Histol Histopathol (2011) 26: 543-549

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Cytotoxic effect of Ochratoxin A on the renal corpuscles of rat kidney: could Ochratoxin A cause kidney failure?

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Summary. To demonstrate that Ochratoxin A can cause kidney failure as the kidney is the primary target for OTA cytotoxicity. Ochratoxin A (OTA) is a mycotoxin found in our food. The cytotoxic effect of a low cumulative dose of OTA on the renal corpuscles of the kidney tissue has been investigated in this report. This study was based on two groups in which weaning albino rats were used: (1) control; (2) OTA-treated rats (289 μg/kg/day). After 28 days of treatment, a significant decrease in body weight, kidney weight and relative weight were detected in OTA treated rats. Serum creatinine and urea level were slightly elevated. These results revealed significant histological as well as ultrastructral lesions in the OTA treated group. The lesions included global congestion in the renal tissue and loss of demarcation between the cortex and medulla. The normal architecture of the renal corpuscles was destroyed and most of the corpuscles lost their ordinary look. The most apparent histopathological changes were urinary space disappearance and hypercellularity. In addition, congested, undifferentiated, atrophied, hypertrophied, fragmented, sclerotic, degenerated, and obliterated renal corpuscles were distinct. The ultrastructural lesions observed in the renal corpuscles in OTA on treated rats included; proliferation and swelling of the endothelial cells with occasional loss of fenestrae; narrowing of the capillary lumen; damaged podocytes with deteriorated secondary foot processes, hypertrophied and proliferated mesangial cells with expanded mesangial matrix. The endothelium was clearly defected and vacuolated, and lost its fenestrations in many glomerular capillaries. In addition, the glomerular basement membrane (GBM) became visibly thickened and tortuous. Necrotic glomerular cells were frequently observed. Pre-apoptotic cells were also seen.

It was concluded that the exposure to relatively low OTA concentrations induced significant lesions to the renal corpuscles. Moreover, it activated oxidative damage and necrosis which can cause extensive damage to the kidney and ultimately kidney failure.

Key words: Cytotoxicity, Glomerular filtration rate, Kidney failure, Ochratoxin A, Renal corpuscles

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Introduction

Mycotoxins are secondary metabolites produced by species of fungi that are capable of causing disease and death in humans and animals. Food contamination by mycotoxins is a dilemma in terms of health, both in humans and animals. They are also responsible for causing deleterious effects and toxicosis in animal and in human populations. Among various mycotoxins is Ochratoxin A (OTA), which was discovered by (Van Der Merwe et al., 1965), mainly produced by *Aspergillus ochraceus*. It is a common contaminant of a variety of food including grain products, nuts, coffee beans, fruits, beer and wine (Aziz and Moussa); (O'Brien and Dietrich, 2005; Selma et al., 2009; Soubra et al., 2009). It has been identified in blood, bile, and in urine of humans and animals after consumption of contaminated food (Okutan et al., 2004; Sangare-Tigori et al., 2006). In addition, Ochratoxin A was found in human milk (Dostal et al., 2008). Due to the wide occurrence of OTA in improperly stored food, the complete avoidance of OTA is unachievable. Many studies with rodents, pigs, poultry and fish have demonstrated that the kidney is the primary target for OTA (Bayman and Baker, 2006). Furthermore, OTA is found to be genotoxic (Pfohl-Leszkowicz et al., 1991), immunotoxic (Alvarez et al., 2004), neurotoxic (Sava et al., 2006), and carcinogenic (Marin-Kuan et al., 2006), with renal toxicity and carcinogenicity being considered the pivotal effects (Marin-Kuan et al., 2008). In humans, exposure to OTA has been linked with endemic Balkan nephropathy and associated urinary tract tumors (Sostaric and Vukelic,

Abbreviations: Glomerular Basement Membrane, GBM; Glomerular Filtration Rate, GFR; Ochratoxin A, OTA

1991; Pfohl-Leszkowicz et al., 2002; Vrabcheva et al., 2004).

Three distinct mechanisms of OTA toxicity have been proposed; inhibition of protein synthesis, induction of lipid peroxidation, and inhibition of mitochondrial ATP production (Marquardt and Frohlich, 1992; Schilter et al., 2005).

Since no study has yet examined the ultrastructural lesions of the renal corpuscles induced by OTA found in our food, this study was undertaken.

Materials and Methods

Materials

Experimental Animals

The experiments were performed with weaning albino male Wistar rats (*Ratus norvegigus*) weighing 35 g to 40 g, obtained from the experimental animal house center, King Abdulaziz University, Jeddah, Saudi Arabia. All animals were given food (rat chow) and water, and were maintained at a relative humidity of 65% to 86% at a temperature of 18°C to 20°C. Appropriate care and hygienic conditions were maintained in order to keep them healthy and free of infections.

Ochratoxin A

Ochratoxin A (Cat. No. 01877) were purchased from Sigma-Aldrich Chemical Company (USA). It was dissolved in 0.5 M $NaHCO_3$ pH 7.4 (Atroshi et al., 2000). OTA was given by gavages through a stomach tube for 28 days.

Methods

The animals were distributed randomly into two groups, with seven rats per group. The groups were treated for four weeks (5 days/week).

Group 1: control animals injected with sodium bicarbonate buffer 0.5 M, pH 7.4.

Group 2: animals treated with OTA (289 μg/kg/day). After four weeks of treatment, a number of parameters in the kidney were determined, including body weight, kidney weight, relative weight, and the area of the urinary space of the renal corpuscle and also the parameters that indicate kidney function, such as serum creatinine and urea concentrations were determined. Histopathological and ultrastructural changes of the renal corpuscles were examined using light and electron microscopy.

Light microscopy.

Animals were killed 24 hours after last dosing by anesthesia with ether and autopsied and the kidneys were removed. Samples from the kidney were fixed in 10% buffered formalin solution, processed through graded alcohols and xylene, and embedded in paraffin blocks (BANCROFT John D., 2007). Tissue sections were cut for 3-5 μm, and stained with haematoxylin and eosin (H&E) for light microscopy examinations.

Electron microscopy.

Small pieces of the kidney tissue were immediately collected into a petri dish containing chilled 2.5% glutaraldehyde in cacodylate buffer pH 7.4 (Robards AW, 1993). The sections were examined and viewed using Philips 100 CM TEM.

Morphometric analysis.

10 renal corpuscles from the cortex of each rat were chosen randomly under a light microscope Olympus DP12 with a digital camera attached to a computer at a magnification of x 40, scale bar: 500 μm. The area of the renal corpuscle and the area of its glomerulus were measured, using a computer image analysis software Olysia. The area of the urinary space was calculated by subtracting the area of the glomerulus from the area of the renal corpuscle.

Biochemical analysis.

Serum creatinine and urea concentrations were measured.

Statistical analysis.

Data were presented as mean values ± SE, and computed statistically by (SPSS software package, version 15) using one-way ANOVA.

Table 1. Morphmetric measurements of control and OTA treated rats.

Variable	Experimental Groups		Control Group G1	OTA treated Group G2
Body weight	Zero day	mean	35.93	36.58
		± S.E	1.37	1.22
	Fourth week (gm)	mean	173.45	165.48*
		± S.E	1.10	3.64
Kidney parameters	Kidney weight (gm)	mean	0.51	0.44*
		± S.E	0.03	0.02
	Relative weight (%) R= K.W/ B.W * 100	mean	0.30	0.26*
		± S.E	0.01	0.01
Measurement study	Area of urinary space of cortical renal corpuscles (μm^2)	mean	1026.27	440.34*
		± S.E	60.97	191.75
	Area of urinary space of juxtamedullary renal corpuscles (μm^2)	mean	1509.32	1258.77
		± S.E	210.93	84.08

*: Significantly different from the control at, $p<0.05$

Results

Morphometric study

In OTA treated rats, no changes were noticed on the morphology and behavior of the animals. In addition, no macroscopical lesions were observed on the kidney. However, a significant decrease was noticed on the body weight, kidney weight and relative weight of OTA treated group compared to the control group. The statistical measurements also indicated a significant decrease in the urinary space area of the cortical renal corpuscles of OTA treated rats compared to the control (Table1). Serum creatinine concentration and urea level were slightly elevated by OTA treatment in comparison with the control (Table 2).

Light Microscopy:

The kidney tissue of the control group revealed

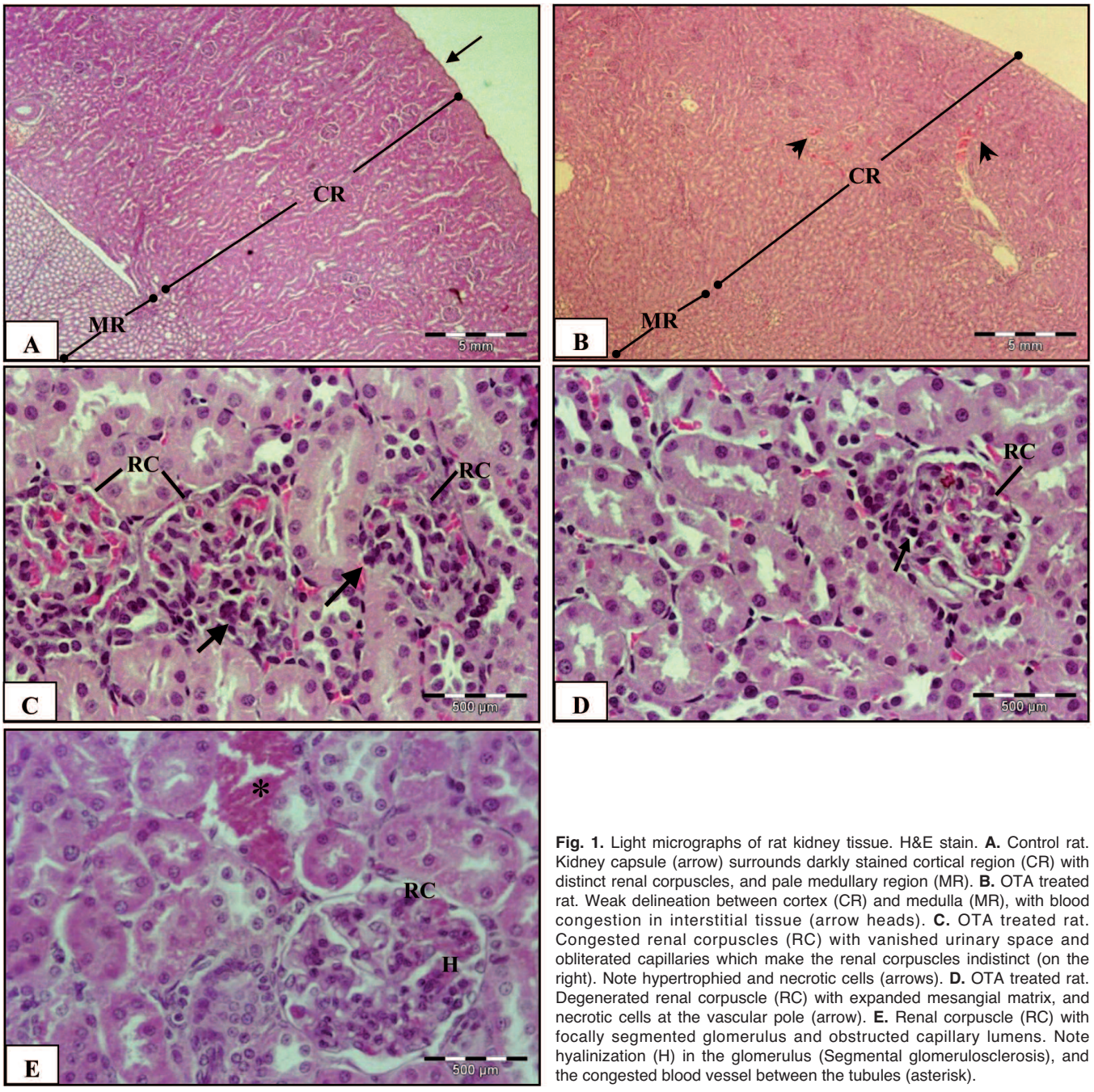


Fig. 1. Light micrographs of rat kidney tissue. H&E stain. **A.** Control rat. Kidney capsule (arrow) surrounds darkly stained cortical region (CR) with distinct renal corpuscles, and pale medullary region (MR). **B.** OTA treated rat. Weak delineation between cortex (CR) and medulla (MR), with blood congestion in interstitial tissue (arrow heads). **C.** OTA treated rat. Congested renal corpuscles (RC) with vanished urinary space and obliterated capillaries which make the renal corpuscles indistinct (on the right). Note hypertrophied and necrotic cells (arrows). **D.** OTA treated rat. Degenerated renal corpuscle (RC) with expanded mesangial matrix, and necrotic cells at the vascular pole (arrow). **E.** Renal corpuscle (RC) with focally segmented glomerulus and obstructed capillary lumens. Note hyalinization (H) in the glomerulus (Segmental glomerulosclerosis), and the congested blood vessel between the tubules (asterisk).

Table 2. Serum creatinine and urea level of control and OTA treated rats.

Variable/Experimental Groups		Control Group G1	OTA treated Group G2
Creatinine μmol/ L	mean	33.43	35.43
	± S.E	1.02	0.43
Urea mmol/L	mean	5.74	5.83
	± S.E	0.16	0.25

normal appearance with well defined renal corpuscles (Fig. 1A). However, in the OTA treated group, our results revealed significant histopathological changes. Global congestion in the renal tissue and loss of demarcation between the cortex and medulla were noticed (Fig. 1B). The normal architecture of the renal corpuscles was destroyed; most of the corpuscles lost their normal appearance, especially in the cortical region. The most apparent changes were congestion, urinary space disappearance, and hypercellularity of the

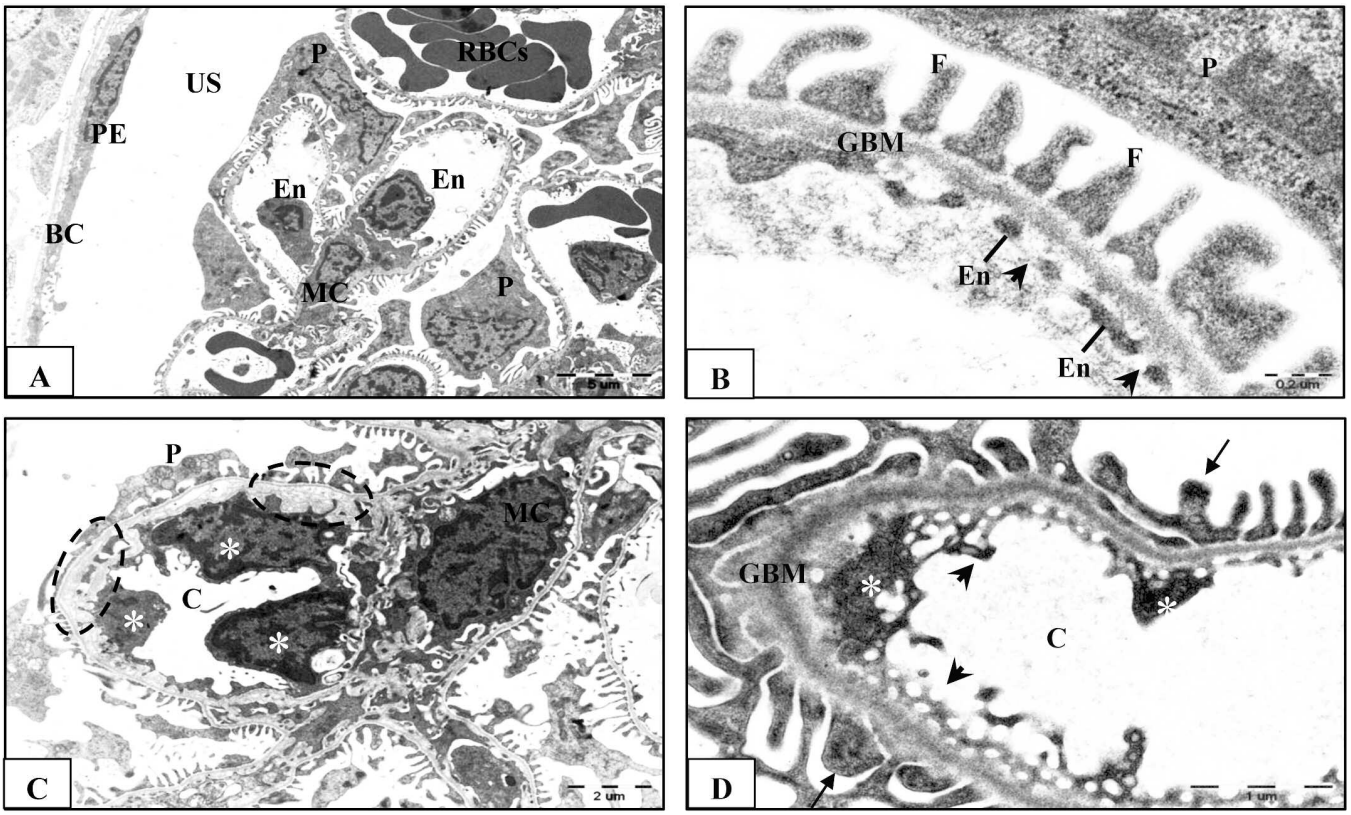


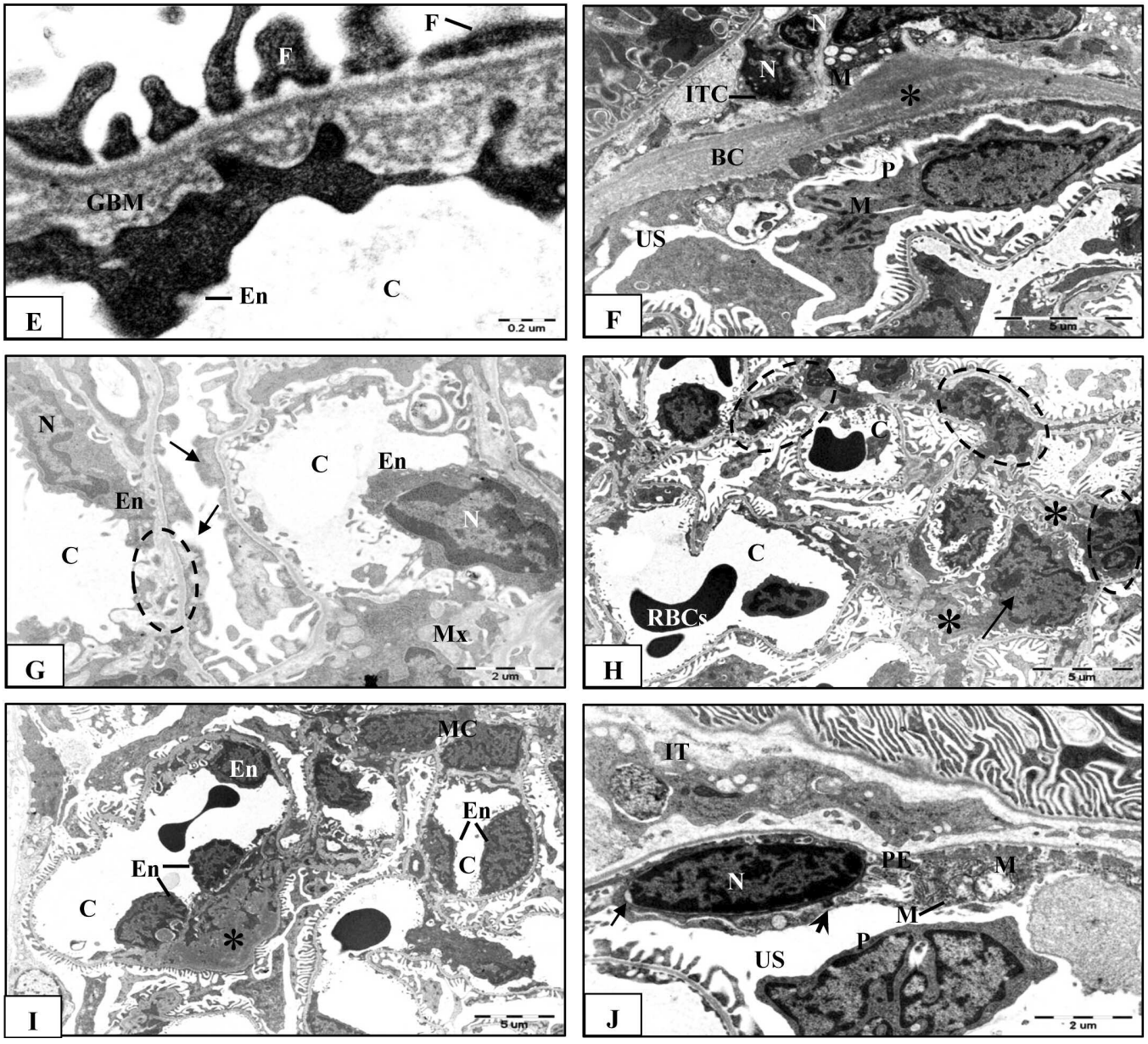
Fig. 2. Electron micrographs of rat renal corpuscle. Uranyl acetate and lead citrate. **A.** Control rat. Parietal epithelial cell (PE) lines Bowman's capsule (BC). The urinary space (US) is distinct with podocytes (P) rest on the capillary walls and endothelial cells (En) line capillaries lumens (C) containing red blood cells (RBCs). Mesangial cells are located (MC) among capillaries. **B.** Filtration barrier of the control rat. The (GBM) appears normal in thickness with clearly fenestrated endothelium (arrow heads) and normally gapped secondary foot processes (arrows) of the podocyte (P). **C.** OTA treated rat. Glomerulus with very conspicuous endocapillary hypercellularity and hypertrophy (asterisks) leading to narrow capillary lumen (C). The (GBM) is markedly thick (detached line).Note the podocyte (P) with damaged mitochondria, and the hypertropheid mesangial cell (MC). **D.** OTA treated rat. Glomerular capillary (C) with significant alteration of the filtration barrier. The endothelium is clearly destructed (arrow heads) showing vacuolation and sub-endothelium deposits (asterisks). The (GBM) is thick, and the secondary foot processes are abnormal (arrows). **E.** OTA treated rat. High magnification of the filtration barrier of the glomerular capillary (C). Severely thickened (GBM) with fused endothelium (En) and broad secondary foot process (F) in some places. **F.** OTA treated rat. A significant membranous thickening of Bowmann's capsule (BC) with fibrous deposits (asterisk). The podocyte (P) has detached foot processes and condensed mitochondria (M) found in a narrow urinary space (US). Note necrotized interstitial cells (ITC) with pyknotic nuclei (N) and degenerated mitochondria (M). **G.** OTA treated rat. Fused secondary foot processes containing a dense material (arrows) closing the filtration slits. The endothelial cells (En) with pyknotic nuclei (N) lined the glomerular capillary (C). The mesangial matrix (Mx) expanded into the capillary lumen creating extremely thick GBM (detached line). **H.** OTA treated art. The mesangial cells appear necrotic (detached line), and hypertrophied (arrow) with fragmented nucleus and expanded matrix (asterisks) in the capillary loops that contain red blood cells (RBCs). **I.** OTA treated rat. The mesangial cells (MC) are enlarged. The capillary walls (C) with proliferated endothelial cells (En) are severely thickened by mesangial matrix interposition into the sub-endothelial zone (asterisk). **J.** OTA treated rat. Necrotic parietal epithelial cell (PE) with pyknotic nucleus (N) and slightly dilated nuclear envelope (arrow) surrounded by electron dense material (arrow head). The mitochondria (M) of the parietal cell are swollen and degenerated. The podocyte (P) appears enlarged with fragmented nucleus in a narrow urinary space (US). interstitial tissue (IT). Scale bars: A, F, H, I, 5 μm; C, G, J, 2 μm; D, 1 μm; B, E, 0.2 μm.

glomeruli. In addition, undifferentiated, atrophied, hypertrophied, fragmented, degenerated and sclerotic renal corpuscles could clearly be seen (Fig. 1C-E). Obliterated capillary lumens were frequently seen. Occasionally, basement membranes of Bowman's capsules, as well as vascular poles, were seen as degenerated. Numerous necrotic cells were observed in the renal corpuscles (Fig. 1C,D).

Electron Microscopy

Group 1: The ultrastructural study showed normal renal corpuscles in the control group (Fig. 2A). The podocytes were normal and remained in contact with the basement membrane by normal foot processes with ordinary gaps. The endothelial cells were also normal in number and size with richly fenestrated endothelium (Fig. 2B). The mesangial cells and matrix were seen in normal position.

Group 2: Significant ultrastructural changes were seen in the renal corpuscles of OTA treated rats compared to the control. These lesions included proliferation and swelling of many endothelial cells exhibited large projections inside the capillary lumens causing stenosis to them (Fig. 2C). The endothelium was



clearly defected and vacuolated, and lost its fenestrations in many glomerular capillaries (Fig. 2D). In addition, the GBM became visibly thickened and tortuous (Fig. 2C,E,H,I). Several podocytes were swollen and proliferated. The secondary foot processes were clearly deteriorated (Fig. 2F) with markedly focal fusion (Fig. 2G). Numerous mesangial cells were also hypertrophied and proliferated as well as the mesangial matrix expanded into the capillary lumens caused focal severe thickening and bending of the GBM (Fig. 2C,H,I). The slit membrane structure was severely deteriorated in many areas (Fig. 2D) compared to the control (Fig. 2B). Propagated and enlarged parietal epithelial cells were also observed. In addition, necrotic endothelial, mesangial and parietal cells were occasionally seen (Fig. 2C,G,H,K). Pre-apoptotic cells were detected and visualized through dilation of the nuclear envelope, margination and condensation of heterochromatin (Fig. 2J). A loss of membrane integrity was detected in most cytoplasmic organelles. Mitochondria were the most affected and disturbed organelles by OTA treatment, exhibiting severe swelling with disintegrated membranes (Fig. 2J).

Discussion

In the present study, using a relatively small daily dose of OTA revealed no behavioral changes and no macroscopic lesions on the rat kidney due to the short duration of the treatment. These data are corroborated by a previous report (Stoev et al., 2001). However, significant histopathological and ultrastructural changes in the renal corpuscles of rat kidney were seen.

Some of our histological findings included swollen glomeruli, and increased mesangial matrix paralleled a previous report on the effect of aluminum on rat glomeruli (Kutlubay et al., 2007). In addition, many renal corpuscles seemed to have disappeared in this study, which coincides with the previous study on cisplatin induced nephrotoxicity (Tarladacalisir et al., 2008).

The cytotoxicity of OTA in the present study was demonstrated by; narrowing and obliteration of many glomerular capillaries, as well as reduction and disappearance of the urinary space in many glomeruli. Narrowing of the capillary lumen owing to proliferation and swelling of the endothelial cells, in addition to mesangial cells propagation and mesangial matrix expansion into capillary lumens. Some of the ultrastructural alterations seen in the present study were previously seen in cadmium effect on rat kidney (Abdel-Moneim and Said, 2007), but the severity of the lesions in this study were much stronger.

Proliferation and activation of mesangial cells are a hallmark and a primary event in a large number of glomerulonephritis (Herrera, 2006; Schwerdt et al., 2009) reported that OTA has only a minor effect on human mesangial cell in primary culture and a risk of mesangial damage by OTA exposure is unlikely. However, in this study the extent of the lesions were aggravated by a remarkable increase in the number of mesangial cells. The expansion of the mesangial matrix into the adjacent glomerular capillary lumens caused severe and focal thickening of the GBM, as well as obstruction of the capillary lumen, which clearly reflected a pathological condition called mesangio-capillary glomerulonephritis (STEVENS Alan, 2002).

Thickening of the (GBM) was detected previously by (Aunapuu et al., 2003; Abdel-Moneim and Said, 2007; Baleato et al., 2008) using losartan, cadmium, and deletion respectively. In this view, thickening of the GBM will definitely impair the glomerular filtration rate (GFR), which was indicated ultrastructurally in the present study by damaged filtration barrier, in which the three components of the filtration barrier were deformed; the fenestrated endothelium, the GBM and the secondary foot processes of the podocytes. In addition, impaired glomerular filtration rate (GFR) was also detected physiologically by increased serum creatinine concentration.

The reduction and disappearance of the urinary space in the present study were caused by proliferation and hypertrophy of many glomerular cells, including parietal epithelial cells, which lead to low (GFR).

Necrosis was detected frequently in the glomerular cells in this study. An earlier report detected tubular necrosis using cisplatin (Tarladacalisir et al., 2008). In addition, pre-apoptosis was visualized in the present study. Apoptosis was a characteristic feature reported in OTA induced kidney tubular damage in rabbits by (Kumar et al., 2007), also in rat kidney (in vivo) and in human kidney (in vitro) (Rached et al., 2006).

OTA exerts its cytotoxic effect by causing inhibition of protein synthesis, peroxidation of membrane phospholipids and inhibition of ATP production. This increases membrane fluidity and permeability, as demonstrated in this study, by hypertrophy of the cells and degeneration of cell membranes and organelles, such as nuclei and mitochondria. The mitochondrial alterations in this report were similar to those described in pigs and rabbits (Kumar et al., 2007) respectively. In this study, both membranes of the mitochondria were degenerated, which greatly reduced ATP production. On the other hand, Meisner and Chan (1974) proposed that OTA inhibits mitochondrial oxidative phosphorylation by acting as a competitive inhibitor of carrier proteins in the inner mitochondrial membrane.

We conclude from the present study that using a relatively small amount of OTA on rat kidney caused lesions in the glomerular structure which can cause severe malfunction of the renal corpuscles, reducing the functional capacity of the kidney and eventually lead to kidney failure.

Acknowledgements. This work was supported by a grant from king Abdullaziz city for science and technology, Riyadh, Saudi Arabia.

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Accepted October 18, 2010