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Preventive role of selenium against toxicological and pathological effects of ochratoxin A in rabbits

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Abstract

Thirty New Zealand white male rabbits weighing 1.2-1.5 Kg, were randomly separated into three experimental groups (10 rabbits each). 1st group (control) fed on the basal diet, 2nd group fed on the basal diet containing Ochratoxin A (OTA) (0.1 ppm) and 3rd group was orally administered selenium (as sodium selenite dissolved in distilled water) at a dosage of 0.05 mg /kg body weight/ day, two hours before dietary OTA at a dose of 0.1 ppm for 21 days. At the end of the experimental period, all rabbits from each treatment were kept off feed for 12 h, then were slaughtered and carcasses were dissected**.** Blood samples were collected and was left to coagulate at ambient temperature for about 2 hours and centrifuged at 3000 rpm for 10 min to separate the serum, which was kept at -20 ° C until biochemical analysis. Liver and kidney were extracted weighed and preserved for biochemical and pathological examination. Ochratoxin A administration induced a significant elevation in serum ALT, AST, ALP enzymes, serum levels of creatinine and urea as well as hepato-renal lipid peroxidation expressed as tissue malondialdehyde (MDA), while evoked a significant reduction in serum total protein levels, albumin levels and albumin/ globulin ratio (A/G) in association with reduced activities of hepato-renal enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH Px) and nonenzymatic antioxidants glutathione (GSH), whereas globulin levels were not altered. The pathological examination to kidney of rabbits treated with OTA revealed atrophic glomerular tufts, and hydropic degeneration to epithelial lining of renal tubules while liver showed congestion of central vein with dilatationin hepatic sinusoid. In addition, Se supplementation appeared to improve liver and kidney function, induced a significant enhancement of hepato-renal antioxidants and reduce lipid peroxidation in rabbits fed on OTA contaminated diet. Histopathological examination revealed that Se ameliorate the severe hepato-renal tissues damage evoked by OTA exposure.

Keywords: *Ochratoxin A; Selenium, hepato-renal toxicity, oxidative stress, histopathology, male rabbit.*

Introduction

Rabbit feed ingredients are derived from different raw materials which contaminated with mycotoxins, such as ochratoxin and would represent an important potential hazard **(Mézes and Balogh, 2009)**. OTA is produced by several fungi of *Aspergillus* and *Penicillium* species, often found in a variety of food commodities, such as cereals and meat products, resulting in continuous human exposure to OTA **(Duarte *et al*., 2012)**. Ochratoxin A (OTA) has been shown to be a potent nephrotoxic and hepatotoxic compound. In farm animals, the intake of feed contaminated with OTA affects animal health and productivity, and may result in the presence of OTA in the animal products.

OTA is considered a nephrotoxic and carcinogenic agent, being the origin of many kidney diseases. OTA is mainly found in cereal and oilseed grains, and to a minor extent in animal products **(Denli and Perez, 2010).** Histopathological changes in kidney and liver of duckling which given OTA are represented by hemorrhages at the cortico-medullary junction with degeneration and coagulative necrosis of tubular epithelium, while liver exhibited vacuolar degeneration and fatty change of hepatocytes **(Bahry, 2012).**

Antioxidants, such as glutathione and selenium regulate the generated reactive oxygen species (ROS). Antiradical is further supported with antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase those exert synergistic actions in removing free radicals **(Uttara *et al*., 2009)**. Selenium (Se) is a micronutrient that is required for a number of biochemical functions in both humans and animals. Selenium could not only have the potential to prevent oxidative damage during heat stress, but could also influence maintenance of cell membrane integrity via phospholipid hydroperoxide glutathione peroxidase **(Mahima *et al*., 2012)**. The reported nutritional requirements of rabbits for Se is only 0.08 mg/kg diet. Selenium is a part of the body's antioxidant defence system, and with dietary Se supplementation, its concentration in the meat can be effectively increased **(Dalle Zotte and Szendrő, 2011)**. The dietary protein and selenium supplementation at appropriate level apparently have the potential to improve male rabbit general performance **(Abdulrashid and Juniper, 2016)**.

The objective of this study was to investigate the potential antioxidant effect of selenium and its protective effect against hepato-nephrotoxicity in rabbits fed on ochratoxin A-contaminated diet.

Materials and Methods

Selenium (as Sodium selenite):

Sodium selenite ≥ 98% powder obtained from Sigma-Aldrich. Linear Formula: Na2O3Se.

Preparation and analysis of Ochratoxin A (OTA):

Aspergillus ochraceus grown on malt peptone (MP) broth using 10% (v/v) of malt extract (Brix 10) and 0.1 % (w/v) bacto peptone (Difco) in 2 ml of medium in 15 ml tubes. The cultures were incubated at 25ºC for 7 days in light/darkness. Finally, OTA was quantified using Refurbished thermo/Dionex Ultimate 3000 Rs UHPLC in Faculty of Agriculture Zagazig University according to **Samson *et al*. (2004)**. The media was sprayed on diet to obtain ochratoxin A (100 ppb).

Animals management and experimental design:

Thirty New Zealand white male rabbits weighing 1.2-1.5 Kg, were individually housed in battery cages at controlled temperature (25±2ºC) and ambient humidity. Lights were maintained on a 12-h light/dark cycle. All rabbits were fed balanced diet and water ad libitum. After two weeks acclimatization period, the animals were randomly separated into three experimental groups (10 rabbits each). 1st group (control) fed on the basal diet formulated to meet the recommended nutrient requirements of rabbits **(NRC, 1994)**, 2nd group fed on the basal diet containing Ochratoxin A (OTA) (0.1 ppm) (the maximum residue limit) according to **(European Commission Recommendation 2006/576/EC)** (0.05-0.1ppm) in animal feed and 3rd group was orally administered Selenium (as sodium selenite dissolved in distilled water) at a dosage of 0.05 mg /kg body weight/ day according to **Swadi (2015)** through stomach tube two hours before dietary OTA at a dose of 0.1 ppm for 21 days. Clinical examination of rabbits of all groups was recorded throughout the experimental period. At the end of the experiment, all rabbits from each treatment were kept off feed for 12 hrs., then were slaughtered and carcasses were dissected and post mortem examination was performed. Blood samples were collected and was left to coagulate at ambient temperature for about 2 hours and centrifuged at 3000 rpm for 10 min to separate the serum, which was transferred to 5 ml eppendroff tubes and kept at -20°C until biochemical analysis. Liver and kidney were extracted and preserved for biochemical and pathological analysis.

Biochemical analysis:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities was measured according to **Reitman and Frankel,**

(1957), alkaline phosphatase (ALP) activity according to **Tietz, (1996)**, serum urea level and serum creatinine level measured according to the method of **Coulombe and Favreau (1963)** and **Larsen (1972)** respectively. Total serum protein **(Doumas *et al.*, 1981)**, albumin **(Drupt, 1974)** were colormetrically determined using commercial Bio-diagnostic kits, Egypt and globulin was calculated as difference between total protein and albumin and then albumin/ globulin ratio (A/G) was calculated.

Hepato-renal antioxidants and lipid peroxide assay:
Specimens from liver and kidney from all groups were washed with saline solution, then minced and homogenized in ice-cold normal saline at a concentration of 0.1g/ml. The homogenates were centrifuged at 3500 rpm for 10 min at- 4C^{o} and the supernatant was obtained and used for estimation of enzymatic and non enzymatic antioxidants activities and lipid peroxidation by spectrophotometric methods. Superoxide dismutase (SOD) activity was estimated as described by **Spitz and Oberley (1989)**. Catalase (CAT) activity was determined according to the method of **Aebi (1984).** GSH px was measured according to **Flohé and Günzler (1984)**, reduced glutathione (GSH) was determined according to **Lin *et al.* (1988)**. Lipid peroxides formation were determined as Malondialdehyde (MDA) according to **(Satoh, 1987)** using chemical kits (Biomed Egypt).

Histopathological Studies:
Specimens from liver and kidney from all groups were collected and fixed in10 % neutral buffered formalin solution then dehydrated, cleared and embedded in paraffin wax. The specimens were sectioned to 4-5 micron thickness and stained with Hematoxyline and Eosin stain (H&E) **(Survarna *et al.*, 2013).**

Statistical analysis:
Data were statistically analyzed using analyses of variance (F-test) followed by Duncan's multiple range test. A probability at level of 0.05 or less was considered significant. Standard errors were also estimated using IBM SPSS statistics program version 20.

Results
Table (1) manifested that dietary administration of Ochratoxin A (0.1 ppm) stimulated a nephrotoxic and hepatotoxic effects in rabbits (2nd group) and induced a significant elevation in serum ALT, AST, ALP enzymes, serum levels of creatinine and urea ($p<0.05$), while evoked a significant reduction in serum total protein levels in association with reduced albumin levels and albumin/ globulin ratio (A/G) ($p<0.05$) whereas globulin levels were not altered in comparison to control group. However, it was demonstrated that oral administration of selenium Se (as sodium selenite) at a dosage of 0.05 mg/kg b.wt. ameliorated liver and kidney function as well as serum total protein and albumin levels in male rabbits fed on OTA-contaminated diet in 3rd group.

The current study exhibited that dietary OTA (0.1 ppm) induced a significant decline in the activity of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH Px) and nonenzymatic antioxidants glutathione (GSH) ($p<0.05$) whereas lipid peroxidation expressed as tissue malondialdehyde (MDA) significantly decreased ($p<0.05$) in both liver and kidney of rabbits fed on dietary ochratoxin (OTA) (2nd group). On the other hand, Se (as sodium selenite) at a dosage of 0.05 mg/kg b.wt. induced a significant enhancement of hepato-renal antioxidants and a significant decline in lipid peroxidation in 3rd group (Tables 2-3).

Table (1). Effect of oral administration of selenium (0.05 mg/kg b.wt.) on liver and kidney function in male rabbits fed on dietary ochratoxin A (0.1 ppm) for 21 days.

Groups / Parameters	Control	OTA	OTA+Se
ALT (U/L)	10.05±0.06 a	34.07±0.15 b	12.08±0.11 c
AST (U/L)	65.44±0.12 a	96.83±0.17 b	72.28±0.14 c
ALP (U/L)	46.91±0.18a	84.41±0.39 b	66.87±0.59 c
Urea (mg/dl)	33.43±0.22 a	77.29±0.71 b	47.72±0.48 c
Creatinine (mg/dl)	0.38±0.01 a	1.69±0.03 b	0.55±0.02 c
T.Protein (g/100 ml)	7.00±0.02 a	4.38±0.04 b	4.94±0.08 c
Albumin (g/100 ml)	3.87±0.02 a	1.06±0.03 b	1.83±0.0 2c
Globulin (g/100 ml)	3.13±0.04 a	3.11±0.05 a	3.03±0.03 a
Albumin / Globulin ratio	1.24±0.01 a	0.34±0.005 b	0.60±0.02 c

Data are represented as means of samples ± S.E. Mean in the same row with different letters are significantly different (Duncan multiple range test $P < 0.05$).

Table (2). Effect of oral administration of selenium (0.05 mg/kg b.wt.) on hepatic antioxidants activities and lipid peroxidation of male rabbits fed on dietary ochratoxin A (0.1 ppm) for 21 days.

Groups / Parameters	Control	OTA	OTA+Se
SOD (U/g liver)	45.52±0.19 a	18.18±0.17 b	41.27±0.21 c
CAT (U/g liver)	1.95±0.03 a	0.57±0.04 b	1.73±0.05 c
GSH (U/g liver)	5.41±0.02 a	1.33±0.05 b	3.97±0.07 c
GSH- Px (U/g liver)	75.88±0.11 a	24.43±0.10 b	47.33±0.14 c
MDA (nmol/g liver)	5.26±0.02 a	17.63±0.08 b	10.77±0.04 c

Data are represented as means of samples ± S.E. Mean in the same row with different letters are significantly different (Duncan multiple range test $P < 0.05$).

Table (3). Effect of oral administration of selenium (0.05 mg/kg b.wt.) on renal antioxidants activities and lipid peroxidation of male rabbits fed on dietary ochratoxin A (0.1 ppm) for 21 days.

Groups / Parameters	Control	OTA	OTA+Se
SOD (U/g kidney)	32.29±0.17 a	11.45±0.10 b	26.40±0.15 c
CAT (U/g kidney)	1.57±0.08 a	0.45±0.02 b	0.88±0.03 c
GSH (U/g kidney)	5.05±0.01 a	1.12±0.05 b	3.48±0.04 c
GSH- Px (U/g kidney)	71.11±0.12 a	22.17±0.09 b	67.61±0.14 c
MDA (nmol/g kidney)	5.87±0.06 a	18.36±0.08 b	12.41±0.02 c

Data are represented as means of samples ± S.E. Mean in the same row with different letters are significantly different (Duncan multiple range test $P < 0.05$).

Clinical Signs:

In the present study rabbits administrated ochratoxin (OTA) (0.1 mg/kg diet) showed depression, reduced food intake, weakness, dullness and diarrhea in few cases followed by emaciation . On the other hand, treated group (3rd group) showed limited apparent signs.

Macroscopic Pathology:

Gross lesions in 2nd group revealed congestion, enlargement, petechial haemorrhage on surface of the liver and kidney **(fig. 1 A, B)** but rabbits treated with ochratoxin and selenium (as sodium selenite) in 3rd group the lesion were mild represented by paleness of liver and kidney **(fig. 2 A, B)** and both organs were normal in consistency.

Microscpic Pathology:

The cytotoxicity of OTA in the present study was demonstrated in rabbits treated with 0.1 mg OTA /kg feed for 21 day where kidneys showed atrophic glomerular tufts, atrophic proximal tubules with hydropic degeneration of their lining epithelial cells **(fig. 3),** glomerular atrophy with dilatation in glomerular space **(fig. 4**). Dilatation of renal tubules which filed with cellular debris and hyaline casts in addition to congested blood vessel in between the tubules (**fig. 5)**. While liver revealed congestion of blood vessels in portal area with dilatation of hepatic sinusoids **(fig. 6).** In respect of rabbits exposed to ochratoxin and selenium (as sodium selenite) in the 3rd group, kidney revealed few focal areas of necrosis in some renal tubular epithelial cells **(fig. 7),** but liver showed mild hydropic dergeneration of the hepatocytes and mildly dilated central vein (**fig. 8)** and mild vacuolization of hepatocytes were seen **(fig. 9)**.

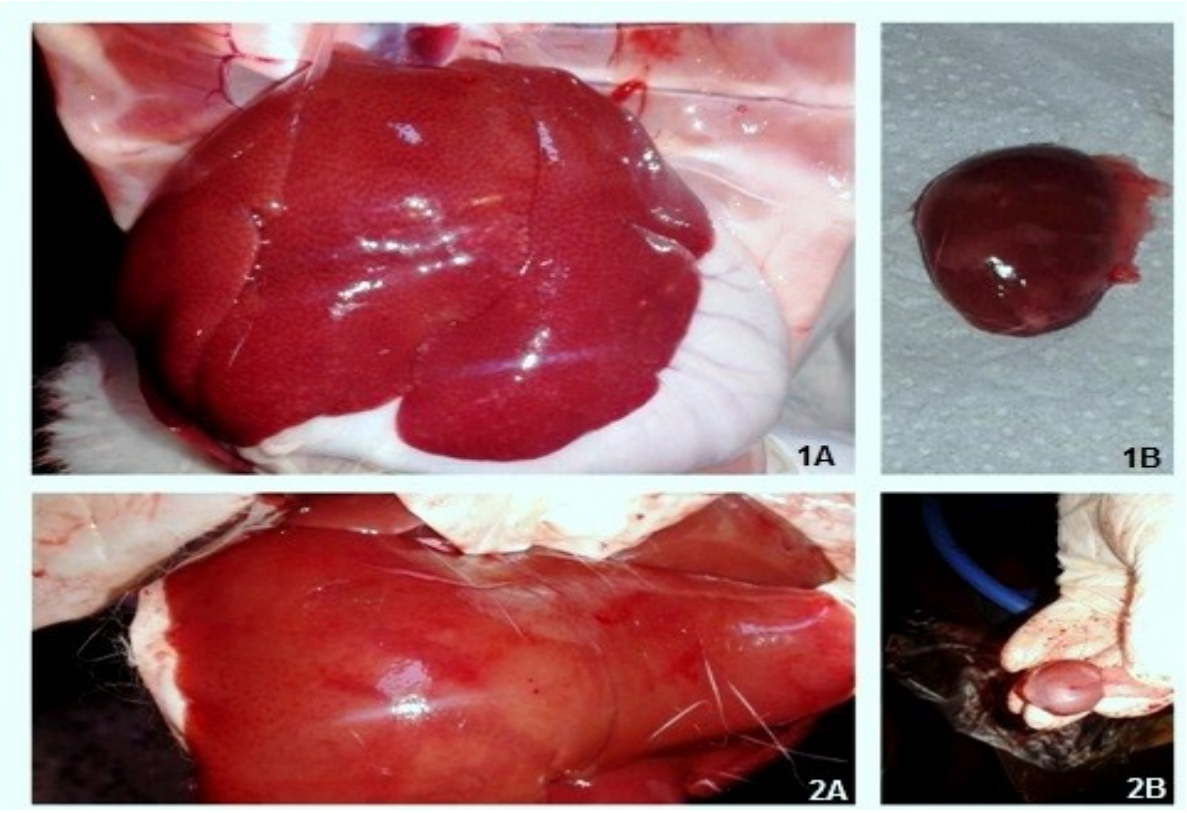

Plate (1).

Fig (1): Rabbit exposed to ochratoxin A showing congestion, enlargement, petechial haemorrhage on surface of the liver (1A) and kidney (1B).

Fig (2): Rabbit exposed to ochratoxin A and selenium showing paleness of liver (2A) and Kidney 2)B).

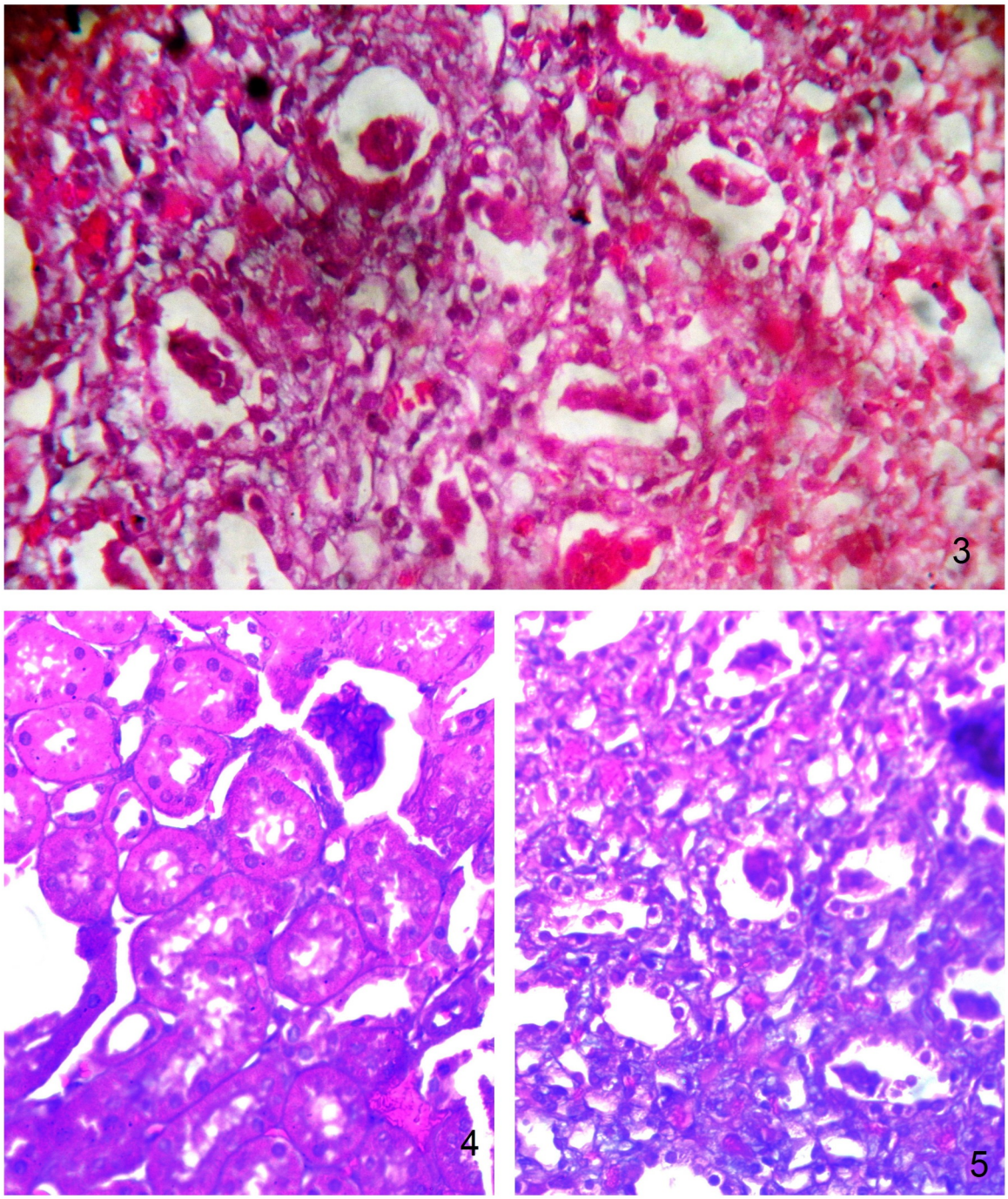

Plate (2).

Fig. (3): Kidney of a rabbit exposed to ochratoxin A showing atrophic glomerular tufts, atrophic proximal tubules with hydropic degeneration of their lining epithelial cells (H&E 200).

Fig. (4): Kidney of rabbit exposed to ochratoxin A showing glomerular atrophy with dilatation in glomerular space (H&E 400).

Fig. (5): Kidney of a rabbit exposed to ochratoxin A showing dilatation of renal tubules which filed with cellular debris and hyaline casts in addition to congested blood vessel in between the tubules.

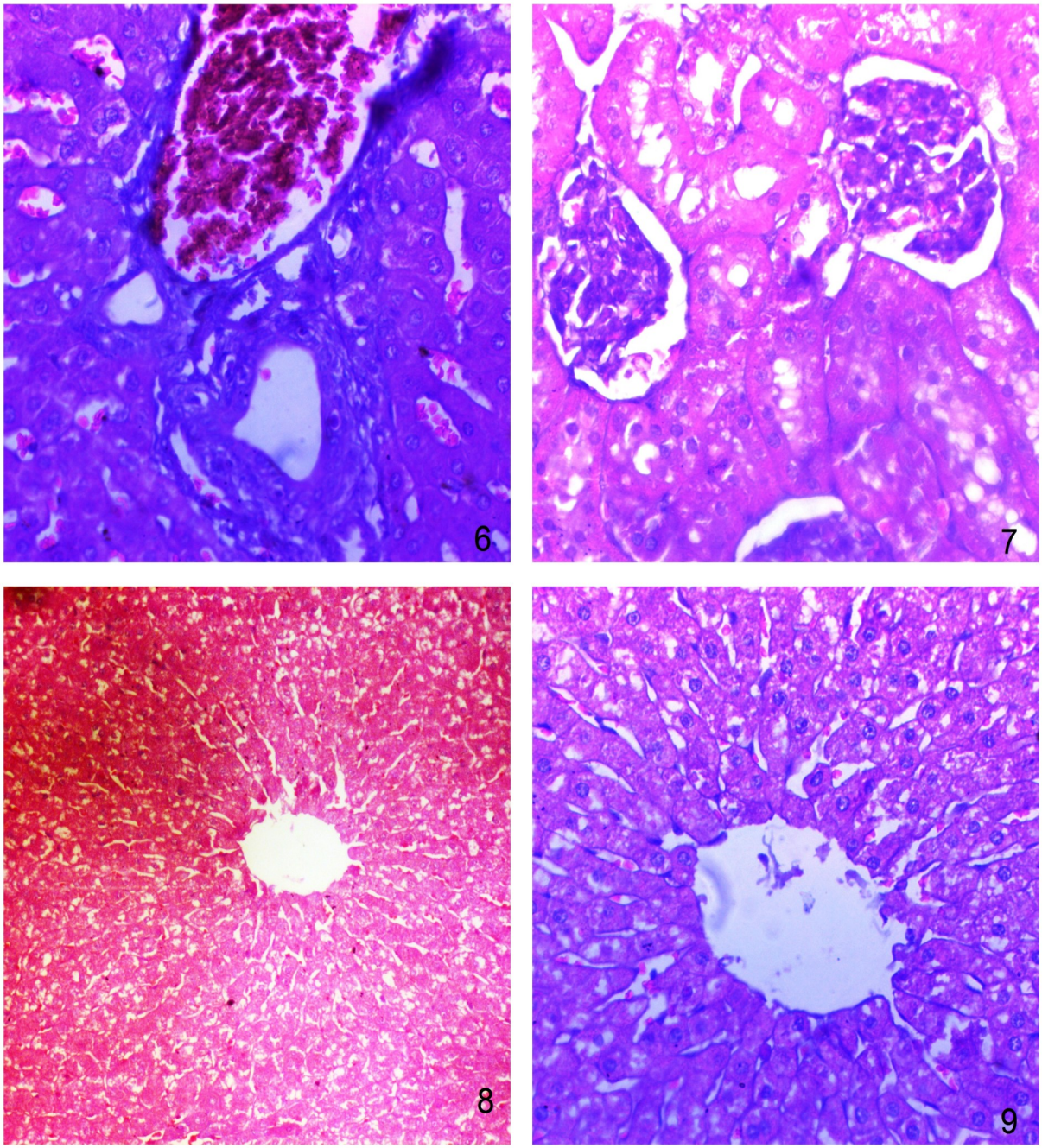

Plate (3).

Fig. (6): Liver of rabbit exposed to ochratoxin A showing congestion of blood vessels in portal area with dilatation of hepatic sinusoids (H&E 400).

Fig. (7): Kidney of rabbit exposed to ochratoxin A and selenium showing few focal areas of necrosis in some renal tubular epithelial cells (H&E 400).

Fig. (8): Liver of a rabbit exposed to ochratoxin A and selenium showing mild hydropic dergeneration of the hepatocytes and mildly dilated central vein (H&E200).

Fig. (9): Liver of rabbit liver exposed to ochratoxin A and selenium showing mild vacuolization of Hepatocytes (H&E400).

Discussion

Ochratoxin A (OTA) is classified as group 1 carcinogens. The toxicity of OTA may be attributed to its isocoumarin moiety and lactone carbonyl group. OTA reduces the expression of antioxidant enzymes and induced renal toxicity **(Boesch-Saadatmandi *et al.*, 2009)**. Interference with metabolic systems and promotion of membrane lipid peroxidation are the mechanisms proposed for OTA toxicity and OTA renal tumor formation. The role for oxidative stress in OTA toxicity and carcinogenicity have been evidenced. OTA exposure results in the overproduction of free radicals, increased reactive oxygen species (ROS) production, as well as oxidative damage (lipids, proteins, and DNA). Protection against OTA-induced DNA damage, lipid peroxidation, as well as cytotoxicity confirming the link between OTA toxicity and oxidative damage (**Sorrenti *et al.*, 2013).**

In the present study, Ochratoxin A administration induced hepatotoxic and nephrotoxic effects in rabbits and resulted in significant elevation in serum ALT, AST, ALP enzymes, serum levels of creatinine and urea, while evoked a significant reduction in serum total protein and albumin levels whereas globulin levels were not altered in 2nd group in comparison to control group. These results were in agreement with **Sivakumar *et al.* (2009)**, **Mir and Dwivedi (2010)** and **Al-Masri *et al.* (2012)**. The increase in the activities of the ALT, AST, and ALP enzymes caused by OTA intoxication could be attributed to degenerative changes in the liver leading to leakage of enzymes into serum. The increase in the levels of creatinine and urea are suggestive of nephrotoxicity. Urea and creatinine, which depend on glomerular filtration for their excretion, accumulate almost in proportion to the number of nephrons that have been destroyed and hence directly reflect the functional status of the kidneys (**Sivakumar *et al.*, 2009)**. The decrease in total protein and albumin levels might have been due to OTA induced chronic liver damage which constitutes the major source of plasma proteins, as well as to proteinuria (**Elaroussi *et al.*, 2008)**. OTA binds very strongly to human and animal albumin. Inhibition of protein synthesis and energy production, induction of oxidative stress, as well as apoptosis/necrosis and cell cycle arrest are possibly involved in OTA toxic action **(Kőszegi and Poór, 2016)**. It was proved that the accumulation of OTA in the kidney is due to strong binding of OTA to plasma proteins and its long half-life in plasma **(Mantle *et al.*, 2015**).

Among ochratoxins, OTA shows the highest toxicity. OTA treatment induced GSH depletion and ROS production **(Palabiyik *et al.*, 2013)**. Oxidation of OTA by cytochrome-p 450 (CYP450) enzymes also produces a reactive electrophilic product called OTA-quinone (OTQ). OTQ can be partially detoxified by conjugation with GSH or it possibly forms OTA-hydroquinone (OTHQ) after reduction **(Reljic *et al.*, 2014)**. Dietary exposure to OTA has been associated with several human and animal diseases including poultry ochratoxicosis, porcine nephropathy. It was demonstrated that antioxidants are able to counteract the deleterious effects of chronic consumption of OTA. Electrophiles generated from OTA metabolism react with reduced glutathione (GSH) to produce GSH-conjugates. In liver quantities of OTAα and OTHQ-GSH in addition to OTB-GSH were generated. Moreover, in kidney, GSH conjugates may be involved in the nephrotoxicity **(Heussner and Bingle, 2015)**. The ability of OTA to generate free radicals which may lead to DNA breakage, inhibition of protein biosynthesis, lipid peroxidation, disruption of oxidative phosphorylation in mitochondria, apoptosis, and interference with signal transduction in some cell types causing different organ pathogenesis **(Abdel-Aziz *et al.*, 2010)**. It was shown in liver cell cultures that OTA significantly increases ROS concentration and expression of several metallothioneins, while reducing superoxide dismutase (SOD) activity and catalase mRNA levels **(Zheng *et al.*, 2013).** It was suggested that low concentrations of OTA induced apoptosis and lipid peroxidation in the rabbit kidney, which appeared to play a major role in the pathogenesis of nephrotoxicity. OTA-induced apoptosis and oxidative stress in rabbit kidneys, leading to the pathogenesis of nephrotoxicity. Since OTA-induced nephrotoxicity is associated with oxidative damage **(Kumar *et al.*, 2014).** In accordance to the present study, OTA induced a

significant increase in lipid peroxidation as well as a significant decrease in activity of enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic antioxidants (glutathione) in the kidney and liver of mice **(Chakraborty and Verma, 2010)**.

Selenium is an essential trace element in animal known as a highly effective antioxidant and its metabolic role in animals is its presence in the active site of the selenoenzyme (GSH-PX). This enzyme, together with superoxide dismutase and catalase, protects cell against damage caused by free radicals and lipoperoxides **(Wang *et al.*, 2013)**. The biological effects of Se are due to its incorporation into the selenocysteine and further into the selenoproteins **(Hoffmann *et al.* 2010)**. Selenoproteins, such as Se-dependent glutathione peroxidase (GPx) and thioredoxinreductase (TR) are involved in the cellular antioxidant defense system **(Cao *et al.*, 2012)**. In agreement with the present study, the oral supplementation of selenium (Se) in rabbits improved some biochemical parameters seems to be a simple and effective antioxidant to reduce the imbalance state between the formation of free radicals and antioxidant systems **(Rouabhi *et al.*, 2015).** In the current study, the increased rate of hepatic lipid peroxidation (MDA), the depletion of GSH, increase free radicals and decrease the enzymatic activity of GPx, these are important factors responsible for tissue damage. Catalase (CAT) is the second step in the enzymatic defense system. It supports the hydrogen peroxide produced previously by the SODs and metabolizes into water. In liver cells, supplementation of selenium resulted in substantial improvement, where the rate of glutathione, MDA, the enzymatic activities of GPx, GST and CAT levels in the liver retrieved. This is due to the antioxidant effect of selenium, which is a cofactor of many antioxidant enzymes such as glutathione peroxidase GPx, the thioredoxin reductase, wherein the activity of these enzymes is very dependent on the intake of selenium **(AL-Rasheed *et al.*, 2013).** Selenium is recognized as an essential trace element that plays an important role in antioxidant system as a component of Se-dependent glutathione peroxidase. Administration of organic selenium, increased plasma total proteins, globulins, alkaline phosphatase. Seminal plasma aspartate aminotransferase, alanine aminotransferase and lipid peroxidation were significantly decreased. while, seminal plasma antioxidant enzymes were significantly increased due to Se supplementation in rabbit **(Kamel, 2012)**. Selenium in combination with α-lipoic acid was found to be effective in prevention and treatment of nephrotoxicity, as well as decrease creatinine and increase antioxidant activity (AOA) **(Tahira *et al.*, 2012)**. Dietary supplementation of organic Se diets elevated serum total antioxioxidant capacity and reduced the lipid peroxidation expressed as serum malondialdehyde which finally translated into enhancing the immune response in the growing rabbits **(Ebeid *et al.*, 2012)**. Se is one of the essential trace mineral serving as an essential co-factor in the antioxidant enzyme glutathione peroxidase (GSH-Px), as well as catalase (CAT) and superoxide dismutase (SOD) in the body, to reduce the damaging effects of reactive oxygen species (ROS) and numerous peroxides in rabbits. Antioxidant capacity of growing rabbits was improved when offered supplementary dietary Se at a rate of 0.24 mg Se/kg DM **(Zhang *et al.*, 2011).**

In the present sudy the clinical signs which represented by depression, reduced food intake, weakness, dullness and diarrhea in few cases followed by emaciation may be due to toxic immunosuppressive effect of OTA which acting as stress factor and affect of internal organs of rabbits and these results were in accordance to **Shimaa (2011).** Clinical signs forms in the toxicated rabbits may be attributed to their immunological status and the amount of consumed OTA contaminated diet **(Gaheen, 2017)**. In addition, gross lesions in 2nd group revealed congestion, enlargement, petechial haemorrhage on surface of the liver and kidney. The enlargement of kidney and liver are attributed to the involvement of these organs in detoxification and elimination. While rabbits treated with ochratoxin and selenium in 3rd group, the lesions were mild represented by paleness of liver and kidney and both organs were normal in consistency. These results are in the same line with the results obtained by **Mir *et al.* (1999)** in rabbits.

In the current study, the cytotoxicity of OTA was demonstrated in rabbits treated with 0.1

ppm OTA in feed for 21 day where kidneys showed dilated renal tubules lined by hydropic degenerated epithelial cells with occasional luminal casts and intervening stromal blood vessels are congested, atrophic glomerular tufts, atrophic proximal tubules, but liver revealed dilated and congested central veins with dilated sinusoids containing extra-vasated RBCs, microsteatosis in addition to dilated bile duct, and the cords of hepatocytes revealed hydropic degeneration.

The histological changes observed in the kidney and liver of rabbits due to Ochratoxin A treatment in the present study may be attributed to the increase in lipid peroxidation level and glutathione depletion **(Baudrimant *et al*., 2001** and **Meki and Hussein, 2001)** that lead to oxidative damage of kidney and liver induced by OTA. Our results agree with **Francisco and Maria (2010)** in rats they mentioned that OTA induce degeneration and necrosis in epithelial cells of proximal convoluted tubules and these lesions could be related to oxidative damage of ochratoxin, and **Abdu *et al*. (2011)** also in rats who reported that OTA exerts its cytotoxic effect by causing inhibition of protein synthesis, peroxidation of membrane phospholipids and inhibition of ATP production in addition to **Gaheen (2017)** in chickens and **Jan *et al.* (2017)** in rabbits who reported that the damage to hepatocytes and accumulation of lipids in the cells, presumably fatty change due to decreased apo-protein synthesis in rabbits.

Furthermore, the proximal tubule of the kidney is the primary site targeted in OTA-induced nephrotoxicity **(Suzuki *et al*., 1975)**. OTA (99%) is bound to albumin, which can't be excreted by glomerular filtrate. However, unbound portion (1%) can be found in the urinary filtrate. The remaining OTA is only execrated via organic Anion Transporter (OAT) route, which prone the proximal tubular epithelial cells to damage, by virtue of depletion of indigenous dicarboxylic acid (glutarate, ketoglutarate) on expense of OTA internalization **(Sekine *et al*., 2006 and Khatoon *et al*., 2016)**. In accordance with our result, **Siddiqui *et al.* (2002)** and **Milicevic *et al.* (2009)** found that ochratoxin A induced moderate and obvious degenerative changes, swelling, and vacuolar degeneration were the main changes in the kidney tubular epithelial cells. Alteration of the morphological structure of the kidney and liver tissues of OTA treated groups may be due to the oxidative damage occurred in the cellular membranes by the accumulation of OTA oxidant metabolites, and by direct or indirect inhibition of antioxidant enzymes, reducing the total antioxidant protection of the cell, which affecting membrane structure and function and altering the physiological processes of these tissues **(Ramirez *et al*., 2007**).

On the other hand, the powerful antioxidant capacity of selenium which appeared in the present investigation in the form of histological recovery of kidney and liver tissues which showed apparent normal sized and shaped of the glomeruli and renal tubules revealed hydropic degeneration of the epithelial cell lining, while liver revealed mild hydropic dergeneration of the hepatocytes and mildly dilated central vein may be attributed to its potential to eliminate free radicals by the donation of electrons**.** Such ameliorative effect could be attributed to the selenium (Se), as trace element that plays a key role in antioxidative defense. Selenoprotein S (SelS) overexpression increased glutathione (GSH) levels and decreased reactive oxygen species (ROS) and malondialdehyde levels in cells, regardless of OTA treatment. SelS overexpression and lowerxpression affect OTA-induced cytotoxicity and apoptosis by modulating the oxidative stress and phosphorylation which demonstrates the relationship between SelS- and OTA **(Gan *et al*., 2017).** It was suggest that selenium alleviates OTA-induced nephrotoxicity by improving seleno-enzyme expression and by promoting antioxidant capacity. Therefore, selenomethionine supplementation may protect humans and animals from the risk of kidney damage caused by OTA **(Gan *et al.* 2015**).

Conclusion

In conclusion, lipid peroxidation and the decline in antioxidants induced by OTA appeared to cause hepato-renal damage. Hepatic vascular congestion induced alterations in the hepatocyte membrane permeability and elevated hepatic enzymes activities. Hepato-renal damage resulted in increased urea, creatinine levels and inhibition of protein synthesis.

In addition, Se supplementation (as sodium selenite) antagonized the adverse effects of free radicals generated by OTA exposure by enhancing the antioxidants activities and reducing lipid peroxidation in liver and kidneys. Se appeared to ameliorate hepato-renal histopathological damage and improve liver and kidneys functions in male rabbits and thus the protective role of selenium against OTA toxicity was emphasized.

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