Aflatoxin induced renal toxicity in Albino rats and the ameliorative effect of green tea aqueous extract: Histological, Morphometric and Immunohistochemical Study

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ABSTRACT

Tea is the most common drink worldwide besides water. It is rich in antioxidant polyphenolic flavonoids. Further, it has anti-apoptotic, anti-inflammatory, and autoantigen-inhibitory properties. To evaluate the effects of oral administration of aqueous extract of green tea against experimental aflatoxicosis on the renal tissues of adult male albino rats. Twenty five adult male rats were included and they were divided equally into 5 groups: Group 1 (aflatoxin group): the rats received aflatoxins (2.5 mg/kg diet for 15 days), group 2 (aflatoxins and green tea aqueous extract group) for 15 days, group 3, the rats received aflatoxins for 15 days and aqueous extract of the green tea for 30 days, group 4 (green tea aqueous extract group) and group 5(control negative group). Detailed non-traditional histological and morphometrical analyses for renal tissues stained with Hematoxylin and Eosin, Masson's trichrome stain, Periodic Acid Schiff’s (PAS), Alizarin red S stains and immunohistochemical detection of Ki-67 were done. The main observed renal lesions in rats received aflatoxins were dilatation of the renal pelvis, increases in glomerular areas (GA), widening of Bowman's spaces and necrosis of the renal lining epithelium and proliferation of fibrous connective tissues. Morphometric analysis revealed the presence of significant difference between group I and the other experimental groups. Meanwhile, groups 2 and 3 revealed a significant improvement in the histological changes and decrease in most of parameters. The highest percentage of PAS positive reaction was significantly found in group 4. Quantitative analysis of positive reactions of Ki-67 revealed the highest positive immunohistochemical reactions were detected in group 1 which significantly different in groups 4 and 5. In conclusion, green tea has a protective effect against the adverse effects of aflatoxins on renal tissues evidenced by improvement of histopathological alterations and regulation of Ki-67 expressions.

Key words: Aflatoxins, protective, green tea, Ki-67 and rats.
INTRODUCTION

Kidney is a vital organ where exposure to chemical substances can cause adverse effects on the kidney, ureter and urinary bladder (Azab et al., 2014). The kidney is usually vulnerable because of its role in clarifying injurious substances from the blood. Toxic damage (either acute or chronic) to the kidney is occur as a result of exposures to halogenated hydrocarbons, heavy metals as well as aflatoxins that can lead to end-stage renal failure or cancer (Khan et al., 2013).

Aflatoxins cause immunosuppression, carcinogenicity, teratogenicity, and mutagenicity (Bhat et al., 2010) and also hinder several metabolic systems, producing hepatic, renal and cardiac damage. Aflatoxicosis was also exhibited in other vital organs such as lung which expressed as carcinogenesis and neural tube defects (Gelineau-van et al., 2009). These toxins have been incriminated as the reason of high mortality in livestock and some cases of death in human being (Wangikar et al., 2005; Walter et al., 2016).

Aflatoxicosis is a food-borne disease due to the consumption of aflatoxin-contaminated foods posing a greatest concern in the food safety especially in poor economic countries (Williams et al., 2004). Aflatoxins are produced by Aspergillus fungus, predominantly Aspergillus flavus and Aspergillus parasiticus (Sabran et al., 2012), where the humid environments favors their growth which can be easily proved in the storing areas of gathered food materials particularly maize (Astoreca et al., 2014).

Aflatoxins comprise aflatoxin B1, B2, G1, G2, M1, and M2. Aflatoxin B1 is the most predominant and poisonous with acute toxicity revealed in all animals’ species (Oliveira et al., 2011). Moreover, the severity of aflatoxins-induced lesions is affected by several factors; age, gender and duration of exposure (Dhanasekaran et al., 2011).

Green tea, made from leaves of Camellia sinensis plant, is the most popular drink worldwide as well as water that is rich in antioxidant polyphenolic flavonoids (Ide et al., 2016). Also it has anti-carcinogenic, anti-apoptotic, and anti-mutagenic roles (Bitu Pinto et al., 2015) and contains extraordinary levels of vitamin C with considerable useful effects to human health (Bitu Pinto et al., 2015). Polyphenols isolated from the water extract of the green tea have been shown in several animal models to prevent chemically-induced carcinogenesis in the organs (Stagos et al., 2012). Recently, a worldwide trend is to return to traditional therapeutic plants, as natural products of herbal origin which have antifungal, anti aflatoxigenic and antioxidant activity against the destructive effects of aflatoxin B1 on some organs (Mohamed and Metwally, 2009).

Currently, computer-assisted image analysis provides a new potent tool for high-precision measurement of several variables characterizing the size and shape of cell nuclei (Deans et al., 1993). Many of these profiles appear to be valuable predictive judges in various human malignancies lesions (Jalava et al., 2001).

Ki-67 is a marker of cell proliferation and expressed in the nuclear matrix of cells during the late G1-, S-, G2- and M phases of the cell cycle, it peaks in the G2- and early M-phases (Yerushalmi et al., 2010).

Few studies have studied the effect of aflatoxins on cellular structure of the kidney. Therefore, the aim of the existing study was to
assess the effect of green tea against experimental aflatoxicosis on the kidneys of albino rats by means of histological, morphometric and immunohistochemical analyses.

MATERIALS AND METHODS

Aflatoxicogenic strains: Aspergillus flavus was used for the production of Aflatoxin and their ability to produce aflatoxins was evaluated at laboratory of Mycology and Mycotoxins, Animal Health Research Institute, Agriculture Research Centre (Gabal et al., 1994).

Preparation and detection of aflatoxin by thin layer chromatography: The aflatoxicogenic isolates of A. flavus were covered from feed were inoculated into flasks containing 50 ml of sterile yeast extract solution 2% and 20% sucrose. Inoculated flasks were incubated at 25 ºC for 15 days. At the end of the incubation period, the flask content was filtered to separate the mycelial mat from YES medium. Both were subjected for estimation of aflatoxins and measured qualitatively by thin layer chromatography (TLC). The positive samples for aflatoxins in TLC method were measured quantitatively by fluorometric method using specific FGisAfla test standards according to the recommended method of (AOAC, 2000; Refai and Hassan, 2013).

Aqueous extract of green tea: AHMAD Green Tea Unilever brand, packed in the United Arab Emirates was used. Green tea was soaked in the drinking water which freshly prepared three times per week and stored at 4°C until use. The content of drinking bottle was renewed every day (Khalaf et al. 2012).

Experimental animals: Twenty-five mature male albino rats, weights “100-120 g” were supplied from the breeding unit of laboratory animal, Faculty of Veterinary Medicine, Beni-Suef University. All animals were properly maintained in well-ventilated animal house, suitable temperature, relative humidity polypolypropylene cages with 12 hours light/dark cycle. Commercial food pellets for rats and water were available ad libitum. The rats were acclimated to the environment for 7 days prior to the experiment. The rules of ethics committee of Faculty of Veterinary Medicine, Beni-Suef University were followed (Institutional Animal Care and Use Committee, Beni-Suef University).

Experimental design: Rats were randomly assigned into equal 5 groups. Group 1 aflatoxicated rats; were fed on diet containing aflatoxins (2.5 mg/kg of diet). Group 2, the rats received Aflatoxins 2.5 mg/kg of diet (5times/week) plus green tea in drinking water at concentration of 5gm/L for 15 days. Group 3, the rats received Aflatoxins 2.5 mg/kg of diet for 15 days (5times/week) plus green tea in drinking water at concentration of 5gm/L. for 30
days (green tea was continually supplied for another 15 days after termination of aflatoxins administration). Group 4, the rats received green tea in drinking water at concentration of 5gm/L. Group 5 (control group), the rats received distilled water during the whole period of experiment (Skrzydlewska et al. 2005). At the end of the experiment, the rats of groups (1, 2, 4 and 5) were weighed, anesthetized with alcohol chloroform ether mixture ACE mixture in a ratio of 1:2:3 respectively and sacrificed. Kidney specimens were taken from each group. In group 3, the rats were maintained for another 15 days and kept on receiving green tea only in drinking water without exposure to aflatoxins.

**Histopathology:** Kidney specimens were prepared and fixed in 10% buffered formalin for 72 hours, routine processing was performed. Microtomy of 4-6 μm sections, followed by their staining with Hematoxylin and Eosin (H&E), Masson's trichrome stain to identify the fibrous connective tissues, Periodic acid Schiff’s (PAS) stain for detection of mucopolysaccharides which highlighted the glomerular capillary basement membranes as well as the tubular lining epithelium and Alizarin red S for detection of calcium deposition (Bancroft and Gamble, 2008).

**Immunohistochemistry of Ki-67 expression in renal tissues:** Immunohistochemical labelling of Ki-67 on 4-μm thick paraffin-embedded sections was performed on the kidneys of all experimental rats. The sections were dewaxed in xylene and rehydrated in graded ethanol. To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with H2O and phosphate-buffered saline (PBS), and then probed with a Ki-67 Antibody (SP6) Rabbit Monoclonal Antibody (Thermo Fisher Scientific, USA) at a dilution of 1:200 for 1 hour at 37°C in a humidified chamber. Then, the sections were washed 3 times with PBS, and incubated with HRP-conjugated secondary antibody (EnVision + System HRP-labelled polymer anti-rabbit; Dako) for 30 minutes at RT. After washing 3 times with PBS, 3,3-diaminobenzidine tetrahydrochloride (Liquid DAB + Substrate Chromogen System, Dako®) was added to the sections. Tissues were counterstained with Mayer's hematoxylin and dehydrated with ethanol and xylene to be prepared for mounting for microscopical examination.

**Morphometric analysis:** Renal Morphometric analyses were carried out by using optical microscope. Images were captured by a digital camera (Leica, DM2500 M). A freeware version of Image-J (1.45s) downloaded from the NIH website was used for image analysis preceded by calibration of the scale was changed to micrometers using set scale analysis as following: 1) Measurements of glomerular area (GA): Measurements of glomerular area (μm²) were carried out by the use of line tool inside a standard measuring frame of a known area using thirty microscopic images for each group routinely stained with H&E stain at x400 lens. 2) Measurements of Bowman's capsule thickness: Bowman's space thicknesses (μm) were measured in similar technique resembling glomerular areas measurements; three readings for each glomeruli were carried out inside a standard measuring frame of a known area using thirty microscopic images for each group routinely stained with H&E stain at x400. 3) Renal tubules diameter: Measurements of renal tubules diameter were performed inside a standard measuring frame of a known area using...
thirty microscopic images for each group routinely stained with H&E stain at x400. 4) Renal pelvis: Dilation in the renal pelvis (μm) was calculated by the use of line tool inside a standard measuring frame of a known area in ten x40 microscopic field images for each group routinely stained with H&E stain. 5) Quantification of collagen fibers area percentages (%) in renal cortex: Quantification was performed by using Masson's trichrome stain; fifty and microscopic fields at x200 magnifications were randomly selected and evaluated using image J software. 6) Quantification of integrated intensities (pixels) and area percentages (%) of mucopolysaccharides using Periodic acid-Schiff (PAS) stain: Twenty five captured microscopic images at x400 magnification fields were evaluated using image J software. 7) Quantification of calcium deposition using Alizarin red S method: Calcium deposition was quantified by using Alizarin S stain; at x400 magnifications. All positive orange to red reactions were captured in different groups. Random fields were selected in the control non-treated group, using image J software.

**Statistical analysis:** The statistical significances were evaluated by SPSS version 21 software package (SPSS, Inc, USA) through one way ANOVA followed by LSD and Dunnett tests for multiple comparison performance among the tested groups. P value ≤0.05 was considered statistically significant. All data were tabulated as means± SD.

![Fig. (1) Sections of rat's renal cortex (1st raw) and renal pelvis (2nd raw) routinely stained with Hematoxylin and Eosin stain from different groups, including G1 (A,a), G2 (B,b), G3 (C,c), G4(D,d) and G5 (E,e). HE x40, 400](image-url)
RESULTS

1. Quantification of histopathological lesions:

1.1. Histopathological lesions using H&E stain: The main observed pathological alterations were focal interstitial nephritis, degenerative changes and necrosis of tubular lining epithelium together with hypercellularity of the glomerular tuft and dilatation of the Bowman's spaces among groups which were clearly detected in aflatoxins-treated group (G1). On the other hand, more or less normal histological structure of the kidneys could be demonstrated in the green tea supplemented (G4) and control negative groups (G5) as shown in (Table 1, Fig.1A-E). The hypercellularity of the glomerular tufts were calculated by the measurement of the glomerular areas (um$^2$). Statistical analysis revealed a significant difference among groups. Glomerular area (GA) was ranged from 3800 to 5000 µm$^2$, the highest calculation was detected in G1 group (5000µm$^2$) with a significant difference with groups 2, 4, 5 ($P<0.0001$). Furthermore, no significant difference could be detected in G4 and G5 ($P=0.590$) as illustrated in (Table 1). The increased GA was associated with increasing in the Bowman's space, ranging from 1.9 to 3.6 µm. A significant increasing of Bowman's space could be detected between G1 and G2, 4, 5 ($P<0.0001$) with no significant difference between G4 and G5 ($P=1.11$). Degenerative changes and necrosis were observed in examined the renal tissues especially in G1, 2 and 3. The renal tubules diameter was ranged from 4.1 to 6.6 µm. Severe necrosis of tubular lining epithelium was detected in G1 when compared with control rats ($P=0.02$) with a significant decrease in G2, 3 and 4 (0.011, 0.046 and 0.0001 respectively). Normal histological structure of renal lining epithelium was observed in G4 and G5 with renal tubule diameters ranged from 5.5 to 6.6 um with no significant difference between them (Figs. 1A-E). The most predominant lesion observed in the kidneys was renal pelvis dilatation which more frequently noticed in G1(860µm). Significant increase of the renal pelvis was found between G1 and other groups ($P<0.0001$) as shown in (Figs. 1a-e).

1.2. Collagen fibers area percentages in renal cortex using Masson's trichrome stain: The lowest collagen fibrous connective tissue area percentages was recorded in G4 (0.1%). On contrast, the highest percentage was detected in G1 (3.2%). A highly significant difference in the renal cortex was found between G1 and other groups, $P<0.0001$) as illustrated in (Table 1 and Figs. 2A-E). By application of Post hoc test; no significant difference of collagen fibers proliferation was detected between G2 and G3 ($P=0.308$) and also between G4 and G5 ($P=0.349$).

1.3. Positive reactions area percentages and integrated intensities of mucopolysaccharides using Periodic acid Schiff's (PAS) stain: Mucopolysaccharides are mainly localized in different portions of the kidneys including; glomerular capillary loop, basement membranes and tubular epithelium. Affected cells stained positively indicating abundant cytoplasmic mucopolysaccharides. No significant difference could be detected between area percentages of PAS positive reactions between G1 and G2 groups ($P=0.975$) and similarly between G1 and G3 ($P=0.815$) as illustrated in (Table 1, Figs. 2a-e).
Fig. (2) Sections of rat’s renal cortex stained with Masson’s trichrome (1st raw) and PAS stain (2nd raw) from different groups, including G1 (A,a), G2 (B,b), G3 (C,c), G4 (D,d) and G5 (E,e). Masson’s trichrome and PAS x400.

Fig. (3) Sections of rat’s renal cortex (1st raw) stained with Alizarin S stain (1st raw) and Ki-67 immunohistochemistry (2nd raw) from different groups, including G1 (A,a), G2 (B,b), G3 (C,c), G4 (D,d) and G5 (E,e). Alizarin R method and IHC x400.
1.4. Calcification area percentages using Alizarin S stain: Orange red granules within renal tissues indicating deposition of calcium salts. The highest level of calcium deposits was found in G1 (0.5%) and a significant increase with other groups G2, 3, 4, 5 (P=0.01, 0.003, 0.001 and 0.001) respectively as shown in (Table 1, Figs 3A-E)

1.5. Quantification of immunohistochemical reaction of Ki-67: Immunoreactivity of Ki-67 was observed in the nucleus of renal tubules (nuclear reactivity). The highest area percentages expression of Ki-67 was detected in G1, G2 and G3 (1.8, 1.3 and 1.0%) respectively with no statistical differences among them (P=0.630 and 0.233). The lowest area percentages of positive immunohistochemical reaction (negative to faint nuclear reactions) were found in G4 and G5 (0.5 and 0.7% respectively) with no statistical differences between them (P=0.580). Significant differences was detected between G1, G4 and G5 (P=0.007, 0.013) as shown in (Table 1 and Figs. 3a-e)


<table>
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<tr>
<th>Measured parameter</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
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<td>GA (µm²)</td>
<td>4979±1771</td>
<td>3909±964*</td>
<td>4713±1040</td>
<td>3653±950*</td>
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<td>Bowman's space (µm)</td>
<td>6.5±3.6</td>
<td>3.7±1.9*</td>
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<td>4.6±2.8*</td>
<td>3.9±2.0*</td>
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<tr>
<td>Renal tubules diameter (µm)</td>
<td>4.1±2.8</td>
<td>5.7±2.4*</td>
<td>5.3±2.9*</td>
<td>6.6±4.0*</td>
<td>5.5±3.6*</td>
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<tr>
<td>Renal pelvis dilatation (µm)</td>
<td>864±358</td>
<td>381±336*</td>
<td>468±181*</td>
<td>307±95*</td>
<td>356±195*</td>
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<td>Fibrous connective tissues area %</td>
<td>3.2±1.7</td>
<td>1.0±1.1*</td>
<td>1.4±0.6*</td>
<td>0.1±0.2*</td>
<td>0.4±0.4*</td>
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<td>PAS stain</td>
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<td>PAS positive reactions area %</td>
<td>35.8±5.4</td>
<td>35.5±3.5</td>
<td>36.2±6.2</td>
<td>38.8±3.8</td>
<td>31.9±4.7*</td>
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<td>Calcification area %</td>
<td>0.55±0.35</td>
<td>0.20±0.25*</td>
<td>0.14±0.11*</td>
<td>0.04±0.006*</td>
<td>0.04±0.004*</td>
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<td>Immunohistochemistry</td>
<td></td>
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<tr>
<td>Ki-67 positive reactions area %</td>
<td>1.81±1.0</td>
<td>1.3±0.3</td>
<td>1.0±0.7</td>
<td>0.5±0.2*</td>
<td>0.7±0.6*</td>
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*Statistically significant

DISCUSSION

Aflatoxicosis in livestock and human is a major economic and health concern (Walte et al., 2016). Aflatoxins are known as animal carcinogens and have been related to cancer of the liver in humans and kidneys in rodents (Wild et al., 2002). Aflatoxins are present in the food chain and they have been found in human cord blood and seemingly can enter the developing fetus in humans and animals (Denning et al., 1990). These toxins have been found in human breast milk (El-Nesami et al., 1995), cow's milk and dairy products (Thirumala-Devi et al., 2002) and infant formula (Aksit et al., 1997). Aflatoxins are immunosuppressive in a diversity of animals, such as sheep, cattle, mice, rats, rabbits, pigs and poultry which making them more liable to infection by numerous
microorganisms (Aflatoxins, 2002). Though the mechanism underlying the toxicity of aflatoxins is not totally understood, numerous reports propose that toxicity may result via the generation of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide (H2O2) during the metabolic processing of AFB1 by cytochrome P450 in the liver. These species may attack soluble cell compounds as well as membranes, eventually leading to the diminishing of cell functioning and cytolysis (Sohn et al., 2003, Berg et al., 2004).

In the existing study, to elaborate the aflatoxin injurious effects on rat’s kidney a histomorphometric analysis was done in addition to immunolabelling of Ki-67. The results revealed that the rats intoxicated with aflatoxins exhibited a significant renal pelvis dilatation compared to other experimental groups which in the same line with (CAST, 2003; Bryden, 2012) who observed hydronephrosis and chlorosis of kidneys. Moreover, aflatoxins-fed group exhibited severe tubular degeneration and necrosis reflected by a significant decrease of the renal tubules diameter (P<0.05). Such findings might be attributed to the shrinkage and necrosis of the lining renal epithelium. Similar pathological findings were detected in the distal tubules in G1, in the form of epithelial swelling and degeneration which is in agreement with (Devendran and Balasubramanian, 2011). Renal lesions could be easily inspected by the use of diverse special stains (Kong et al. 2017).

In the current work, staining of kidney sections using Masson’s trichrome and PAS was supportive in monitoring the improvement in the histological structure in kidneys groups supplemented with green tea. In renal tissues stained with Masson’s trichrome, minimal area percentages of fibrous connective tissues proliferation could be detected in these groups with a highly significant decrease in aflatoxins-fed group (P<0.05) and no significant difference in the control negative group (G1). These findings were in harmony with (Elgawish et al., 2015) who noticed traces of fibrous connective tissues proliferation in the green tea-treated animals compared to excessive proliferation of fibrosis in CCl4 treated groups.

In PAS stained sections, a positive reaction was detected due to the presence of glycoproteins which mainly noticed in the glomerular basement membranes and tubular epithelium especially the proximal portions. The highest area percentages and integrated intensities of positive reaction could be clearly found in the green tea-fed group (G4), with a high significant increase compared to the other groups. Such finding might be due to increased level of mucopolysaccharides and glycoproteins. Moreover, G2 and G3 showed a high significant increase of PAS positive reactions compared to G5, reflecting the higher accumulation of glycoproteins within renal tubules. These cellular alterations were improved in the green tea-treated groups (G2 and G3). Tofovic et al., (2007) examined the early nephropathic lesions associated with the caffeine administration and proteinuria occurred.

Alizarin S staining was mainly detected in rats fed on aflatoxins containing diet which indicating presence of areas of necrosis favoring calcium deposition. Even though, lower staining tendency was observed in rats supplemented with green tea.
The expression of Ki-67 is strongly related with tumor cell proliferation and progression, and is broadly used in routine pathological investigation as a proliferation marker (Lian et al., 2014). Expression of the Ki-67 protein is an indicator of tumor aggressiveness (Kloppel et al., 2004). The predictive worth of Ki-67 has been inspected in a number of studies with its potential as a dependable marker in cancers of the breast, soft tissue, lung, prostate, cervix and central nervous system (Josefsson et al., 2012). In the current study, the highest expression levels of Ki-67 were detected in the renal tissues sectioned from rats fed on aflatoxicated diet. The expression was decreased in G2 and G3 groups that might be attributed to the use of aqueous extract of the green tea. So, acute aflatoxicosis resulted in increasing the proliferation rate of tubular epithelium demonstrated by strong nuclear expressions of Ki-67 which return to its normal level in rats supplemented with green tea. Therefore, chronic exposure to diet containing aflatoxins may result in chronic irreversible changes in renal tissues or even kidney cancer. Numerous earlier studies indicated that the green tea extract has a heavy-duty antioxidant and free-radical-scavenging properties (Crespy and Williamson, 2004). The defensive effect of the green tea is principally due to the existence of polyphenols, especially catechins which prevent lipid peroxidation induced by different chemicals in the liver and kidneys (Franca et al., 2005). (Tulayakul et al., 2007) showed that green tea extract increased AFB1 detoxification, decreased P450 enzyme activity in liver, increased GST activity in intestine and increased Conversion of AFB1 to aflatoxicol in the liver of female piglets. Toxicological studies inspected the beneficial effects of the green tea usage against the adverse effects of variable damaging agents on different internal organs including liver, kidneys and brain disclosed that green tea administration significantly decreases lead-induced low concentration of SOD activity and reduced glutathione in rats’ brain tissues (Khalaf et al., 2012) and can attenuate oxidative stress and lead load on kidneys function (Abdel-Moneim et al., 2014). Also, green tea modulates signaling pathway including NFkB and ERK pathways, conserves mitochondrial membrane potential, obstructs caspase-3 activity, down-regulates pro-apoptotic proteins, and induces the phase II detoxifying pathway (Chen et al., 2017).

CONCLUSION
In the existing work, renal damage characterized by marked renal dilatation, focal interstitial nephritis, necrosis of tubular lining epithelium together with hypercellularity of the glomerular tuft and dilatation of the Bowman's spaces was mainly observed in the aflatoxins-treated group (G1) and greatly diminished in the green tea supplemented groups (G2 and G3). Acute aflatoxicosis, induces up regulation of Ki-67 expression indicating cellular proliferation to the degree of permanent cellular damage or renal carcinogenesis upon chronic exposure. To our knowledge, no previous report investigated Ki-67 expression in rats exposed to aflatoxins. Green tea supplementation, regulated Ki-67 expression by renal tubular cells, diminished fibrous tissue proliferation and percentage of calcification.

Thus, the aqueous extract of the green tea was able to guard rat’s kidneys against experimental aflatoxicosis.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.
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