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Protective effect of lycopene against ochratoxin A induced renal oxidative stress and apoptosis in rats

S. Sezin Palabiyik[a,b], Pinar Erkekoglu[a], N. Dilara Zeybek[c], Murat Kizilgun[d], Dilek Ertoy Baydar[e], Gonul Sahin[a,f], Belma Kocer Giray[a,*]

[a] Hacettepe University, Faculty of Pharmacy, Department of Toxicology, 06100 Ankara, Turkey
[b] Ataturk University, Faculty of Pharmacy, Department of Toxicology, 25240 Erzurum, Turkey
[c] Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, 06100 Ankara, Turkey
[d] Department of Biochemistry, Diskapi Children's Health and Diseases, Hematology, Oncology Training and Research Hospital, 06100 Ankara, Turkey
[e] Hacettepe University, Faculty of Medicine, Department of Pathology, 06100 Ankara, Turkey
[f] Eastern Mediterranean University, Faculty of Pharmacy, Department of Toxicology, 10 Mersin, Turkey

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ABSTRACT

This study was designed to investigate the possible protective effect of lycopene against the renal toxic effects of OTA. Male Sprague-Dawley rats (<200 g, $n=6$) were treated with OTA (0.5 mg/kg/day) and/or lycopene (5 mg/kg/day) by gavage for 14 days. Histopathological examinations were performed and apoptotic cell death in both cortex and medulla was evaluated by TUNEL assay. Besides, biochemical parameters and activities of renal antioxidant selenoenzymes [glutathione peroxidase 1 (GPx1), thioredoxin reductase (TrxR)], catalase (CAT), superoxide dismutase (SOD); concentrations of total glutathione (GSH), and malondialdehyde (MDA) levels were measured. OTA treatment was found to induce oxidative stress in rat kidney, as evidenced by marked decreases in CAT (35%) activity and GSH levels (44%) as well as increase in SOD activity (22%) vs control group. Furthermore, TUNEL analysis revealed a significant increase in the number of TUNEL-positive cells in cortex (49%) and medulla (75%) in OTA administrated group compared to control ($p<0.05$). Lycopene supplementation with OTA increased GPx1 activity and GSH levels, and decreased apoptotic cell death in both cortex and medulla vs. control. The results of this study showed that at least one of the mechanisms underlying the renal toxicity of OTA is oxidative stress and apoptosis is the major form of cell death caused by OTA. Besides, our data indicate that the natural antioxidant lycopene might be partially protective against OTA-induced nephrotoxicity and oxidative stress in rat.

1. Introduction

Ochratoxins are toxic fungal toxins that are produced by several species of *Aspergillus* and *Penicillium* families as secondary metabolites. Ochratoxin A (OTA), a potent nephrotoxin, is one of the most abundant mycotoxins. It is present in wide variety of foods (grains, wine, dried grapes, coffee) due to improper storage conditions (EFSA, 2006; WHO, 2001) and can also accumulate in the meat of animals, human blood and breast milk (EFSA, 2006; Erkekoglu et al., 2010; WHO, 2001).

OTA was shown to induce renal cell adenomas and carcinomas in both sexes of rats and mice (Cavin et al., 2009; Marin-Kuan et al., 2011). Besides, OTA also has hepatotoxic, immunotoxic, mutagenic and genotoxic properties (EFSA, 2006; WHO, 2001). It has been demonstrated that OTA inhibits protein synthesis in hepatoma tissue cultured cells (Renzulli et al., 2004), disturbs mitochondrial respiration in isolated rat liver mitochondria (Ringot et al., 2006) and causes increase of lipid peroxidation (LP) in different tissues of rodents (Mally et al., 2005; Palabiyik et al., 2012). OTA exposure is also associated with Balkan endemic nephropathy (BEN) and with the etiology of human urinary tract tumors (Cavin et al., 2009; Stefanović and Polenaković, 2009) although a clear mechanism of

Abbreviations: $^1O_2$, singlet oxygen; BEN, Balkan endemic nephropathy; BUN, blood urea nitrogen; CAT, catalase; DTNB, 5,5′-dithiobis-(2-nitrobenzoic) acid; DTPA, diethylenetriamine pentaacetic acid; GPx1, glutathione peroxidase 1; GR, glutathione reductase; GSH, total glutathione; GSSG, oxidized glutathione; HPLC, high performance liquid chromatography; LP, lipid peroxidation; MDA, malondialdehyde; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; $NADP^+$, nicotinamide adenine dinucleotide phosphate; OTA, ochratoxin A; PMSF, phenylmethanesulphonyl fluoride; ROS, reactive oxygen species; SD, Sprague Dawley; SEM, standard error of mean; SOD, superoxide dismutase; SPSS, Statistical Package for Social Sciences Program; TNB, 5-thio-2-nitrobenzoic acid; TrxR, thioredoxin reductase; TdT, terminal deoxynucleotidyl transferase; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling.

* Corresponding author. Tel.: +90 3123092958; fax: +90 3123092958.
E-mail addresses: bgiray@hacettepe.edu.tr, belmagiray@yahoo.fr (B.K. Giray).

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carcinogenicity of OTA is not clearly demonstrated. OTA is classified as Group 2B carcinogen (possible carcinogenic to humans) by The International Agency for Research on Cancer (IARC) with sufficient carcinogenicity evidence in experimental animals (IARC, 1993).

High levels of reactive oxygen species (ROS) are shown to contribute to protein, lipid or DNA damage causing cellular injury, apoptosis and cancer (Marin-Kuan et al., 2011). Currently, there is a growing interest in natural toxins that can cause ROS production (Manda et al., 2009; Petrik et al., 2003; Soory, 2009). It is not yet prominent whether the renal toxicity of OTA is related to its potential to produce oxidative stress and to its effects on cellular antioxidant system. Both carcinogenicity and cytotoxicity of OTA have been linked to free radical-mediated oxidative cell damage *in vivo* and *in vitro* (Gautier et al., 2001; Marin-Kuan et al., 2011; Schaaf et al., 2002). Moreover, the doses and application periods of those studies differ from each other and chronic and acute effects seem to be discrete (Rached et al., 2007; Yenilmez et al., 2010; Hibi et al., 2011; Meki and Hussein, 2001).

Apoptosis was suggested to be involved in the etiology of BEN. OTA was shown to cause apoptosis in kidney cell lines and in rat kidneys whereas necrosis plays only a minor role in its toxicity (Petrik et al., 2003; Schwerdt et al., 1999). Primary lesions of proximal tubules, followed by spontaneous damage of glomeruli and involution of the interstitia are usually related to the chronic effects of OTA (Yenilmez et al., 2010).

Supplementation of antioxidants was shown to reduce oxidative damage caused by aflatoxins (AFs) and OTA (Rauscher et al., 1998; Tang et al., 2007). Lycopene, the most prevalent carotenoid in the western diet, is majorly present in tomato. The consumption of tomatoes and/or tomato products is associated with increased lycopene blood levels and reduced oxidative damages of lipids, proteins, and DNA (Rao and Agarwal, 1998). Lycopene has been suggested to have strong antioxidant potency in vitro, almost being 100 times more efficient in quenching singlet oxygen ($^1O_2$) than vitamin E (Mordente et al., 2011). Lycopene may act as a chemopreventive agent against certain types of cancers (i.e. cancers of prostate, stomach, breast and lung) and was found to be protective against chemotherapeutic-induced renal damage in several studies (Atessahin et al., 2005; Dogukan et al., 2011; Mein et al., 2008; Wang et al., 2010).

Based on this background and taking into account the frequency of high OTA exposure, this study was designed to investigate the possible protective effect of lycopene against the oxidative stress caused by OTA in rat kidney.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). Lycopene was a gift from Micro-Gen (Ankara, Turkey). Colorimetric assay kit for thioredoxin reductase (TrxR) was also from Sigma–Aldrich. Entellan, Cell-Death Detection Kit (AP), terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) kit, TUNEL dilution buffer and fast red tablets were obtained from Roche Applied Science (Roche; Mannheim, Germany).

2.2. Animals and treatment

Male Sprague-Dawley (SD) rats (<200 g), supplied from Hacettepe University Experimental Animals Laboratory, were used in the experiments. Animals were divided randomly in four groups, and each group was housed in plastic cages with stainless-steel grid tops. The cages were placed in a room with controlled temperature (23 °C), humidity (50%) and a 12-h light–dark cycle. Animals were fed with standard laboratory chow and allowed to access ad libitum feed and drinking water. The dose chosen for OTA was 1/40 of LD_{50} of OTA (JECFA, 2001). Body weights were monitored throughout the experiments including before the first dose of OTA treatment. The animals were treated humanely and with regard for alleviation of suffering, and the study was approved by Hacettepe University Ethical Committee.

2.3. Experimental groups

The study consisted of four groups with six animals each: Control group (Control) received a corresponding amount of corn oil (including 10% DMSO) for 14 days by intragastric lavage (i.g.); Lycopene Group (L) received 5 mg/kg bw lycopene (dissolved in 10% DMSO and then scaled to required volume with corn oil) for 14 days by i.g.; OTA group (OTA) received 0.5 mg/kg bw OTA (dissolved in 10% DMSO and then scaled to required volume with corn oil) for 14 days by i.g.; OTA plus Lycopene group (OTA-L) received 0.5 mg/kg bw OTA and 0.5 mg/kg bw lycopene for 14 days by i.g.

Twenty-four hours after the last dose of OTA treatment or vehicle administration, animals were weighed, and sacrificed by decapitation under thiopental anesthesia. Venous blood samples were taken in heparinized tubes and both kidneys were removed. Right kidneys were frozen immediately in liquid nitrogen, divided into pieces and stored at −80 °C until the preparation of tissue homogenates. Left kidneys were put into 10% formalin for overnight fixation to perform histopathological evaluation and staining for apoptosis.

2.4. Preparation of plasma samples

Venous blood samples were centrifuged at 800 × *g* for 10 min. Plasma was separated and used for the measurement of plasma biochemical parameters.

2.5. Biochemical parameters

Blood urea nitrogen (BUN), creatinine, Ca^{2+}, Na^+, K^+ and Cl^- levels were measured by an automatic analyzer (Perkin Elmer, Waltham, MA, USA).

2.6. Histopathological examination

After overnight fixation in formalin, the left kidney from each animal was processed for routine light microscopy. Paraffin blocks were prepared and 5 μm thick sections were obtained from each block. Routine hematoxylin and eosin method was used to stain slides. The sections were examined for the determination of morphological alterations using Leica® DM6000B (Wetzlar, Germany) microscope.

2.7. Preparation of kidney homogenates

Kidney homogenates were prepared in a volume of ice-cold buffer containing Tris (10 mM), diethylenetriamine pentaacetic acid (DTPA, 1 mM), and phenylmethanesulphonyl fluoride (PMSF, 1 mM; adjusted to pH 7.4) using a Teflon pestle homogenizer to obtain 10% (w/v) whole homogenate. After centrifugation at 1500 × *g*, 4 °C, for 10 min, malondialdehyde (MDA) concentration was measured in the supernatant. The rest of the supernatants were recentrifugated at 9500 × *g*, 4 °C for 20 min, and the antioxidant enzyme activities were determined in the supernatant. For the measurement of total GSH (oxidized glutathione [GSSG] plus reduced GSH) levels, the whole homogenate was diluted (5:1) with

metaphosphoric acid (6%), centrifuged at 1500 × g, 4 °C, for 10 min, and the supernatant was used.

2.8. Determination of antioxidant enzyme activities

The activity of glutathione peroxidase (GPx1) was measured in a coupled reaction with glutathione reductase (GR) as described earlier (Gunzler et al., 1974; Flohe and Gunzler, 1984). One unit of enzyme was defined as the amount of GPx1 that transformed 1 μmol of NADPH to $NADP^+$ per min at 37 °C.

TrxR activity was determined colorimetrically using the Thioredoxin Reductase Assay kit. As described previously, the method was based on the reduction of 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) with NADPH into 5-thio-2-nitrobenzoic acid (TNB) the concentration of which was measured at 412 nm (Arner et al., 1999). One unit of TrxR activity was defined as the amount of enzyme that caused an increase in absorbance of 1.0 per min and per ml at pH 7.0 at 25 °C.

Catalase (CAT) activity was determined according to Aebi (1974) (Aebi, 1974). The enzymatic decomposition of H_2O_2 was followed directly at 240 nm. One unit of CAT activity was defined as the amount of enzyme required to decompose 1 μmol H_2O_2 in one min.

The total superoxide dismutase (total SOD) activity was determined by monitoring the auto-oxidation of pyrogallol at 420 nm (Marklund and Marklund, 1974). One unit of total SOD activity was defined as the amount of enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%.

2.9. Determination of total glutathione levels

Kidney GSH content was assessed by a kinetic assay in which catalytic amounts of GSH caused a continuous reduction of DTNB to TNB at 412 nm (Akerboom and Sies, 1981). Quantification was achieved by parallel measurements of a standard curve of known GSH concentrations, and results were expressed in nmol/mg protein.

2.10. Determination of lipid peroxidation

As an indicator of LP, MDA levels were determined by high performance liquid chromatography (HPLC) after reaction with thiobarbituric acid (Templar et al., 1999), and the level of MDA was expressed as nmol/L after quantification by parallel measurements of a standard curve of known MDA concentrations.

2.11. Protein determination

Protein concentrations were determined by the standard method of Lowry et al. (1951).

2.12. TUNEL assay

Apoptosis in kidney tissues was detected by enzymatic labeling of DNA strand breaks using TUNEL method with Cell Death Detection kit according to manufacturers' instructions. Briefly paraffin sections of 5 μm thickness from tissues were deparaffinized. After deparaffinization and rehydratation slides were washed twice in PBS for 5 min. Following the incubation of slides with the permeabilisation solution (0.1% Triton X-100 in 0.1% sodium citrate) for 8 min at 4 °C and washing twice with PBS for 5 min, the labeling reaction was performed using 50 μl TUNEL reagent for each sample, except negative control, in which reagent without enzyme was added and incubated for 1 h at 37 °C. After washing with PBS slides were incubated with converter reagent for 30 min at 37 °C. Following washing, color development for localization of cells containing labeled DNA strand breaks was performed by incubating the slides with Fast Red substrate solution for 10 min. A positive control for detection of DNA fragmentation was included in each experiment, by adding deoxyribonuclease I solution grade I [1500 U/ml in 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 1 mg/ml bovine serum albumin] and incubating for 30 min at 37 °C, previously to the terminal deoxynucleotidyl transferase (TdT) reaction. A negative control was also performed in which no TdT enzyme was present in the reaction mixture and sections were incubated in label solution only for 1 h at 37 °C. All slides were examined and photographed by using Leica DM6000B microscope (Wetzlar-Germany) with a DC490 digital camera (Leica, Wetzlar-Germany). Red-labeled TUNEL-positive apoptotic cells were counted in ten random fields for each renal region including cortex and medulla at a microscopic magnification of 400×.

2.13. Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). The differences among the groups were evaluated with Kruskal–Wallis test, followed by Mann–Whitney *U* test using a Statistical Package for Social Sciences Program (SPSS) version 17.0. *p*-values < 0.05 were considered as statistically significant.

3. Results

3.1. Body weight, absolute and relative kidney weights

Body weights, mean and relative kidney weights of study groups are given Table 1. Throughout the 14 days of OTA exposure, we have observed a time-dependent decrease in the body weight of rats in the OTA group. In the first week of OTA application, we have observed a ~11% decrease in the body weights and at the 10th day of exposure, the decrease was ~15%. At the termination of the experiments, body weights of OTA group were significantly lower than control group (20%). Mean kidney weights in OTA-treated rats showed a marked decrease of 25% while relative kidney weights of OTA-administered rats showed a significant decrease of 10% vs. control ($p < 0.05$, both). Lycopene supplementation affected neither body weights nor absolute kidney weights of rats; however caused a decrease of 16% in relative kidney weights vs. control ($p < 0.05$). Lycopene administration along with OTA did not cause any significant alteration in body weights and absolute kidney weight compared to OTA group; however a significant elevation of 10% was observed in relative kidney weight in OTA-L group compared to Control.

3.2. Biochemical parameters

Plasma biochemical parameters are given in Table 2. In OTA-treated rats, both plasma BUN levels (20%) and creatinine levels (50%) increased significantly vs. control ($p < 0.05$, both). There were also marked increases in plasma electrolytes after OTA application. Ca^{2+} (16%), Na^+ (14%) K^+ (34%) and Cl^- (11%) levels showed significant elevations in OTA group compared to control ($p < 0.05$, all). Lycopene alone did not affect any of the biochemical parameters measured. Lycopene with OTA treatment provided significant changes in blood biochemistry, when compared to OTA treatment alone, taking the BUN and electrolyte (except K^+) levels to control values. However, creatinine levels showed marked increases in OTA-L group compared to both OTA and Control groups.

3.3. Histopathological examination

OTA treatment caused several histopathological changes, including tubules with epithelial cell desquamation and

Table 1
Body weight, absolute and relative kidney weights in study groups.

	Body weight (g)	Absolute kidney weight (g)	Relative kidney weights (g/100 g BW)
Control	271.2 ± 3.89[a]	1.18 ± 0.01[a]	0.44 ± 0.01[ac]
L	277.57 ± 6.22[a]	1.03 ± 0.03[a]	0.37 ± 0.02[b]
OTA	216.82 ± 2.88[b]	0.88 ± 0.02[b]	0.40 ± 0.01[c]
OTA-L	190.03 ± 6.4[b]	0.85 ± 0.04[b]	0.44 ± 0.02[a]

Control group (Control); Lycopene group (L); OTA group (OTA); OTA plus Lycopene group (OTA-L). All results were given as mean ± SEM of $n = 6$ animals. Columns that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

Table 2
Plasma biochemical parameters in study groups.

Groups	BUN (mg/dl)	Creatinine (mg/dl)	Ca^{2+} (mg/dl)	Na^{+} (mmol/L)	K^{+} (mmol/L)	Cl^{-} (mmol/L)
Control	49.17 ± 2.26[a]	0.24 ± 0.02[a]	8.23 ± 0.19[a]	121.00 ± 3.22[a]	6.63 ± 0.78[a]	83.50 ± 2.50[a]
L	51.50 ± 2.23[a]	0.29 ± 0.02[a]	8.84 ± 0.17[a]	132.80 ± 1.18[a]	6.23 ± 0.29[a]	88.17 ± 2.30[a]
OTA	58.83 ± 0.01[b]	0.36 ± 2.57[b]	9.54 ± 0.14[b]	138.00 ± 9.87[b]	8.86 ± 1.27[b]	93.00 ± 4.53[b]
OTA-L	53.17 ± 0.03[a]	0.42 ± 0.03[c]	9.33 ± 0.12[a]	127.60 ± 1.60[a]	7.88 ± 0.33[c]	89.40 ± 2.13[a]

Control group (control); Lycopene group (L); OTA group (OTA); OTA plus Lycopene group (OTA-L). All results were given as mean ± SEM of $n = 6$ animals. Columns that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

detachment (Fig. 1A), hyaline casts in the tubules (Fig. 1B) and mononuclear inflammatory cells in the interstitium (Fig. 1C). Histological examination of the kidney samples both in the control and lycopene group showed no pathological changes (Fig. 2A and B). There were rare areas of inflammatory cell infiltration, which consisted of a few mononuclear cells while tubular degeneration, mild congestion and hemorrhages were also observed (Fig. 2C). Lycopene supplementation was only partially protective against OTA-induced histopathological changes. Degenerated tubules and mononuclear cell infiltration was also observed in OTA-L group (Fig. 2D).

3.4. Antioxidant enzymes

By OTA exposure, no differences were found in selenoenzyme [GPx1 (Fig. 3A) and TrxR (Fig. 3B)] activities in comparison to control group ($p > 0.05$, both). On the other hand, a significant decrease of 35% in CAT activity ($p < 0.05$) (Fig. 3C) and a significant increase of 20% in total SOD activity (Fig. 3D) were determined in OTA group compared to control ($p < 0.05$). Lycopene supplementation caused increases in all antioxidant enzyme activities measured [GPx1 (41%), TrxR (30%), CAT (17%) and SOD (60%)] compared to control and other than TrxR, all the changes were statistically significant ($p < 0.05$). When lycopene was administered along with OTA, it provided significant increases in GPx1 (37%); while not affecting TrxR, CAT and SOD activities vs. OTA group.

3.5. Total glutathione, and lipid peroxidation levels

GSH and MDA levels were given in Fig. 4. Renal GSH levels showed a decrease of 44% in OTA group vs. control significantly ($p < 0.05$) (Fig. 4A). Lycopene supplementation alone did not supply any increase in renal GSH, while lycopene along with OTA exposure (73%) provided significant elevations in GSH levels compared to OTA treatment. However, no significant alterations were observed in renal MDA levels in the study groups when compared to control ($p > 0.05$) (Fig. 4B).

3.6. TUNEL assay

The micrographs of TUNEL labeling of kidney in Control, L, OTA and OTA-L groups were given in Fig. 5. The mean number of TUNEL positive apoptotic cells in cortex and medulla were given in Fig. 6. TUNEL analysis revealed a significant increase in the number of TUNEL-positive cells in cortex (~10-fold) and medulla (~3-fold) in OTA administrated group compared to control ($p < 0.05$). Lycopene administration alone did not cause a significant difference in the mean number of TUNEL positive cells. The mean number

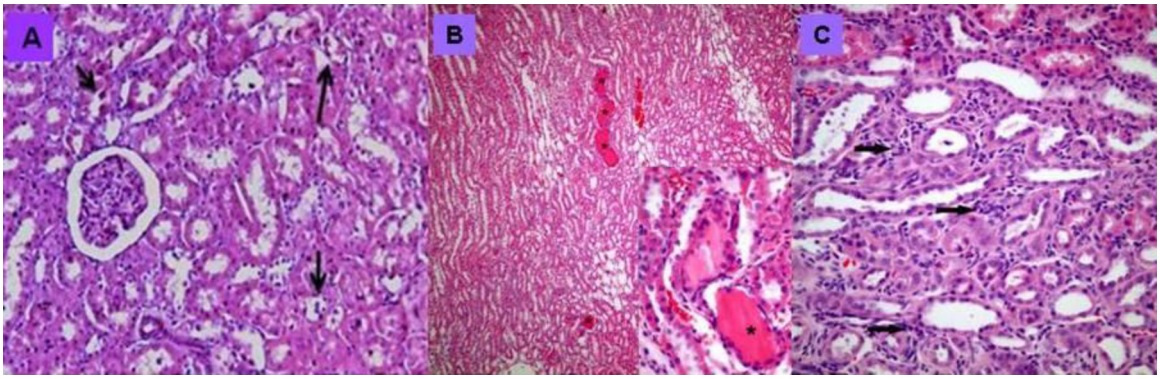

Fig. 1. Kidneys from OTA treated rats. (A) Tubules with epithelial cell desquamation (H–E 200×). (B) Hyaline casts in the tubules (H–E × 100; inset H–E 400×). (C) Mononuclear inflammatory cells in the interstitium (H–E 400×).

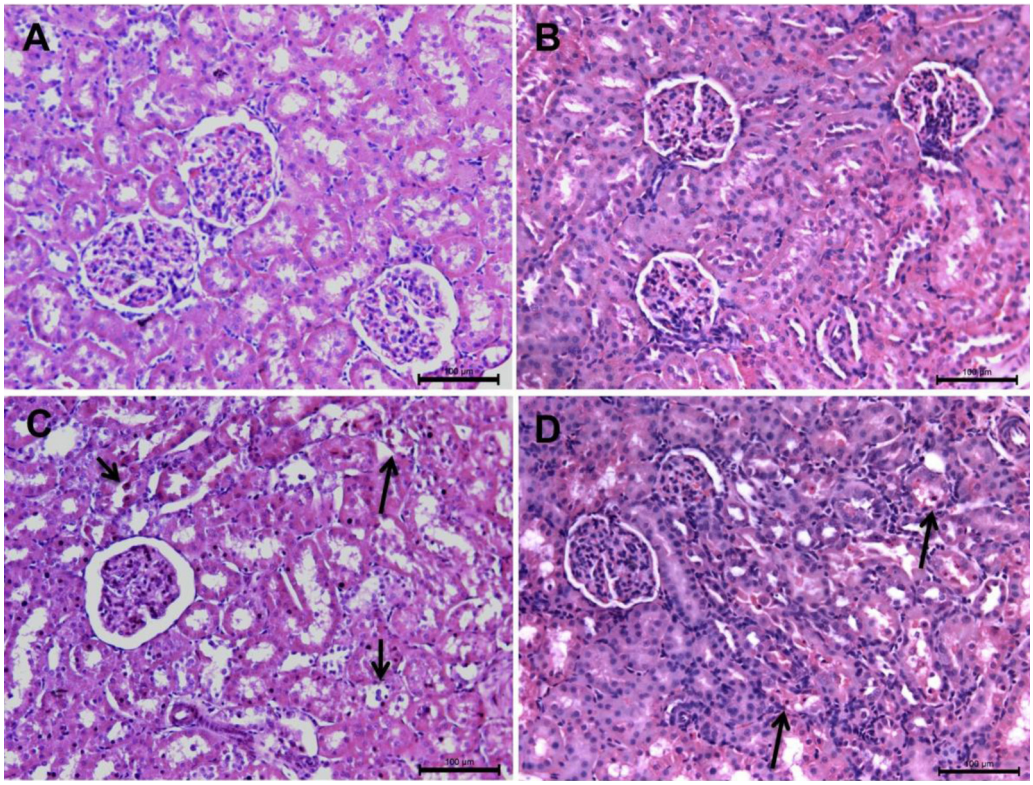

Fig. 2. Renal histopathological evaluation of study groups. (A) Control. (B) L. (C) OTA. (D) OTA-L. Control group (Control); Lycopene Group (L); OTA group (OTA); OTA plus Lycopene group (OTA-L). Tubular damages (arrows) are seen in OTA and OTA-L14 treated groups (hematoxylin–eosin 200×).

of apoptotic cells was significantly decreased in OTA-L group when compared to OTA group.

4. Discussion

OTA is a stable nephrotoxic and possibly carcinogenic fungal toxin (EFSA, 2006; IARC, 1993; WHO, 2001). It was shown to induce cytotoxicity and ROS production in rat and monkey kidney cell lines (Schaaf et al., 2002; Kamp et al., 2005). Besides, OTA was suggested to cause an imbalance between oxidant/antioxidant parameters in both rat kidney and liver (Marin-Kuan et al., 2011; Gautier et al., 2001; Palabiyik et al., 2012).

Several antioxidants have been applied to provide protection against OTA-induced renal toxicity (Aydin et al., 2003; Gautier

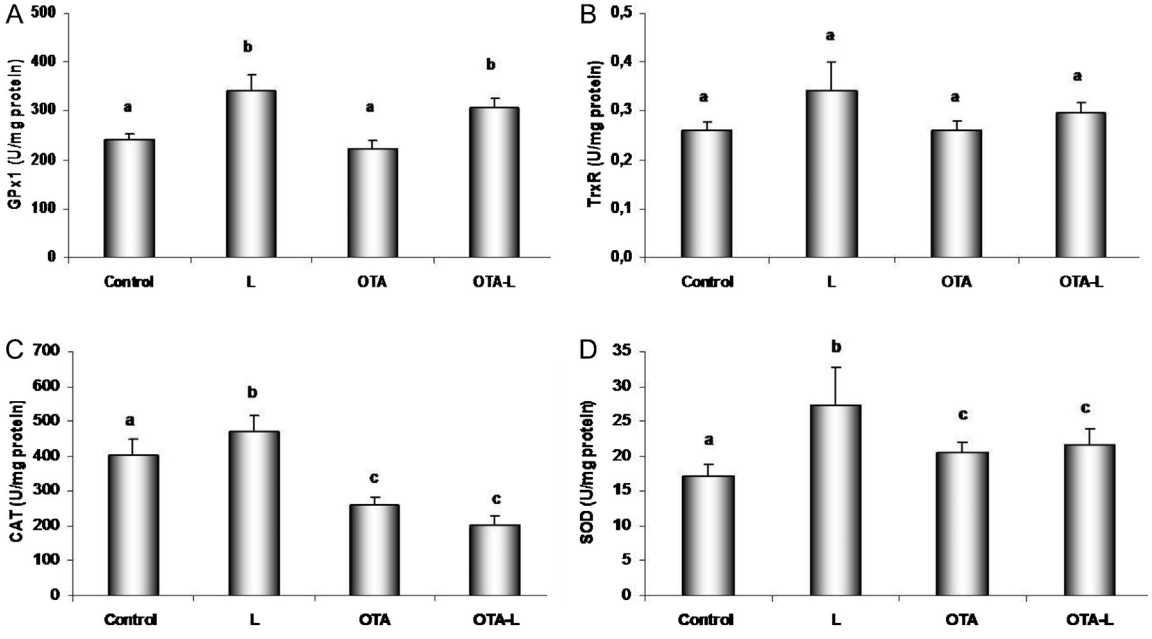

Fig. 3. Renal antioxidant enzyme activities in study groups. (A) Renal GPx1 activity. (B) Renal TrxR activity. (C) Renal CAT Activity. (D) Renal SOD activity. Control group (Control); Lycopene Group (L); OTA group (OTA); OTA plus Lycopene group (OTA-L). All results were given as mean ± SEM of $n = 6$ animals. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

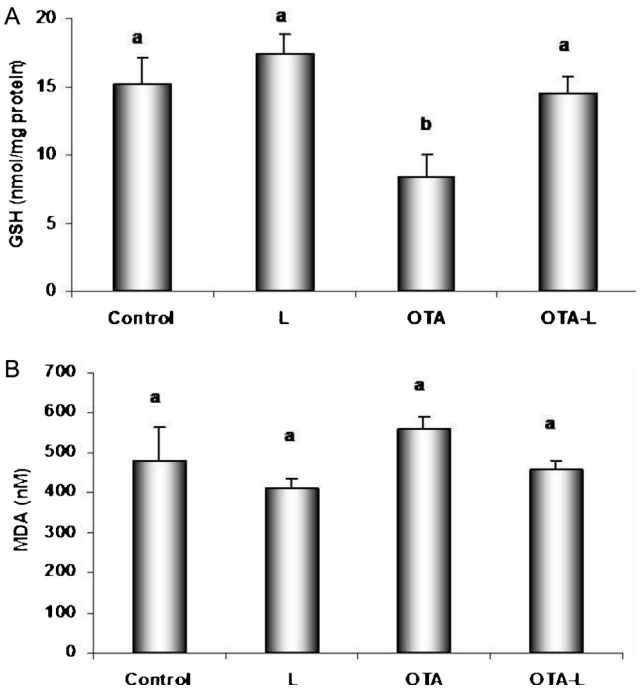

Fig. 4. Renal total glutathione and malondialdehyde levels in study groups. (A) Renal GSH levels. (B) Renal MDA levels. Control group (Control); Lycopene Group (L); OTA group (OTA); OTA plus Lycopene group (OTA-L). All results were given as mean ± SEM of $n = 6$ animals. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

et al., 2001; Ozcelik et al., 2004; Yenilmez et al., 2010). Lycopene, an efficient free radical scavenger, was not applied against OTA toxicity before although it has been tested against the toxicity of AFs (Rao and Rao, 2007; Rauscher et al., 1998; Stahl and Sies, 2003; Tang et al., 2007; Wertz et al., 2004). Breinholt et al. (2000) demonstrated that lycopene (1–100 mg/kg bw/day for 14 days) induced erythrocyte GPx1, GR, SOD and CAT activities of female rats and the induction for all four enzymatic activities peaked at 5 mg/kg bw/day. Therefore, we chose 5 mg/kg bw/day dose of lycopene herein.

In the present study, the renal toxicity of OTA was evidenced by decreased mean and relative kidney weights as also observed by Abdu et al. (2011) by applying 289 μg/kg/day OTA (i.g.) in rats for 28 days and by Hibi et al. (2011), after administering 5 ppm OTA in diet for 4 and 13 weeks. Lycopene administration with OTA changed neither body weights nor absolute kidney weights compared to OTA group; however a marked increase (10%) was observed in relative kidney weight in OTA-L group vs. control.

Our data confirm and extend the data obtained from previous studies which showed that OTA induces renal functional disorders such as abnormal creatinine and BUN levels (Abdu et al., 2011; Bertelli et al., 2005; Rached et al., 2007). The marked enhancements in plasma Na^+, Cl^- and K^+ levels in the OTA group may be an indicator of kidney failure. The loss of normal Ca^{2+} homeostasis is an early and critical event in the development of toxic cellular injury and the entrance of Ca^{2+} to nucleus can trigger activation of nucleases which cause DNA damage (Ringot et al., 2006; Meki and Hussein, 2001). In agreement with our results, Rached et al. (2007) also observed that chronic OTA administration caused significant increases in K^+ and Ca^{2+} levels in rat serum. On the other hand, lycopene can be beneficial in providing normal kidney function. In a study by Ateşşahin et al. (2007), lycopene (10 mg/kg, 21 days, i.g.) with cyclosporine (15 mg/kg, 21 days, i.g) provided decreases in BUN and creatinine levels in rat serum when compared to cyclosporine alone, so as the study of Dogukan et al. (2011), in which rats were exposed to cisplatin (7 mg/kg, single dose, i.p.) and/or tomato lycopene complex treatment (6 mg/kg, 10 days, i.g.).

Several studies demonstrated that OTA treatment caused apoptosis and pre-neoplastic lesions in rat kidney and it was reported that the increase in apoptosis could play a significant role in the development of chronic changes in kidney parenchyma (Stemmer et al., 2009). We observed tubular degenerative changes (epithelial cell desquamation and detachment) by OTA treatment herein. Moreover, we determined significant increases in the number apoptotic cells of cortex and medulla by OTA administration. In the forementioned study, Abdu et al. (2011) also observed lesions like global congestion of the renal tissue and loss of demarcation between the cortex and medulla, with pre-apoptotic cells. Kamp et al. (2005) determined a dose-dependent increase in apoptosis in CV-1 cells by OTA treatment. Yenilmez et al. (2010) showed OTA (2 mg/kg bw, oral, single dose) caused limited tubular degeneration and, desquamation, without any significant increase in apoptosis. On the other hand, in the present study lycopene was found to be protective against the histopathological and apoptotic alterations induced by OTA in rat kidney. In line with the observations of our study, Ateşşahin et al. (2007) and Dogukan et al. (2011) also reported that lycopene supplementation with cyclosporine provided significant protection against the tubular necrosis, atrophy and dilation induced by cisplatin treatment in rats (Dogukan et al., 2011).

The results of several studies on rat renal oxidant/antioxidant status performed using OTA are inconclusive. Our results showed no difference in the activity of GPx1 in the kidney of OTA-treated rats compared to control group. However, both Sutken et al. (2007) (289 μg/kg bw OTA treatment for 4 weeks) and Meki and Hussein (2001) (with 250 μg/kg bw OTA, 10 days) determined significant decreases in the activity of GPx1 in rat kidney and suggested that this may be attributable to the interference of OTA with the absorption of selenium or it may be explained by the association of GPx with OTA or its metabolites (Gautier et al., 2001). In the present study, lycopene treatment alone or with OTA, provided increases in GPx1 activity when compared to both control and OTA-exposed group in agreement with the results of Ateşşahin et al. (2007). We observed significant decreases in CAT activity and significant increases in total SOD activity in OTA group vs. control. Meki and Hussein (2001), observed that OTA exposure caused decreases in both renal CAT and SOD activities in rats, suggesting that these reductions may be due to the repressive action of OTA on protein synthesis rate. Bertelli et al. (2005) observed significant decreases in renal SOD activity by OTA exposure (289 μg/kg bw, 14 days, oral) in rats. Petrik et al. (2003) observed insignificant increases in rat renal SOD activity after short term exposure to oral OTA treatment while long term exposure caused significant decreases. Domijan et al. (2007) did not find any change in CAT and SOD activity with low OTA doses (5 and 50 ng/kg bw, for 15 days, oral) in rat kidney. Concerning all the available data on the antioxidant parameters by OTA exposure, it can be suggested that dose, application route and exposure time are substantial factors in the alterations of crucial antioxidant enzymes.

In the present study, lycopene provided significant increases in both renal CAT and SOD activities compared to control as also observed by Ateşşahin et al. (2005); however in OTA-L group we did not observe any significant changes in CAT and SOD activities vs. OTA group. Some studies in literature show that different antioxidants may be beneficial against OTA-induced changes in antioxidant enzyme systems. Both Meki and Hussein (2001) and Abdel-Wahhab et al. (2005) showed that in rat kidney, melatonin with OTA treatment provided significant increases in GPx1 and SOD activities vs. OTA application alone. Bertelli et al. (2005) determined that red wine application along with OTA (289 μg/kg bw, i.g.) provided increases in renal SOD activity vs. OTA exposed rats.

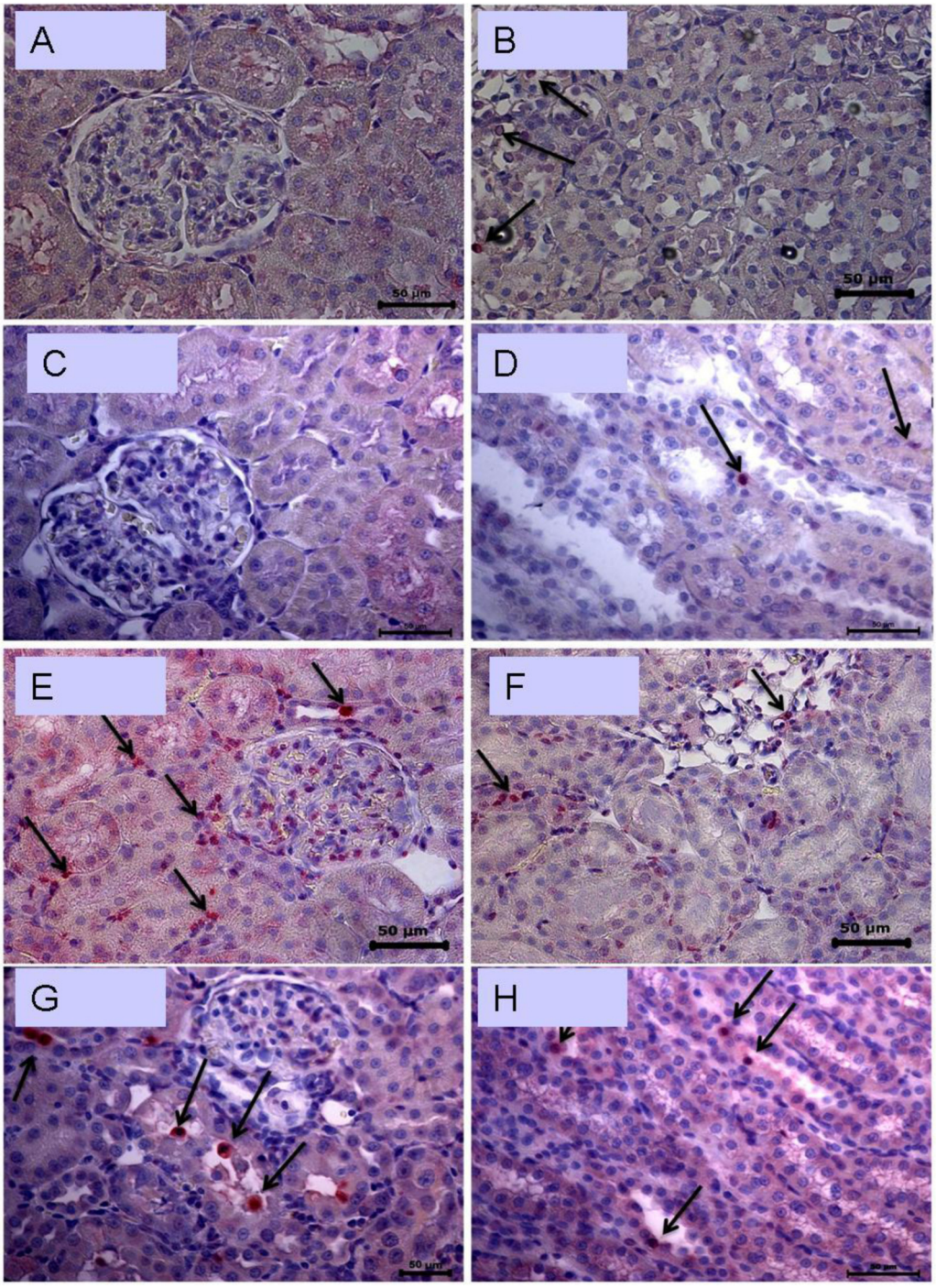

Fig. 5. Effect of OTA on apoptosis in the kidney. TUNEL-positive cells/field for cortex in Control group. TUNEL-positive cells/field for medulla in Control group. TUNEL-positive cells/field for cortex in L group. TUNEL-positive cells/field for medulla in L group. TUNEL-positive cells/field for cortex in OTA group. TUNEL-positive cells/field for medulla in OTA group. TUNEL-positive cells/field for cortex in OTA-L group. TUNEL-positive cells/field for medulla in OTA-L group. Control group (Control); Lycopene Group (L); OTA group (OTA); OTA plus Lycopene group (OTA-L). After OTA administration, there was an increase in the number of TUNEL-positive cells in both cortex and medulla. Arrows indicate TUNEL-positive cells which are observed as red dots in the figure (TUNEL assay 400×). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

In the current study, GSH levels decreased significantly in OTA-treated group in line with similar animal studies and cell cultures (Gautier et al., 2001; Schaaf et al., 2002). Bertelli et al. (2005) observed that OTA administration significantly decreased GSH/GSSG ratio and postulated that the decrease in GSH levels might be due to the toxin's ability to covalently react with GSH after undergoing biotransformation and forming a reactive intermediate [OTQ (6)] (Dai et al., 2002). Lycopene administration along with OTA caused significant elevations in renal GSH levels indicating the protective role of lycopene against OTA toxicity.

The results of several studies concerning the effects of OTA on renal LP are inconsistent. Yenilmez et al. (2010) found significant increase in plasma MDA levels and significant decrease in whole blood GSH levels in rats by single dose of OTA (2.2 mg/kg, oral). However, both Gautier et al. (2001) with single oral dose of OTA (0.3, 1, 2 mg/kg bw, respectively) and Mally et al. (2005)

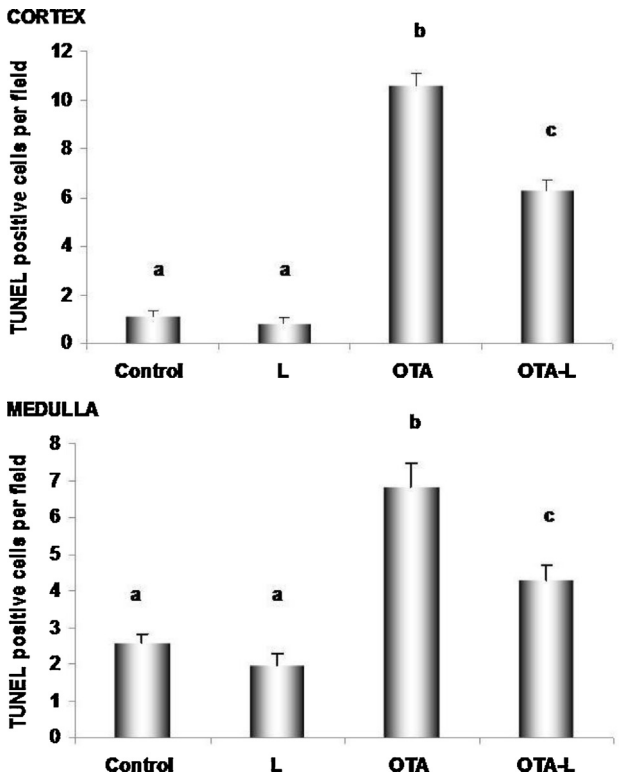

Fig. 6. Quantification of TUNEL labeling of kidney in study groups. CORTEX: quantification of mean TUNEL-positive cells/field for cortex in study groups. MEDULLA: quantification of mean TUNEL-positive cells/field for medulla in study groups. Control group (Control); Lycopene Group (L); OTA group (OTA); OTA plus Lycopene group (OTA-L). Red-labeled TUNEL-positive apoptotic cells were counted in ten random fields for each renal region including the cortex and the medulla at a microscopic magnification of 400×. All results were given as mean ± SEM of $n = 6$ animals. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

with 1 mg/kg OTA (2 weeks, 5 days per week) did not find any increase in renal LP levels in rats. Petrik et al. (2003) reported that short term exposure to oral OTA treatment (120 μg/kg bw) did not increase renal LP levels while long term exposure (60 days) caused significant enhancements. On the other hand, the administration coenzyme Q10 and melatonin with OTA provided significant decreases in plasma and/or renal MDA levels (Yenilmez et al., 2010; Malekinejad et al., 2011; Meki and Hussein, 2001). The unchanged MDA levels in the present study imply that dose and treatment period are important factors in the toxicity of OTA. Although lycopene is an important antioxidant and it was shown to suppress LP (Ateşşahin et al., 2007), we did not observe any alterations in LP with or without OTA exposure. However, Dogukan et al. (2011) observed that in rats, lycopene along with cisplatin provided decreases in renal MDA levels when compared to cisplatin alone. These results suggest that lycopene exert selective protection against LP induced by different chemical agents (Dogukan et al., 2011).

We can conclude that OTA mainly causes histopathological changes in kidneys as evidenced by tubular epithelial changes, and enhancement of apoptosis. Although its toxicity mechanism/s is not well defined, we can suggest that oxidative stress can be implicated as one of the mechanisms involved in OTA toxicity. We can state that lycopene is partially protective against the renal toxicity of OTA, as evidenced by partial recovery in histopathology, apoptosis and antioxidant parameters. Our future aim will be to investigate different antioxidant supplementations to provide full protection against OTA toxicity in rodent models.

Conflict of interest

The authors declare no conflicts of interest.

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