the cells. Previously, Xu *et al.* (2006) reported that activity of EFX is based on the inhibition of DNA-gyrase that leads to an unstable condensation of the DNA configuration during cell division. Moreover, the inhibition of protein synthesis by EFX phenomena is in agreement with what have been previously described by several investigators (Schlosberg *et al.,* 1995; Vancutsem and Babish, 1996; Abo El-Sooud, 2003; Vaccaro *et al.,* 2003). Likewise, other enzymes like cytochrome P450 isoenzymes were found to be inhibited in various animal species and in humans after the metabolism of EFX (Bergan *et al.,* 1988; Fuhr *et al.,* 1990; Mayeaux and Winston, 1998). Additionally, these compounds are sufficiently lipid-soluble and able to penetrate all organs and tissues (Brown, 1996).

The present study showed a decrease in glycogen content in the renal tubules as well as in glomerular structures following exposure to EFX. A marked decrease in polysaccharides may be due reduction in the cellular respiration as well as lower metabolic activity and reduced ATP. The dramatic effects of EFX on polysaccharides may be attributed to what have been suggested before by Abd-Allah *et al.* (2000a) and Selvakumar *et al.* (2005). These authors suggested that the polysaccharides breakdown in testes may be attributed to phosphorylase enzyme or decrease in tricarboxylic acid cycle enzyme activities. Moreover, Kumar *et al.* (2005) reported that decreased ATP synthesis is frequently associated with both hypoxic and chemical toxic injury, added that high-energy phosphate is required for many synthetic and degradative processes including membranes transport, protein synthesis, lipogenesis and reactions necessary for phospholipid turnover. This is obviously present when PAS +ve reaction decreased indicating lower metabolic activity appeared in the cortical cells especially the proximal convoluted tubules means that impaired of intracellular energy in the form of ATP supported the metabolic

activities. Furthermore, Coux *et al.* (2001) reported that the reabsorption of Na+ ions by renal proximal tubular is considered to be the major function of the kidney. So, transports of EFX intracellular might provide organelles damages consequently dysfunction because it's small molecular size and easily filtered through the glomerular membrane and taken by renal tubular cells.

In contrast to that reported by Intorre *et al.* (1997) the pharmacokinetic behavior of the drug indicated its safety and efficiency for the treatment of various systemic bacterial infections. From the results, it seems that EFX is a real inhibitor of several enzymes especially that related to proteins and glycogen metabolism. This has been speculated from the decreased metabolic activity which leads to severe damages to the cortical components. This is in agreement with Vaccaro *et al.* (2003).

Co exposure to EFX with GTE resulted in considerable overturn of renal lesions that arised in structures of glomeruli and proximal and distal tubules. This is particularly related to ruptured membranes, recovered cells and unbroken brush border microvilli. The observed improvements in the histological structure of kidneys cortex may be explained by the direct interactions between toxic substances and GTE or by indirect effect of GTE. The main components of green tea are catechins, which have a polyphenol structure; are considered effective scavengers of superoxide, hydroxyl, and peroxyl radicals (Khan *et al.,* 1992; Guo *et al.,* 1996; Jovanovic *et al.,* 1996). A free radical-scavenging mechanism has generally been described as the basis of their antioxidative activity. In addition, catechins are known to chelate metal ions; this is another possible contributory mechanism in their protective action against the destructive effect of free radicals on the components of the cells (Guo *et al.,* 1996). Catechins suppress the formation of lipid peroxidation products in biological tissues and subcellular fractions (Terao *et al.,* 1994). It has been demonstrated that the chemically induced lipid peroxidation in rat liver and kidney could be inhibited by the intake of tea catechins (Sano *et al.,* 1995). In addition, catechins incorporated in cell membrane were shown to prevent or reduce the morphological and biochemical alterations of hepatocytes induced by hepatotoxic agents (Varga and Buris, 1990). It was investigated that the protective effects of EGCG on CCl4-induced hepatic fibrogenesis. Zhen *et al.* (2007) added that green tea may be an optional therapeutic and preventive measure against oxidative stress-induced liver injury and hepatic fibrosis.

The current results showed that carbohydrates as well as total proteins increased after treatment with EFX and GTE especially in glomeruli, basement membrane of the tubules and brush border of PCT compared to EFX alone. This elevation could be due to an increase in the re-absorption of kidney tubules or might be due to an increase in glycogen synthesis or a decrease in glycogen breakdown by phosphorylase, which causes a glycogen recovery in the tissue. Previously it was reported that GTE can influence EFX turnover or modify mutual toxic effects interactions, including disturbances in the metabolism of organic compounds such as carbohydrates and proteins (Elasrag, 2010; El-Daly, 2011).

However, it is imperative to distinguish that cytoprotective defence action of GTE showed dose-dependent effects in kidney tissue. The existing results demonstrated a strong recovery of proteins and carbohydrates in the cells of the cortex when treated with GTE. These results are in accordance with previous findings (El-Dally, 2011).

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التأثيرات الهستولوجية و الهستوكيميائية للشاى الأخضر ضد التأثير الضار لعقار الإنروفلوكساسين على كلية الجرذ الابيض

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لقد تم ملاحظة التأثير الحيوى لعقار الانروفلوكساسين بجرعة 75مل جرام لكل كجم من وزن الجسم يوميا لمدة عشرة أيام والهدف من هذا البحث هو دراسة تأثير تناول مستخلص الشاى الأخضر ضد التأثير الضار لعقار الانروفلوكساسين على قشرة الكلي للجرذ الأبيض. تم استخدام 5 مجموعات (كل منها 6 جرذان). المجموعة الاولى هى الضابطة , المجموعة الثانية تناولت العقار فقط والثالثة تناولت العقارمع الشاى الأخضر وقسمت هذه المجموعة الى تحت ثلاث مجموعات كلا منها تناولت الشاى بتركيز 1% و 1,5% و 3% مع العقار بالجرعة السابقة لمدة 10 أيام . وبعد هذه المدة تم تحضير العينات للفحص الهستولوجي والهستوكيميائي بالمجهر الضوئى. وقد أظهرت النتائج أن كلية الجرذان التى أخذت العقار حدثت فيها تغيرات ضارة بالخلايا فى كل من الأنيبيبات الملتوية القريبة والبعيدة والكبيبات الكلوية مثل تورم الخلايا وتنقرس عضيات الخلية وأنويتها، ترشيحات خلوية بين تلك التراكيب وأحتقان دموي، ونقص الجليكوجين والبروتين من الخلايا. أما بعد أخذ جرعات الشاى مع العقارفقد ظهر تحسن فى التركيب الخلوى لنسيج قشرة الكلية وكان ذلك واضحا وممثلا بزيادة الجليكوجين والبروتين. نستنتج من هذا أن عقار الانروفلوكساسين له تأثير ضار على خلايا قشرة الكلية للجرذ الأبيض, وأن الشاى الأخضر لة دور فعال فى تحسين هذا التأثير الضار للعقار خصوصا فى التركيزات الأعلى(3%) للشاى وخلال هذه المدة القصيرة (10 أيام).

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