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From Cell Biology to Tissue Engineering

Alleviative effect of myricetin on ochratoxin A-induced oxidative stress in rat renal cortex: histological and biochemical study

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Summary. Ochratoxins (OTA) are secondary metabolites of *Aspergillus* and *Penicillium*. The detoxification of OTA has been of major interest due to its widespread threat to human health. We aimed to investigate the possible alleviative effect of myricetin (MYR) against OTA-induced damage in renal cortex of rats. Thirty adult male albino rats were randomized into five equal groups: control (untreated), vehicle control (0.5 ml corn oil/day including dimethylsulfoxide [DMSO]), MYR (100 mg MYR/kg b.w./day in distilled water), OTA (0.5 mg OTA/kg b.w./day; dissolved in 10% DMSO and then corn oil) and OTA + MYR group (received OTA and MYR at similar doses). All treatments were given by oral gavage for 2 weeks. At the end of the experiment, renal cortices were processed for light and electron microscope examinations. Immunohistochemical staining for localization of proliferating cell nuclear antigen (PCNA), p53 and transforming growth factor beta 1 (TGF-β1) was carried out. Biochemical analysis of tissue glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) were determined to evaluate oxidative stress. OTA administration induced deleterious renal injury evidenced by the structural and ultra-structural changes. Immunohistochemical expression of p53, PCNA and TGF-β1 were significantly up regulated compared with control. Alterations in antioxidant parameters supported that oxidative stress was one of the mechanisms involved in OTA toxicity. On the contrary, co-administration of MRY partially ameliorated OTA-induced renal injury. We suggest the potential effectiveness of MYR to counteract OTA-induced toxic oxidative stress on the renal cortex.

Key words: Myricetin, Ochratoxin A, Oxidative stress, Rat, Renal cortex

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Introduction

Ochratoxin A (OTA) is a mycotoxin produced by several fungi, such as *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus ochraceus* or *Penicillium verrucosum* (Sokolić-Mihalak et al., 2012). Food commodities susceptible to OTA contamination include mainly cereal grains (wheat, corn), dry fruits (figs, raisins), coffee, soy, wine and beer (Tjamos et al., 2006; Zinedine et al., 2007; Marin et al., 2009; Haighton et al., 2012). While mycotoxin exposure is primarily food borne, airborne and transdermal routes of exposure also exist (Hope, 2013). OTA can enter the food chain through contaminated cereals and foodstuffs (e.g., milk, meat and eggs) of animals fed contaminated grains (Bozzo et al., 2009; Giancarlo et al., 2011; Martins et al., 2012; Sorrenti et al., 2013). Inhalation exposure of OTA has been recorded in studies of water-damaged buildings from air (Wang et al., 2008) and wallpaper (Polizzi et al., 2009).

OTA exerts several toxicological effects such as nephrotoxic (Zaied et al., 2011), hepatotoxic (Gagliano et al., 2006), immunotoxic (Al-Anati and Petzinger,

2006), neurotoxic (Zhang et al., 2009), carcinogenic (Schilter et al., 2005) and teratogenic (Benford et al., 2001). It has been related to Balkan endemic nephropathy (Pfohl-Leszkowicz, 2009; Hope and Hope, 2012).

Different methods were developed to counteract the toxicity of contaminated products and improve food safety (Gholampour Azizi et al., 2012). However, the detoxification process is associated with a loss of palatability and nutritional values. One of the nutritional approaches to decrease mycotoxin toxicity is addition of nutrients with protective properties to contaminated foodstuffs (Sorrenti et al., 2013).

Interest in the use of plant extracts that possess widespread biological functions has increased in recent years. Myricetin (MYR; 3,5,7,3',4',5' hexahydroxyflavone) is a major flavonol that is widely distributed in fruits, vegetables, tea, berries, red wine and medical plants (Harnly et al., 2006). The dietary intake of MYR (0.98-1.1 mg per day) is much higher than some other flavonols (Lin et al., 2006). Moreover, it is thought to possess a larger antioxidant property than other flavonoids due to its unique chemical structure (Chobot and Hadacek, 2011).

MYR has antioxidant, anti-inflammatory and potent anticancer effects (Jung et al., 2008; Sun et al., 2012; Weng and Yen, 2012). Several studies confirmed the hypoglycemic and anti-hyperlipidemic effects of MYR (Li and Ding, 2012). Godse et al. (2010) suggested that MYR could prevent the development of hypertension. Effects exerted by MYR may be explained by its ability to reduce ROS production and enhance metabolism of free radicals (Özcan et al., 2012). Moreover, MYR protect against ROS-induced cell death (Wang et al., 2007).

Based on this background, the aim of our experimental study was to investigate the possible alleviative effect of MYR against OTA-induced damage in the renal cortex of adult male albino rats.

Materials and methods

Chemicals

OTA (powder; CAS No. 303-47-9; purity of ≥98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

MYR (crystalline; CAS No. 529-44-2; purity of ≥96%) was purchased from Sigma-Aldrich.

Experimental animals

Thirty adult male albino rats (180-200g weight) were obtained from the Breeding Animal House of the Faculty of Medicine, Zagazig University, Egypt. The animals were housed in plastic cages with stainless steel wire-bar lid at a controlled temperature (23±1°C) and humidity (55±5%) in an artificially illuminated room (12:12 h light: dark cycle), free from any source of chemical contamination. The animals were fed with standard laboratory chow and allowed to access ad libitum feed and drinking water. All rats received humane care in compliance with the guidelines of the Ethical Committee of Zagazig University.

Experimental Protocol

After 1-week acclimatization period, animals were randomly divided into 5 groups (6 rats for group). Each group was housed in a separate cage. All treatments were given by oral gavage for 2 weeks.

Group I (control) served as untreated control group. Group II (vehicle control) received corn oil (0.5 ml/day including the same amount of dimethylsulfoxide [DMSO] as the OTA groups). DMSO and corn oil was used as a vehicle to dissolve OTA. Animals of group III (MYR group) were given MYR (100 mg MYR /kg b.w. /day) dissolved in distilled water (Jayakumar et al., 2014). Group IV (OTA group) received OTA (0.5 mg OTA /kg b.w. /day; dissolved in 10% DMSO and then corn oil; Palabiyik et al., 2013). The dose chosen was 1/40 of LD50 values of OTA for rats (22 mg/kg for males and 20 mg/kg for females; NTP, 1989). Group V (OTA + MYR group) was treated with OTA along with MYR at similar doses.

At the end of the experimental period, (i) animals were sacrificed using anesthesia with ether and (ii) both kidneys were removed; (iii) the right kidneys were frozen immediately in liquid nitrogen; (iv) the renal cortices were divided into pieces and stored at -80°C until the preparation of tissue homogenates for biochemical analysis; (v) the left kidneys were processed for light and electron microscope examinations.

Histological study for Haematoxylin Eosin (H&E) stain

Specimens for light microscopy were fixed in 10% saline formalin and processed to prepare 5-µm-thick paraffin sections for Haematoxylin Eosin (H&E) stain (Kiernan, 2000).

Ultrastructure study

Specimens for electron microscopy were immediately fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7,4), post fixed in 1% osmium tetroxide in the same buffer at 4°C, dehydrated, and embedded in epoxy resin. Ultrathin sections were obtained (Leica ultra-cut UCT), stained with uranyl acetate and lead citrate (Ayache et al., 2010), examined and photographed (JEOL JEM 1010 electron microscope; Jeol Ltd, Tokyo, Japan) in the Histology and Cell Biology Department, Faculty of Medicine, Zagazig University, Egypt.

Immunohistochemical study

Immunohistochemical staining for localization of

proliferating cell nuclear antigen (PCNA), p53 and transforming growth factor beta 1(TGF-β1) was carried out by means of the avidin biotin-peroxidase complex method (Dako ARK™ , Peroxidase, Code No. K3954, Dako, Glostrup, Denmark) following the manufacturer's instructions.

Paraffin sections (4 μm) were dewaxed, hydrated and microwave-treated (0.01M Trisodium citrate). Endogenous peroxidase was eliminated by incubation in 10% H_2O_2 in phosphate-buffered saline (PBS), pH 7.4. After washing, the specimens were blocked in a normal mouse serum at room temperature. Then, the sections were incubated with the specific primary antibody overnight (4°C).

The primary antibodies were specific for PCNA (mouse monoclonal antibody; Clone PC-10; Code No. M0879; diluted 1:200, Dako), p53 (mouse monoclonal antibody; Clone: DO-7; Code No. M 7001; diluted 1:25; Dako) and TGF-β1 (rabbit polyclonal antibody; No. AV37156; diluted 1:50; Sigma-Aldrich). The sections were incubated with biotinylated secondary antibodies followed by Streptavidin conjugated-horseradish peroxidase. Staining was completed by incubation with 3,3'-diaminobenzidine (DAB), substrate-chromogen that resulted in a brown-colored precipitate at the antigen site. Sections were counterstained with haematoxylin. Omission of the primary antibodies served as negative controls. Stained slides were analyzed by light microscopy (Ramos-Vara et al., 2008).

Biochemical analysis of tissue antioxidant enzymes

Renal cortex homogenates were prepared in cold ice 0,1M Tris-HCl buffer (pH 7.4) by a Teflon pestle homogenizer to obtain 10% (w/v) whole homogenate. The homogenate was centrifuged at 8000×g for 30 min at 4°C to remove the cell debris. The antioxidant enzymes were determined in the supernatant using commercial kits (Bio Diagnostic Company, Dokki, Giza, Egypt). All enzymatic activities were calculated as U/mg protein.

Glutathione peroxidase (GPX)

GPX activity was determined according to Flohe and Gunzler (1984). One unit of enzyme was defined as the amount of GPX that transformed 1 μmol of NADPH to NADP + / min at 37°C.

Catalase (CAT)

CAT activity was measured as described earlier by Aebi (1974). One unit of CAT activity was represented by the decomposition of 1 μmol H_2O_2/min/ml.

Superoxide dismutase (SOD)

SOD activity was assessed as described by Paoletti and Mocali (1990). One unit of SOD activity was equal to the amount of enzyme that inhibits the oxidation of NADH by 50% at 37°C.

Morphometrical study

The Leica QWin 500 image analyzer (Leica Ltd, Cambridge, UK) was used. Positive cells in anti-p53 and anti-PCNA immune stained sections were counted. In addition, the area percentage of positive TGF-β1 immune reactivity in anti-TGF-β1 immune stained sections was measured. All measurements were taken at 400 magnifications in ten non-overlapping fields from five sections for each rat in each group using the interactive measure menu.

Statistical analysis

SPSS statistical software version 20 for Windows was used to evaluate the data. Values were expressed as means ± standard error of means (SEM). Data were statistically analyzed by using ANOVA test and Tukey test; for post hoc multiple comparisons. Rejection of the null hypothesis was set at $P<0.05$.

Results

Histopathological results

The light and electron microscopic examinations of control, vehicle control and myricetin (MYR) groups revealed similar results, consequently, only morphological results of the control group will be presented.

Histological results of H&E-stain

Light microscope examination of renal cortex of control group revealed normal renal corpuscles with glomeruli, Bowman's capsules lined by simple squamous epithelium and narrow Bowman's space. Proximal convoluted tubules (PCT) had eosinophilic cuboidal epithelium and narrow lumen, whereas distal convoluted tubules (DCT) had wide lumen (Fig. 1a).

Examination of OTA group showed distorted and dilated tubules. Some were lined by flat cells. Others had hyaline casts or exfoliated cells in their lumen. Some tubular cells had dark-stained pyknotic nuclei or vacuolated cytoplasm. Mitotic figures were also present. The interstitium showed many inflammatory cellular infiltrations, hemorrhages and dilated congested peritubular capillaries. The renal corpuscles revealed thick glomerular basement membrane. Some glomeruli appeared segmented, atrophied or enlarged with hyper cellularity and dense nuclei of extra mesangial cells. The Bowman's spaces of some glomeruli were narrow but others were wide and Eosinophilic exudate was also present (Fig. 1b-g).

OTA + MYR group showed partial alleviation with mild degeneration in the PCT and nearly normal

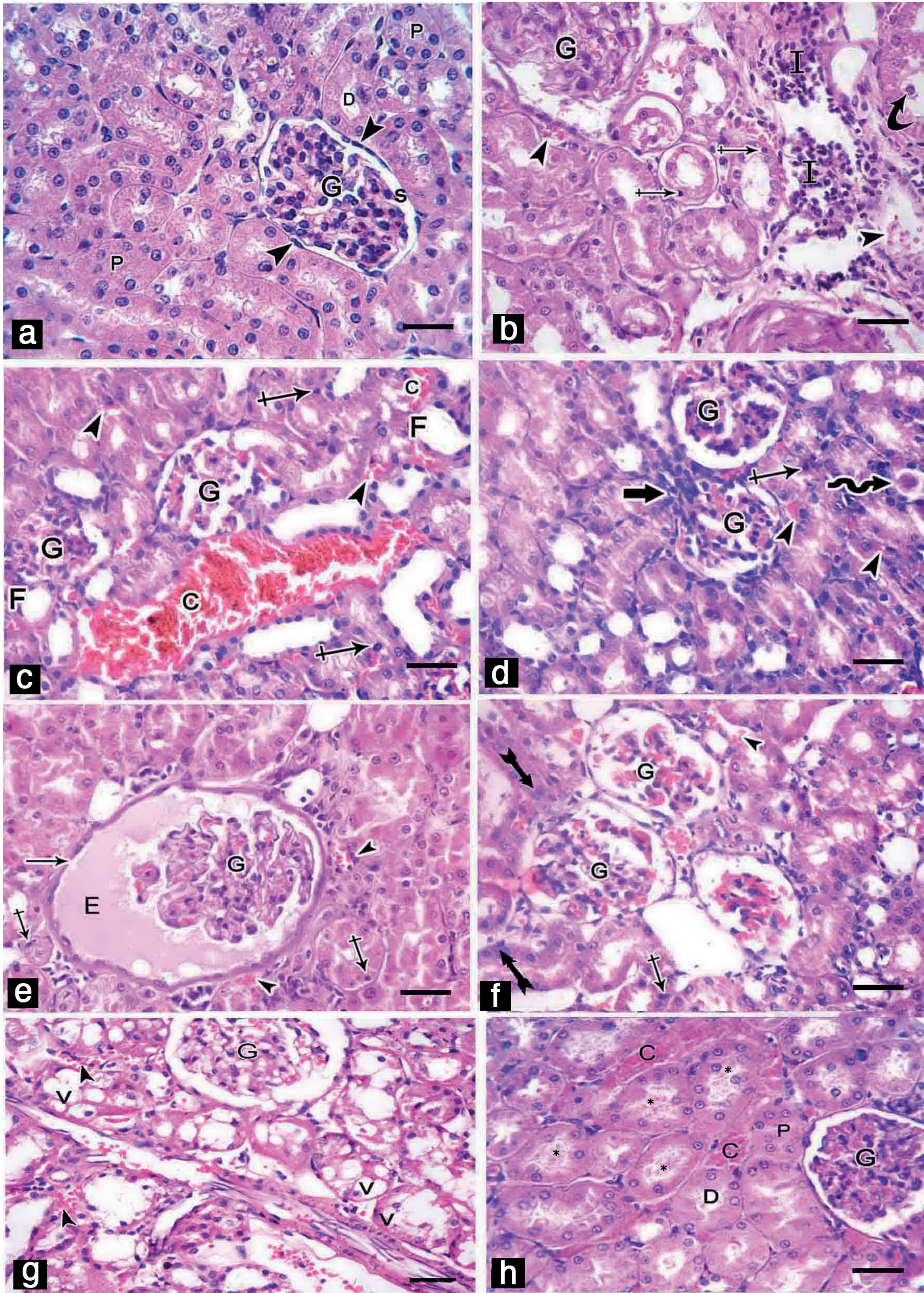

Fig. 1. A photomicrograph of sections in the renal cortex of adult male rats of study groups. **a.** Control group. Normal glomeruli (G), Bowman's capsules are lined by simple squamous cells (arrow heads), narrow Bowman's space (s), Proximal convoluted tubules PCT (P), distal convoluted tubules DCT (D). **b-g.** OTA group. Tubular cells with dark-stained pyknotic nuclei (crossed arrows), interstitial hemorrhage (arrow heads), many inflammatory cellular infiltrations (I), distorted tubules with exfoliated cells (curved arrows), dilated congested peritubular capillaries (C), distorted glomeruli (G) with narrow or wide Bowman's space, dilated tubules lined by flat cells (F) with dark nuclei, hypercellularity and dense nuclei of extramesangial cells (thick arrows), distorted tubules with hyaline casts (wavy arrows) in the lumen, thick basement membrane of Bowman's capsule (arrows), segmented glomerulus with eosinophilic exudates (E) in the Bowman's space, tubular cells with mitotic figure (bifid arrows), tubular cytoplasmic vacuolations (v). **h.** OTA + MYR group. Nearly normal glomeruli (G), mild degeneration in PCT (P), DCT (D), with debris in the tubular lumen (*), slightly congested peritubular capillaries (C). Scale bar: 50 μm.

structure of the glomeruli. Debris in the lumen and slightly congested peritubular capillaries were still noticed (Fig. 1h).

Immunohistochemical results

Immunohistochemically stained sections for p53 of control group showed faint nuclear reaction in few PCT and DCT tubular cells, while the glomerular cells appeared negative (Fig. 2a). OTA group revealed strong positive nuclear reaction in many tubular cells (Fig. 2b). The sections of OTA + MYR group showed faint cytoplasmic reaction in few tubular cells (Fig. 2c).

The sections stained for proliferating cell nuclear antigen (PCNA) antibodies showed negative immune reaction in glomerular, PCT and DCT cells in the control group (Fig. 2d). OTA group sections showed strong positive nuclear reaction in many glomerular cells and some tubular cells (Fig. 2e). OTA + MYR group revealed nuclear reaction in few glomerular cells and negative immunoreaction in PCT and DCT cells (Fig. 2f).

Immunohistochemically stained sections for transforming growth factor beta 1 (TGF-β1) antibodies in the renal cortex of control group showed negative cytoplasmic reaction in glomerular, PCT and DCT cells (Fig. 2g). The sections of OTA group revealed strong positive reaction in the cytoplasm of PCT and DCT cells and some glomerular cells (Fig. 2h). OTA + MYR group stained sections showed negative reaction in glomerular, PCT and DCT cells (Fig. 2i).

Ultrastructure results

Electron microscope examination of sections in the renal cortex of adult male rats of control group showed

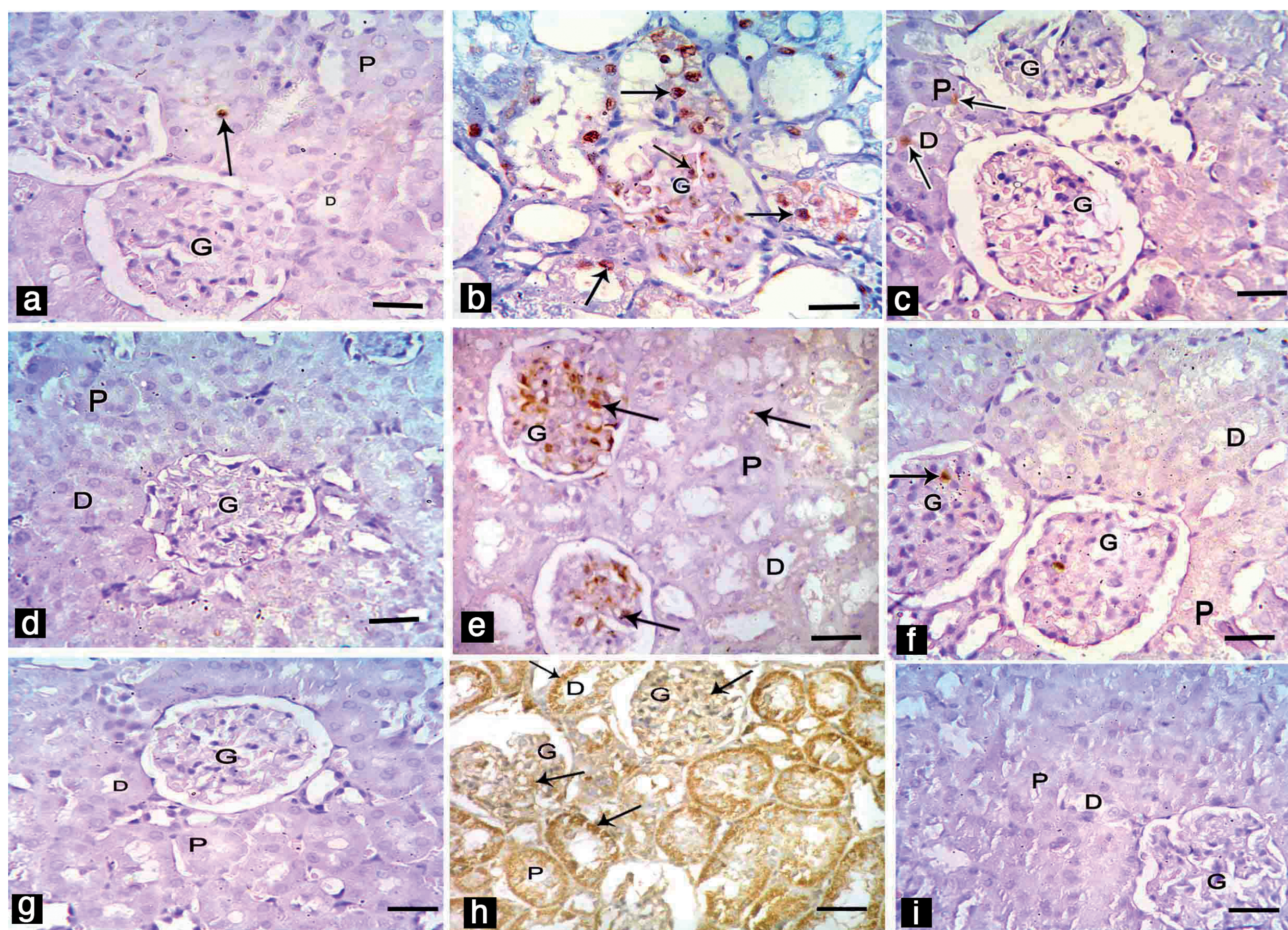

Fig. 2. Immunohistochemically stained sections in the renal cortex of albino rats of different groups. Glomerulus (G), PCT (P), DCT (D), positive immune reaction (arrows). **a-c.** Nuclear immune reaction for p53. **a.** Control group. **b.** OTA group. **c.** OTA + MYR group. **d-f.** Nuclear immune reaction for PCNA. **d.** Control group. **e.** OTA group. **f.** OTA + MYR group. **g-i.** Cytoplasmic immune reaction for TGF-β1. **g.** Control group. **h.** OTA group. **i.** OTA + MYR group. Scale bar: 50 μm.

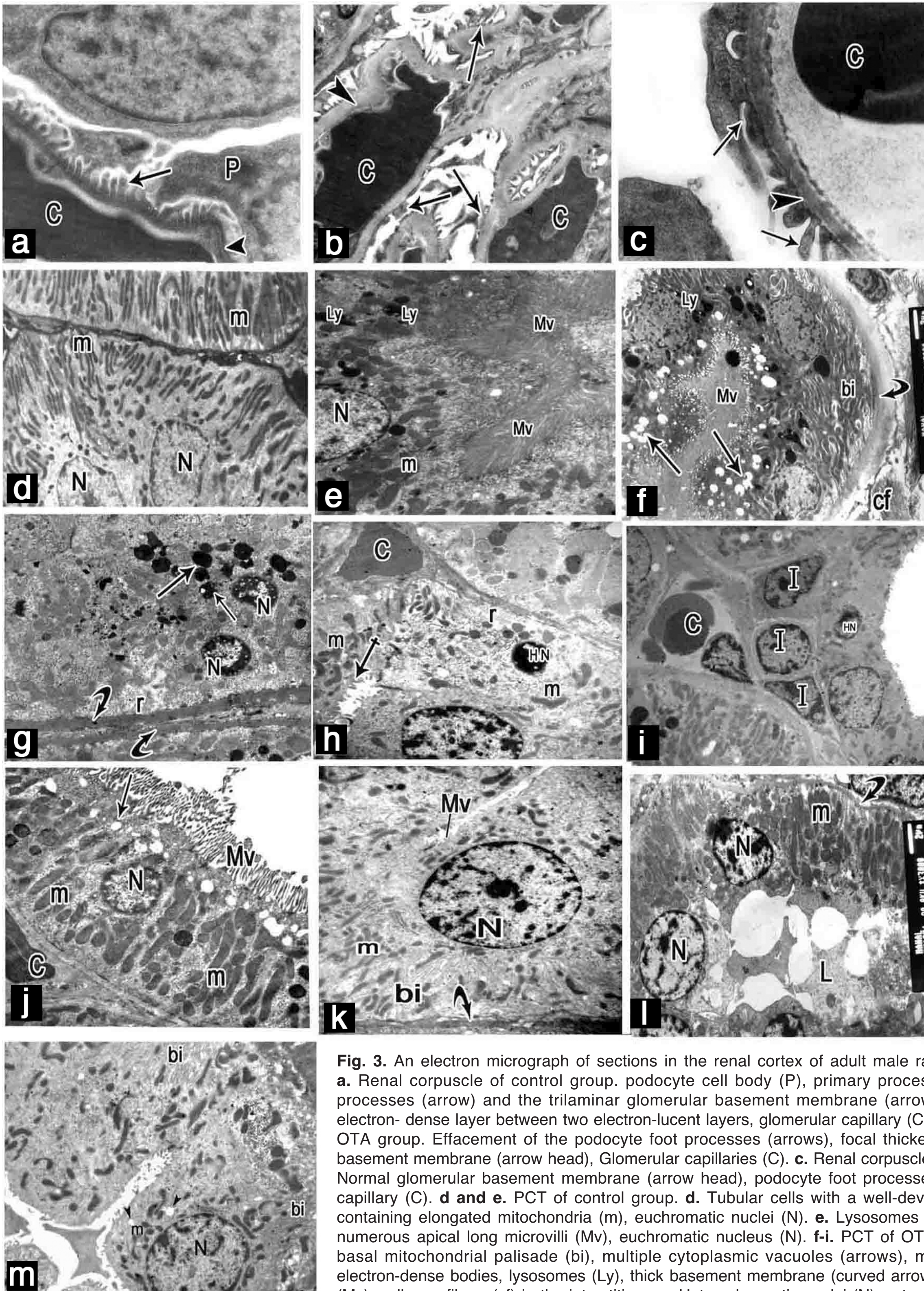

Fig. 3. An electron micrograph of sections in the renal cortex of adult male rats of all study groups. **a.** Renal corpuscle of control group. podocyte cell body (P), primary process, numerous thin foot processes (arrow) and the trilaminar glomerular basement membrane (arrow head) with a central electron- dense layer between two electron-lucent layers, glomerular capillary (C). **b.** Renal corpuscle of OTA group. Effacement of the podocyte foot processes (arrows), focal thickening of the glomerular basement membrane (arrow head), Glomerular capillaries (C). **c.** Renal corpuscle of OTA + MYR group. Normal glomerular basement membrane (arrow head), podocyte foot processes (arrows), glomerular capillary (C). **d and e.** PCT of control group. **d.** Tubular cells with a well-developed basal enfolding containing elongated mitochondria (m), euchromatic nuclei (N). **e.** Lysosomes (Ly), mitochondria (m), numerous apical long microvilli (Mv), euchromatic nucleus (N). **f-i.** PCT of OTA group. **f.** Disoriented basal mitochondrial palisade (bi), multiple cytoplasmic vacuoles (arrows), multiple heterogeneous electron-dense bodies, lysosomes (Ly), thick basement membrane (curved arrows), destroyed microvilli (Mv), collagen fibers (cf) in the interstitium. **g.** Heterochromatic nuclei (N), cytoplasm with some rarified areas (r), many electron dense bodies (arrows), thick basement membrane (curved arrows). **h.** Partial loss of brush border (crossed arrows), disorganized, bizarre shaped mitochondria (m), area of rarified cytoplasm (r), heterochromatic nucleus (HN), dilated blood capillary (C). **i.** Mononuclear inflammatory cells (I) close to the blood capillary (C) in between renal tubules, heterochromatic tubular cells nuclei (HN). **j.** PCT of OTA + MYR group with long apical microvilli (Mv), multiple elongated mitochondria (m), euchromatic nucleus (N), minimal apical cytoplasmic vacuolations (arrows), blood capillary (C). **k.** DCT of the control group with elongated mitochondria (m), basal enfolding (bi), few short apical microvilli (Mv), euchromatic nucleus (N), regular basement membrane (curved arrows). **l.** DCT of OTA group with euchromatic nuclei (N), mitochondria (m), irregular basement membrane (curved arrows) and casts in the tubular lumen (L). **m.** DCT of OTA + MYR group with euchromatic nuclei (N), preserved basal enfoldings (bi), mitochondria (m), cytoplasmic vacuolization (arrowheads). a, x 10,000; b, x 6,000; c, x 20,000; d, x 3,500; e, i, m, x 4,000; f, j, k, l, x 3,000; g, x 4,500; h, x 6,000

normal renal corpuscles with podocyte bodies, primary processes, numerous thin foot processes and the trilaminar glomerular basement membrane with a central electron-dense layer between two electron-lucent layers. Glomerular capillaries were also present (Fig. 3a). PCT cells revealed euchromatic nuclei and well developed basal enfolding with elongated mitochondria. Lysosomes and numerous apical long microvilli forming the brush border were observed (Fig. 3d,e). DCT cells were seen with euchromatic nuclei, elongated mitochondria, basal enfolding, few short apical microvilli, and regular basement membrane (Fig. 3k).

Examination of the ultrathin sections of OTA group showed glomeruli with effacement of the podocyte foot processes and focal thickening of the glomerular basement membrane (Fig. 3b). PCT cells rested on thick basement membrane and their cytoplasm exhibited many electron dense bodies, multiple cytoplasmic vacuoles, multiple heterogeneous electron-dense bodies and lysosomes and areas of rarified cytoplasm. Disoriented basal mitochondrial palisade with disorganized, bizarre shaped mitochondria, partial loss of brush border and heterochromatic nuclei were observed. Dilated blood capillaries were noticed in between renal tubules with mononuclear inflammatory cells infiltration (Fig. 3f-i). DCT cells appeared with irregular basement membrane. The lumen contained casts (Fig. 3L).

The ultrathin sections of OTA + MYR group showed glomeruli with normal appearance of the trilaminar glomerular basement membrane and podocyte foot processes (Fig. 3c). PCT appeared with long apical microvilli, multiple elongated mitochondria, euchromatic nuclei and minimal apical cytoplasmic vacuolization (Fig. 3j). DCT had euchromatic nuclei and preserved basal infoldings and mitochondria. Some cytoplasmatic vacuolizations were seen (Fig. 3m).

Table 1. Glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) activity in different groups.

	Control	Control (vehicle)	MYR	OTA	OTA+MYR
GPX	0.95±0.05	0.9±0.0.02	1.05±0.08	0.52±0.03**[a]	0.88±0.04*[b]
CAT	0.55±0.02	0.53±0.02	0.63±0.03	0.29±0.02**[a]	0.48±0.02*[b]
SOD	26.83±1.5	27.67±1.82	32.17±2.02**[a]	8.83±1.18**[a]	25.83±1.26**[b]

MYR, myricetin; OTA, ochratoxin. Values are expressed as mean± standard error of means (SEM) of n=6 animals; [a]: P value compared with control group; [b]: P value compared with OTA group; *: significant difference (P<0.05); **: highly significant difference (P<0.001).

Table 2. Number of anti-p53 and anti- PCNA immunostained cells and the area percentage of positive anti-TGF-β1 immunoreactivity in different groups.

	Control	Control (vehicle)	MYR	OTA	OTA+MYR
p53	2±0.89	2.33±1.86	1.66±1.21	21.66±4.32**[a]	4.66±1.75**[b]
PCNA	2.5±0.62	1.83±0.54	2.66±0.84	16.17±1.35**[a]	5.83±.79**[b]
TGF-β1	3.83±1.01	3.67±1.23	3±1.03	37.33±1.63**[a]	6.83±1.25**[b]

MYR, myricetin; OTA, ochratoxin. Values are expressed as mean± standard error of means (SEM) of n=6 animals; [a]: P value compared with control group; [b]: P value compared with OTA group; *: significant difference (P<0.05); **: highly significant difference (P<0.001).

Biochemical results

Assessment of the activities of GPX, CAT and SOD revealed a non-significant difference between groups: vehicle control and MYR compared with control group. Whereas there was a highly significant decrease in OTA group compared with control. The MYR + OTA group showed a significant increase in GPX and CAT assay and a highly significant increase in SOD assay compared with OTA group. Further, there was a non-significant decrease compared with control (Table 1).

Morphometric results

The number of anti-p53 and anti- PCNA immune stained cells and the area percentage of positive anti-TGF-β1 immune reactivity showed a non-significant difference between vehicle control, MYR and control group. There was highly significant increase in OTA group compared with the control, but MYR+OTA group showed a highly significant decrease compared with OTA group and a non-significant increase compared with control (Table 2).

Discussion

The kidney is the main target organ for ochratoxin (OTA) toxic effects (Gekle and Silbernagl, 1996). The high sensitivity of kidneys could be attributed to the toxicokinetics of OTA (Petrik et al., 2003). OTA renal toxicity, lowest observable effect level (LOEL) is 8 μg/kg b.w. Based on this assessment, the tolerable weekly intake of OTA is 120 ng/kg b.w. (EFSA, 2010).

The present study showed that cortical tubules revealed dilatation and distortion with exfoliation of their lining cells. These findings were in consensus with earlier reports (Sutken et al., 2007; Yenilmez et al., 2010). Our ultrastructure examination of the cortical tubules showed degenerated mitochondria, multiple cytoplasmic vacuoles, secondary lysosomes, apoptotic nuclei, in addition to disoriented basal mitochondrial palisade and partial loss of brush border in the proximal convoluted tubules (PCT). Similar results were observed by Solcan et al. (2013). Kumar et al. (2007) attributed these changes to increase in membrane fluidity and permeability with degeneration of organelles. Highly destructive hydroxyl radicals attack cellular fatty acids causing changes in the cell membrane and many subcellular structures (Gautier et al., 2001).

Mononuclear cell infiltration was recorded in this

work (Aydin et al., 2003; Palabiyik et al., 2013). OTA causes an increase in inflammatory mediators and fibrosis, as well as loss of epithelial tightness (Sauvant et al., 2005). In agreement with Malekinejad et al. (2011), the findings of our study showed congested blood vessels and peritubular hemorrhage in animals exposed to OTA. This might be explained by hepatotoxic OTA effect on genes encoding for coagulation factors (Hundhausen et al., 2008).

Regarding the renal corpuscles in the current work, variable degrees of damage were found in OTA treated rats, including distortion, segmentation, enlargement and atrophy. Our results confirmed and extended the data obtained from previous studies (Abdu et al., 2011). The ultrastructure evaluation showed podocytes effacement of the foot processes and this lesion was explained by interference with structural components of the slit diaphragm, interference with the actin-cytoskeleton or interference with podocyte-glomerular basement membrane interaction (Mundel and Shankland, 2002). Moreover, irregular thickening of the tubular and glomerular basement membrane, as detected by electron microscope, could be related to increased deposition of glycoproteins (Hotta et al., 2001).

The glomerular enlargement observed in our study could be attributed to proliferation of endothelial and mesangial cells with mesangial matrix expansion (Abdu et al., 2011). In the same context, we recorded mitotic figures in tubular cells of OTA treated rats. The enhanced proliferation of glomerular and tubular cells was evident by the increased number of positive immunoreactions for proliferating cell nuclear antigen (PCNA). Our results were in consistence with Mally et al. (2005) who reported that OTA induced prominent karyomegaly and polyploidy. The previous authors added that mitotic figures were increased in the affected tubules by about 6-fold. OTA is a proven carcinogen in animals and is classified as a class 2B, possible human carcinogen by the International Agency for Research on Cancer (Reddy and Bhoola, 2010). In contrast, we determined an increase in the number of apoptotic cells in the renal cortex by OTA administration. This was confirmed by immunohistochemical expression of p53 protein which was significantly up regulated compared with the controls. In agreement with our results, previous studies showed that OTA exposure activates apoptotic processes in kidney cells (Gekle et al., 2000; Rached et al., 2006). Using the TUNEL staining method, higher incidences of apoptotic bodies were observed (Yenilmez et al., 2010).

It was reported that OTA treatment caused apoptosis and pre-neoplastic lesions in rat kidney (Stemmer et al., 2009). OTA may disrupt mitotic and apoptotic signaling pathways through perturbation of cytoskeletal organization or loss of cell adhesion (Scibelli et al., 2003; Gennari et al., 2004). Stemmer et al. (2009) added that cell death by apoptosis may stimulate cell proliferation to compensate cell loss similar to what occurs in response to tubular cell necrosis.

In the current experimental study, strong expression of transforming growth factor beta-1 (TGF-β1) was observed in OTA exposed rats. Similar findings were reported by Hermenean et al. (2013) after carbon tetrachloride treatment in mouse kidney.

The authors reported that TGF-β1 protein is localized within the cytoplasm of mesangial cells of the glomeruli and tubule interstitial areas. TGF-β1 is one of the most fibrogenic growth factors implicated in the pathogenesis of renal fibrosis (Yamamoto et al., 1994; Goumenos et al., 2001). TGF-β1 is able to activate interstitial fibroblasts, induce apoptosis and mediate the differentiation of tubular epithelial cells into myofibroblasts (Shimizu and Yamanaka et al., 1993; Fan et al., 1999). The extent of TGF-β1 expression is related to the degree of tubule interstitial injury (Goumenos et al., 2002).

In agreement with O'Brien and Dietrich (2005), OTA has been called *the continuing enigma*. The authors declared that the primary toxic OTA mechanism is still controversial whether it is of a genotoxic or epigenetic nature (such as induced cytotoxicity or increased cell proliferation). However, both have been related to oxidative cell damage (Gautier et al., 2001; Schaaf et al., 2002). The production of oxygen radicals is likely to produce macromolecular damage including lipid peroxidation and DNA-damage (Petrik et al., 2003).
In the same respect, the current study showed a highly significant decrease in enzymatic antioxidants (glutathione peroxidase [GPX], catalase [CAT] and superoxide dismutase [SOD]) in OTA exposed rats compared with control. In line with our observations, other researchers suggested that OTA caused an imbalance between oxidant/antioxidant parameters in both rat kidney and liver (Sutken et al., 2007; Marin-Kuan et al., 2011; Palabiyik et al., 2012).

Several studies illustrated that antioxidants were able to counteract the adverse effects of OTA exposure (Sorrenti et al., 2013). MYR is a major flavonol found in edible plants and is one of the most potent antioxidants of plant origin (Erdman et al., 2007). Our results indicated an overall improvement of the structural and ultrastructural changes of the renal cortex upon co-administration of MRY. Examination of the renal cortex of MRY + OTA group revealed mild degenerative changes in PCT. Immunohistochemical expression of p53 protein, PCNA and TGF-β1 were significantly down regulated compared with OTA group. In agreement with our findings, several studies recorded the protective effect of MYR against reactive oxygen species (ROS) - induced DNA damage and apoptosis which was mainly due to its capacity to increase Bcl-2/Bax ratio and inhibit the activation of caspase-3 (Aherne and O'Brien, 1999; Wang et al., 2007).

In the present study, MYR effectively counteracted the OTA-induced oxidative stress by conserving the tissue antioxidant enzymes. MYR co-administration provided a significant increase in GPX, CAT and SOD activities compared to OTA group. Similarly, Petrik et

al., (2006) reported that "flavonoids possessed scavenging activity against free radicals, but to different degrees. The maximum antiradical activity was recorded for MYR and quercetin". MYR has strong anti-oxidative properties due to a large number of active hydroxyl groups that can scavenge ROS (Ding et al., 2012). Further, direct anti-oxidative effects are executed by inhibiting the formation of hydroxyl radicals through chelation of redox-active metal ions (Chobot and Hadacek, 2011).

In conclusion, OTA induced renal injury as evidenced by histopathological and immunohistochemical changes. Alterations in antioxidant parameters supported that oxidative stress was one of the mechanisms involved in OTA toxicity. On the contrary, co-administration of MYR partially ameliorated these changes. We suggest the potential effectiveness of MYR to counteract OTA-induced toxic oxidative stress on the renal cortex.

Conflicts of interest. There are no conflicts of interest.

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