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Oxidative Stress of *in-Ovo* Ochratoxin A Administered during Chick Embryonic Development

ABSTRACT

The present work was carried out to study the effect of *in-ovo* injection of ochratoxin A (OTA) as an oxidative stress and its consequences on hepatic and kidney functions, thyroid activity, and histological examination of brain and liver in chicken embryos and subsequently in the hatching chicks. On the 10th day of incubation, one hundred and sixty-two fertile eggs were randomly divided into two equal treatments. Control treatment, (injected by 50 µl sodium carbonate) and OTA treatment (injected by 12.5 ng OTA dissolved in 50 µl sodium carbonate). OTA treatment group significantly reduced glutathione (GSH) and significantly increased thiobarbituric acid reactive substances production (TBARS) in embryonic and hatched chicks regarding livers, spleen, bursa of Fabricius, heart, and brain as an indicator of oxidative stress. OTA injection increased TBARS and decreased GSH levels in both allantoic and amniotic fluids. On the 14th and 16th days of incubation and at the hatch, a significant lower concentration in cholesterol and higher concentrations of alanine amino transferase, aspartate amino transferase, alkaline phosphatase, gamma glutamyl transferase, acid phosphatase enzymes activities and triglycerides in the hepatic tissues of the OTA group were observed. Histological examination of OTA group of brain and liver tissues showed some degenerative changes through the experimental period. In conclusion, *in-ovo* OTA treated had teratogenic and embryotoxic effects.

INTRODUCTION

Ochratoxins are a group of structurally related, secondary metabolites produced by several toxigenic species of *Aspergillus* and *Penicillium*; they contaminate various raw agricultural commodities and have very diversified toxic effects in humans and animals (Battacone *et al.*, 2010).

The most relevant toxic effect of OTA in animal cells is the production of reactive oxygen species (ROS) that leads to the inhibition of protein synthesis; lipid peroxidation and DNA damage, all resulting in OTA-mediated oxidative damage (Ringot *et al.*, 2006). Evidence for a role of oxidative damage in the toxicological mutagenic, teratogenic, embryotoxic, hepatotoxic, and nephrotoxic properties of OTA has been collected and described (Zahoor-ul-Hassan *et al.*, 2012). Antioxidants can counteract the deleterious effects of chronic consumption or exposure to OTA confirming the link between OTA toxicity and oxidative stress (Sorrenti *et al.*, 2013). OTA within a wide range of contamination (130 µg - 3.9 mg OTA/Kg) in poultry diets lead to the reduction in growth rate, feed consumption, feed efficiency, reduced immunity and increased mortality (Elaroussi *et al.*, 2006). Chickens that consume sub-lethal doses of OTA lead to accumulate the toxin in their tissues and eggs, a dose response relationship was observed (Elaroussi *et al.*, 2008). OTA was observed in all body tissues with the highest level in the kidney



(124 ppb) after laying hens were fed OTA-contaminated ration with 0.5 or 5 mg for 14 days (Frye & Chu, 1977). Niemiec *et al.* (1994) detected OTA in the eggs, blood serum, liver and kidney of hens, roosters and one-day-old chicks of birds fed diets containing 2.1 or 4.1 mg OTA. They found that, as OTA content in the feed increased, the percentage of hatched eggs decreased and survival time of embryos dying during incubation declined. Also, the mass of embryos weighted on the 6th and 8th days of incubation and the hatched chicks were lower in the OTA treated group than the control group (Niemiec *et al.*, 1994).

Therefore, the present work was designed to investigate the effect of *in-ovo* injection of ochratoxin A (OTA) as an oxidative stress and its consequences on hepatic and kidney functions, thyroid activity, and histological examination of brain and liver in chicken embryos and subsequently in the hatching chicks.

MATERIALS AND METHODS

This study was conducted at both the poultry research unit and physiology and biochemistry unit of the Biological Applications Department of the Nuclear Research Center, Abou-Zaabal, Egypt. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Ochratoxin

Ochratoxin A from *Aspergillus ochraceus* (303-47-9) Ec No. 206-143-7 lot 80 K4054 O was purchased from Sigma Company, Cat No. 1877.

Eggs and experimental design

One hundred and eighty Hy-line layer eggs were a generous gift from Miser Poultry Company (MIPCO). Eggs were incubated in Victoria incubator (Via Lardirago, 4, PAVIA (PV), ITALY) at 37.5°C and 60% RH, air sac upside (vertically) and turned 12 times daily at a 45 angle during the first 19 days of incubation. On the 10th day of incubation, the eggs were examined by light candling to remove infertile eggs and to mark air cell border. One hundred and sixty-two fertile eggs were randomly divided into two equal groups with three replicates for each group (27 fertile eggs for each replicate). The first group was injected in the air cell above the mark with 50 µl sodium bicarbonate and served as a control. While the second group was injected with 12.5 ng OTA/egg dissolved in 50 µl sodium bicarbonate and served as a treatment group. The injection site sealed with tape and eggs returned to the incubator.

Samples collection and analysis

Blood samples and analysis

On the 14th and 16th days of incubation, nine blood samples from each group (three blood samples per replicate) were collected from the vitelline vein using a 27 gauge insulin syringe (blood samples were collected from five embryos). At hatch, the blood samples were collected from the jugular vein of nine chicks per each group (three hatchlings per replicate). Then the blood samples were allowed to clot at room temperature for 30 min and then centrifuged to separate the serum, which was frozen and stored at -20°C until hormonal analyses. Serum total thyroxine (T₄) was assayed by radioimmunoassay (RIA) depending on solid phase RIA (Izotop) using a kit (produced by Institute of Isotopes Co., Ltd. Konkoly-Thege Miklós út 29-33, H-1211 Budapest, Hungary).

Allantoic and amniotic fluids

On the 12th, 14th and 16th days of incubation, allantoic and amniotic fluids were collected by 23 gauge needles syringes to determine thiobarbituric acid reactive substances (TBARS) (Yoshioka *et al.*, 1979) and glutathione (GSH) levels (Beutler *et al.*, 1963) in both fluids. While, uric acid (White *et al.*, 1970) and creatinine (Faukemar & King, 1976) concentrations were determined in the allantoic fluid.

Organs samples and analysis

On the 14th, 16th days of incubation and at the hatch, brain, liver, spleen, bursa of Fabricius and heart were extracted and washed in ice cold saline and blotted individually on filter paper. Then the tissues homogenized separately in phosphate buffer, pH 7.4 and kept at -20°C for further estimation of biochemical assays. Organs thiobarbituric acid reactive substances (TBARS) (Yoshioka *et al.*, 1979) and glutathione (GSH) levels (Beutler *et al.*, 1963) were determined. In addition, alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined in hepatic tissue (Reitman & Frankel, 1957). Gamma glutamyl transferase (GGT) (Szasz, 1969), alkaline phosphatase (ALP) (Beliefld & Goldberg, 1971) and acid phosphatase (ACP) (Moss, 1984) activities in hepatic tissue were estimated. Finally, cholesterol (Allain *et al.*, 1974) and triglycerides (Fossati & Principe, 1982) concentrations were determined in hepatic tissue.

Histological examination

Small pieces of the brain and liver were quickly removed and fixed in 10% buffered formalin solution.



After fixation, specimens were dehydrated in graded ethanol, embedded in wax, sectioned at 5 microns thickness. Then, the sections were stained with Haematoxylin and Eosin (Banchraft *et al.*, 1996) and examined under Olympus light microscope.

Statistical analysis

The data were statistically analyzed by one-way analysis of variance using the General Linear Model Procedure of the SAS software (SAS, 2002). Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) at $p < 0.05$. The model applied was:

$$Y_i = \mu + T_i + E_j$$

Where: Y_i = any value from the overall population.

μ = overall mean.

T_i = the effect of the i^{th} treatment ($i=1$, control; 2, OTA).

E_j = the random error associated with the j^{th} individual.

RESULTS

Effect of OTA on TBARS and GSH levels

The effect of OTA as an oxidative stress was demonstrated by measuring TBARS and GSH levels in the brain, liver, spleen, bursa of Fabricius and heart of embryos and hatchlings. It was found that on the 14th and 16th days of incubation and at the hatch, *in-ovo* OTA injection significantly increased ($p < 0.05$) TBARS levels (Table 1) and significantly decreased ($p < 0.05$) GSH levels (Table 2) in the previous organs as compared to the control. Similarly, on the 12th, 14th and 16th days of incubation, *in-ovo* OTA injection significantly increased ($p < 0.05$) TBARS level in both embryonic allantoic and amniotic fluids which was accompanied with a significant decrease ($p < 0.05$) in GSH level in both fluids as compared to the control group (Table 3).

Table 1 – Effect of *in-ovo* OTA injection on thiobarbituric acid reactive substances (TBARS nmol/g wet tissue) in some organs of embryos and hatchlings.

Organs	Treat.	Age			Overall means \pm SEM
		Day 14	Day 16	At hatch	
Brain	Cont.	39.60 \pm 2.94 ^b	67.33 \pm 0.64 ^b	87.88 \pm 6.19 ^b	64.93 \pm 5.25 ^b
	OTA	96.09 \pm 1.32 ^a	107.43 \pm 4.70 ^a	117.28 \pm 7.06 ^a	101.37 \pm 4.82 ^a
Liver	Cont.	77.32 \pm 2.83 ^b	115.88 \pm 1.44 ^b	174.5 \pm 5.71 ^b	109.89 \pm 8.30 ^b
	OTA	101.65 \pm 2.91 ^a	132.51 \pm 3.89 ^a	298.44 \pm 1.05 ^a	156.04 \pm 16.60 ^a
Spleen	Cont.	32.85 \pm 0.53 ^b	40.22 \pm 0.22 ^b	35.74 \pm 1.10 ^b	36.13 \pm 0.95 ^b
	OTA	39.3 \pm 1.06 ^a	60.18 \pm 2.61 ^a	43.16 \pm 1.09 ^a	46.47 \pm 2.53 ^a
Bursa	Cont.	32.24 \pm 0.38 ^b	46.66 \pm 1.85 ^b	56.95 \pm 0.91 ^b	46.97 \pm 3.11 ^b
	OTA	60.40 \pm 0.91 ^a	56.59 \pm 3.36 ^a	60.66 \pm 0.95 ^a	59.64 \pm 0.95 ^a
Heart	Cont.	77.16 \pm 0.75 ^b	96.40 \pm 0.75 ^b	147.21 \pm 4.13 ^b	112.87 \pm 7.93 ^a
	OTA	80.23 \pm 0.68 ^a	113.18 \pm 4.16 ^a	190.13 \pm 4.31 ^a	139.37 \pm 12.03 ^a

*Values are expressed means \pm standard error of the mean.

a, b, Means with different superscripts within the same age indicate significant differences between treatments by Duncan's Multiple Range Test ($p < 0.05$).

N = 9 per treatment. TBARS = Thiobarbituric acid reactive substances.

Table 2 – Effect of *in-ovo* OTA injection on glutathione level (GSH) (mg/g wet tissue) in some organs of embryos and hatchlings.

Organs	Treat.	Age			Overall means \pm SEM
		Day 14	Day 16	At hatch	
Brain	Cont.	18.44 \pm 0.31 ^a	20.84 \pm 1.08 ^a	19.72 \pm 0.95 ^a	19.66 \pm 0.52 ^a
	OTA	14.59 \pm 0.98 ^b	16.64 \pm 0.44 ^b	16.74 \pm 0.58 ^b	15.99 \pm 0.45 ^b
Liver	Cont.	20.04 \pm 0.48 ^a	21.98 \pm 0.48 ^a	21.06 \pm 0.98 ^a	21.29 \pm 0.38 ^a
	OTA	17.58 \pm 0.38 ^b	16.69 \pm 0.45 ^b	18.30 \pm 0.48 ^b	17.34 \pm 0.29 ^b
Spleen	Cont.	24.81 \pm 0.12 ^a	20.35 \pm 0.49 ^a	28.62 \pm 0.12 ^a	24.59 \pm 1.20 ^a
	OTA	17.38 \pm 1.95 ^b	16.75 \pm 1.34 ^b	18.02 \pm 1.59 ^b	17.38 \pm 0.84 ^b
Bursa	Cont.	24.38 \pm 2.32 ^a	23.53 \pm 0.36 ^a	25.23 \pm 0.61 ^a	22.75 \pm 0.93 ^a
	OTA	17.38 \pm 0.73 ^b	18.45 \pm 1.34 ^b	20.35 \pm 0.73 ^b	20.35 \pm 1.31 ^a
Heart	Cont.	20.35 \pm 0.49 ^a	21.41 \pm 0.61 ^a	21.2 \pm 0.39 ^a	21.04 \pm 0.27 ^a
	OTA	16.33 \pm 0.12 ^b	14.29 \pm 0.35 ^b	16.61 \pm 1.01 ^b	15.95 \pm 0.56 ^b

*Values are expressed means \pm standard error of the mean.

a, b, Means with different superscripts within the same age indicate significant differences between treatments by Duncan's Multiple Range Test ($p < 0.05$).

N = 9 per treatment. GSH = Glutathione.



Table 3 – Effect of *in-ovo* OTA injection on thiobarbituric acid reactive substances (TBARS nmol/dl) and glutathione (GSH) (mg/dl) levels in allantoic and amniotic fluids of developing chick embryos.

Parameters	Treat.	Age			Overall means ± SEM	
		Day 12	Day 14	Day 16		
Allantoic	TBARS (nmol/dl)	Cont.	0.65±0.07 ^b	3.20±0.34 ^b	5.26±1.02 ^b	3.03±0.48 ^b
		OTA	2.24±0.34 ^a	6.15±0.95 ^a	11.28±0.91 ^a	6.55±0.99 ^a
	GSH (mg/dl)	Cont.	23.00±0.88 ^a	27.33±1.07 ^a	52.45±0.57 ^a	34.26±3.18 ^a
		OTA	19.35±0.41 ^b	22.32±0.48 ^b	38.36±0.80 ^b	26.67±2.05 ^a
Amniotic	TBARS (nmol/dl)	Cont.	0.38±0.05 ^b	14.72±0.44 ^b	16.1±1.14 ^b	10.40±1.76 ^b
		OTA	1.3±0.34 ^a	25.62±0.56 ^a	27.81±2.42 ^a	18.24±3.01 ^a
	GSH (mg/dl)	Cont.	25.21±0.87 ^a	43.97±1.61 ^a	60.26±3.21 ^a	43.14±3.66 ^a
		OTA	18.93±0.31 ^b	22.07±0.73 ^b	21.81±0.72 ^b	20.93±0.48 ^b

*Values are expressed means ± standard error of the mean.

a, b, Means with different superscripts within the same age indicate significant differences between treatments by Duncan's Multiple Range Test ($p < 0.05$).

N = 9 per treatment.

TBARS = Thiobarbituric acid reactive substances.

GSH = Glutathione.

Effect of OTA on some biochemicals analysis in hepatic tissues

The effect of *in-ovo* OTA injection on some biochemical analysis in the hepatic tissues of embryos and hatchlings are demonstrated in Table 4. Results

demonstrated that *in-ovo* OTA injection significantly increased ($p < 0.05$) hepatic tissues contents of ALT, AST, ALP, GGT, ACP, and triglycerides while it significantly decreased ($p < 0.05$) cholesterol level as compared to the control group (Table 4).

Table 4 – Effect of *in-ovo* OTA injection on some biochemicals in hepatic tissues of embryos and hatchlings.

Parameters	Treat.	Age			Overall means ± SEM
		Day 14	Day 16	At hatch	
ALT (U/g wet tissue)	Cont.	1.95±0.54 ^b	2.08±0.21 ^b	2.83±0.05 ^b	2.17±0.19 ^b
	OTA	4.19±0.64 ^a	3.12±0.31 ^a	6.62±0.03 ^a	4.03±0.41 ^a
AST (U/g wet tissue)	Cont.	4.48±0.64 ^b	6.59±0.50 ^b	10.19±0.69 ^b	6.93±0.65 ^b
	OTA	8.98±0.81 ^a	10.84±0.70 ^a	15.79±0.93 ^a	11.32±0.77 ^a
ALP (IU/g wet tissue)	Cont.	4.38±0.68 ^b	3.34±0.28 ^b	3.02±0.26 ^b	3.43±0.26 ^b
	OTA	7.45±0.79 ^a	4.44±0.19 ^a	4.37±0.39 ^a	5.04±0.43 ^a
GGT (U/g wet tissue)	Cont.	3.91±0.44 ^b	2.88±0.27 ^b	6.82±0.16 ^b	4.31±0.45 ^b
	OTA	6.83±0.45 ^a	7.98±0.64 ^a	12.22±0.14 ^a	8.23±0.65 ^a
ACP (U/g wet tissue)	Cont.	2.61±0.08 ^b	1.73±0.13 ^b	1.82±0.04 ^b	2.02±0.13 ^a
	OTA	2.99±0.02 ^a	2.31±0.20 ^a	2.14±0.08 ^a	2.33±0.11 ^a
Cholesterol (mg/g wet tissue)	Cont.	4.73±0.55 ^a	6.42±0.71 ^a	5.59±0.45 ^a	5.41±0.33 ^a
	OTA	1.85±0.46 ^b	3.07±0.93 ^b	3.75±0.22 ^b	3.10±0.34 ^b
Triglycerides (mg/g wet tissue)	Cont.	7.84±0.22 ^b	7.98±0.29 ^b	9.65±0.78 ^b	8.36±0.31 ^b
	OTA	11.44±0.78 ^a	9.80±0.57 ^a	13.83±1.52 ^a	11.42±0.71 ^a

*Values are expressed means ± standard error of the mean.

a, b, Means with different superscripts within the same age indicate significant differences between treatments by Duncan's Multiple Range Test ($p < 0.05$).

N = 9 per treatment.

ALT = Alanine transaminase. ALP = Alkaline phosphatase. ACP = Acid phosphatase. AST = Aspartate transaminase. GGT = Gamma glutamyl transferase.

Effect of OTA injection on serum thyroid hormones

Results showed that serum T₄ levels significantly increased ($p < 0.05$) in the OTA group as compared to the control group (Table 5).

Effect of OTA on the excretory system

Data demonstrated that *in-ovo* OTA injection caused a significant increase ($p < 0.05$) in allantoic fluid, uric acid and creatinine concentrations on the 12th, 14th and 16th days of incubation as compared to the control group (Table 6).



Table 5 – Effect of *in-ovo* OTA injection on serum thyroxin concentration of embryos and hatchlings.

Thyroid	Function	Treat.	Age			Overall means ± SEM
			Day 14	Day 16	At hatch	
T ₄ (µg/ dl)		Cont.	0.26±0.01 ^b	0.91±0.63 ^b	1.53±0.06 ^b	0.90±0.25 ^b
		OTA	2.03±0.09 ^a	2.97±0.23 ^a	5.90±0.41 ^a	3.66±0.61 ^a

*Values are expressed means ± standard error of the mean.

a, b, Means with different superscripts within the same age indicate significant differences between treatments by Duncan's Multiple Range Test ($p < 0.05$).

N = 9 per treatment. T4 = Thyroxin hormone.

Table 6 – Effect of *in-ovo* OTA injection on some kidney functions as measured in allantoic fluid of developing chick embryos.

kidney function	Treat.	Age (days)			Overall means ± SEM
		Day 12	Day 14	Day 16	
Uric acid (mg/dl)	Cont.	1.6±0.02 ^b	1.84±0.03 ^b	2.36±0.05 ^b	1.94±0.08 ^b
	OTA	1.78±0.02 ^a	2.23±0.03 ^a	2.79±0.05 ^a	2.27±0.10 ^a
Creatinine (mg/dl)	Cont.	0.35±0.006 ^b	0.56±0.01 ^b	0.62±0.01 ^b	0.51±0.03 ^b
	OTA	0.49±0.006 ^a	0.65±0.01 ^a	0.88±0.01 ^a	0.67±0.04 ^a

*Values are expressed means ± standard error of the mean.

a, b, Means with different superscripts within the same age indicate significant differences between treatments by Duncan's Multiple Range Test ($p < 0.05$). N = 9 per treatment.

Histopathological examination

Prominent histological changes related to the *in-ovo* OTA injection were observed in liver and brain tissues.

In Figures (1, 2 and 3) slight cytoplasmic vacuolization of hepatocytes could be observed in liver sections in the control group on the 14th and 16th days of incubation and at hatch.

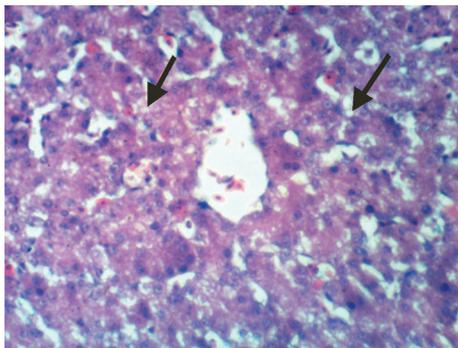


Figure 1 – Liver Control on the 14th day.jpg

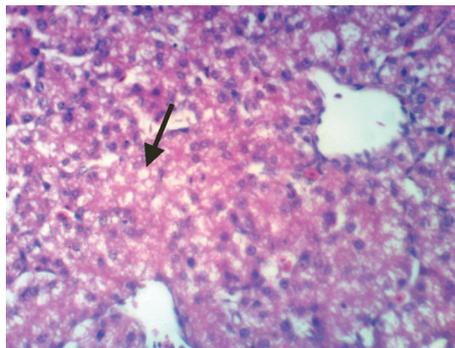


Figure 2 – Liver Control on the 16th day.jpg

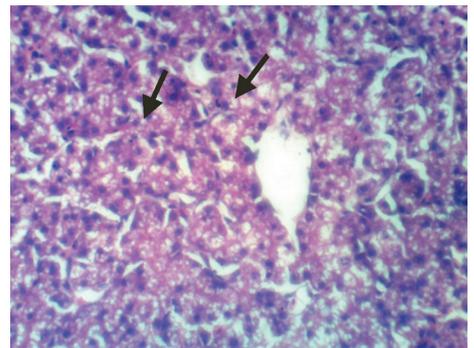


Figure 3 – Liver Control at hatch.jpg

On the 14th day of incubation, cytoplasmic vacuolization of hepatocytes showed steatosis of hepatocytes and oval cells proliferation, portal infiltration with massive inflammatory cells in the OTA group (Figure 4). On the 16th day of incubation, OTA induced cytoplasmic vacuolization of hepatocytes and other sections showed steatosis

of hepatocytes, hyperplasia of epithelial lining bile duct and collagen fibers deposition in portal tract (Figure 5). Finally, at hatch, OTA treated induced steatosis of hepatocytes and collagen fibers deposition in the portal tract and other sections showed steatosis of centrilobular hepatocytes and chronic cholangitis (Figure 6).

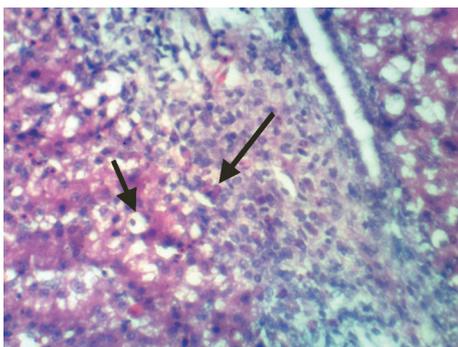


Figure 4 – Liver OTA treatment on the 14th day.jpg

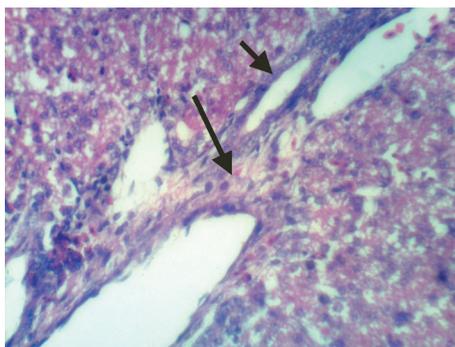


Figure 5 – Liver OTA treatment on the 16th day.jpg

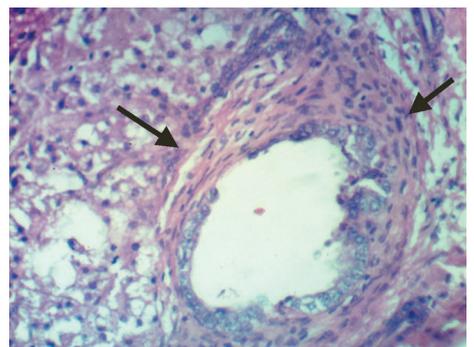


Figure 6 – Liver OTA treatment at hatch.jpg



Figures (7, 8 and 9) shows brain sections of the control chicks of the embryos. No histopathological

changes were observed on the 14th and 16th days of incubation and at hatch.

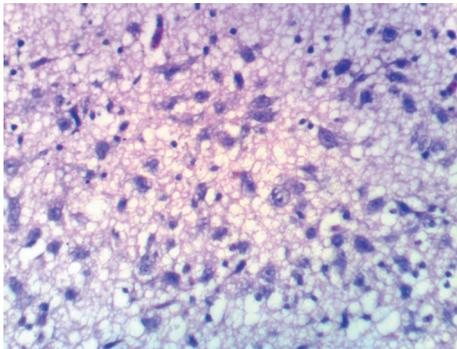


Figure 7 – Brain Control on the 14th day.jpg

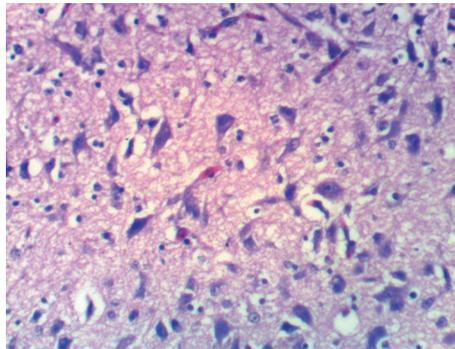


Figure 8 – Brain Control on the 16th day.jpg

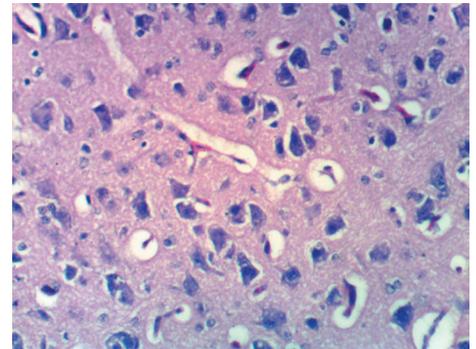


Figure 9 – Brain Control at hatch.jpg

Figure (10) shows embryonic brain sections of the OTA group on the 14th day of incubation. Results observed that some sections had an edema and few inflammatory cells infiltration in the meninges, while others showed a pyknosis of neurons. On the 16th day of incubation, *in-ovo* OTA treatment showed a focal

cerebral hemorrhage in the embryonic brain sections (Figure 11).

Finally, figure (12) shows embryonic brain sections of OTA group at hatch. There is a necrosis of neurons, while in the other sections dilatation and congestion of cerebral blood vessel are seen.

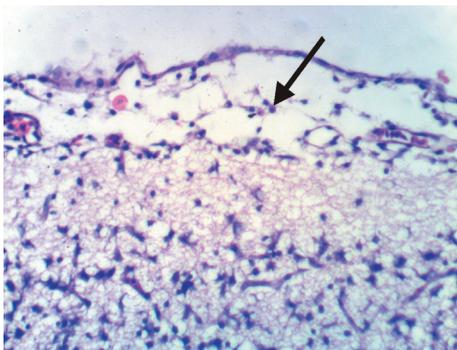


Figure 10 – Brain OTA treatment on the 14th day.jpg

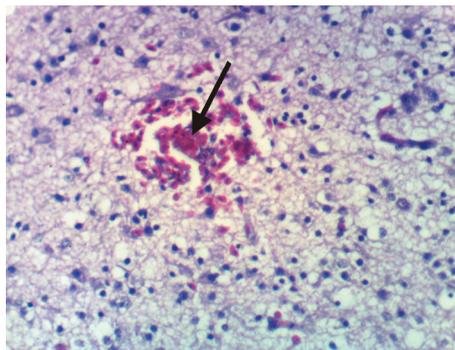


Figure 11 – Brain OTA treatment on the 16th day.jpg

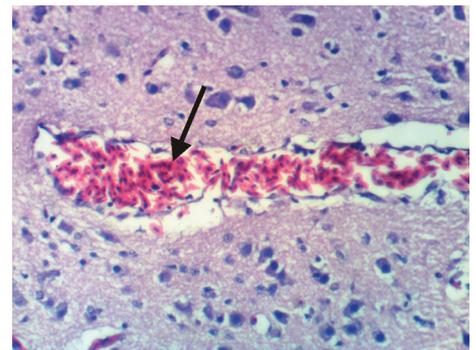


Figure 12 – Brain OTA treatment at hatch.jpg

DISCUSSION

Results of the present study clearly showed that *in-ovo* injection of OTA resulted in a significant oxidative damage, as demonstrated by impairment of antioxidant defenses (GSH) and increased TBARS in the embryonic organs and in both allantoic and amniotic fluids. These results may be attributed to the OTA effects on the cell antioxidant defense, which makes the cells more sensitive to oxidation (Cavin *et al.*, 2007).

The high levels of TBARS in the embryonic livers, bursa of Fabricius, spleen, brain, and heart following OTA inclusion *in-ovo*, probably ascribed to the attack of reactive oxygen species (ROS) which are released from mitochondria (Rached *et al.*, 2007). Furthermore, the antioxidant defenses of livers, bursa of Fabricius, spleen, brain, and heart also compromise by the OTA as manifested by the reduction in GSH. Hoehler *et al.* (1997) indicated that, in stress conditions,

the stimulation of lipid peroxidation by mycotoxins (Ochratoxin A) was higher as compared to the free peroxidation. The highest susceptibility to lipid peroxidation was observed in the brain tissue with the lowest vitamin E concentration and high levels of polyunsaturated fatty acids. This reflects the low antioxidant potential of the brain (Surai, 2002).

Sulfhydryl groups, especially GSH serve as a crucial component of cellular antioxidant defenses and participate in the elimination of reactive oxygen species (ROS) by acting as a nonenzymatic oxygen radical scavenger and a substrate for various enzymatic reactions (Wang *et al.*, 2009). A change in GSH status could be detrimental and could represent the most important targets for mycotoxins in embryonic development. It is possible that this depletion might be a consequence of OTA conjugation with GSH and/o increased lipid peroxidation (LPO) which is known to generate a reactive intermediate, such as



α , or β - unsaturated aldehydes that covalently bind to GSH (Glaab *et al.*, 2001). Since chicken embryo tissues contain high levels of polyunsaturated fatty acids (PUFA), they are vulnerable to peroxidation, and oxidative stress caused by mycotoxins could be lethal. Stimulation of lipid peroxidation (LPO) and consequent apoptosis are important mechanisms of the toxicity of various mycotoxins (Surai, 2002).

In the present study, the elevated activities of hepatic enzymes namely ALT, AST, ALP, ACP and GGT in the OTA group might indicate recent organ damage (Lumeij, 1997). Therefore, the continued rise in AST, ALT, ALP, and GGT was indicative of hepatocellular destruction and dysfunction (Rizvi & Shakoori, 2000). However, elevated AST activities are not solely specific for liver damage but also a cardiac marker. Following toxin administration, the increase degree in ALT activity correlates with the number of hepatocytes damaged (Joo *et al.*, 2013). This association may be useful in evaluating the extent of hepatic damage. Furthermore, the increase in ALP activity is known to be indicative of hepatobiliary disease (Gentles *et al.*, 1999).

Our results are in agreement with Joo *et al.* (2013) who reported that, *in-ovo* OTA injection caused a significant decrease in hepatic tissue cholesterol level and a significantly increase in hepatic tissue triglycerides levels as compared to the control group. This reduction in hepatic tissue cholesterol levels may be attributed to the impaired liver metabolism (Kalorey *et al.*, 2005) and could be one of the OTA hepatotoxicity indicators (Gentles *et al.*, 1999). OTA was able to induce slight degenerative changes in the hepatocytes which consequently led to the decreased cholesterol in blood concentrations (Stoiev *et al.*, 2000). All these metabolic disorders may be responsible for the structural changes in the liver, which is the primary organ involved in mycotoxin detoxification and elimination of OTA from blood, which demonstrates why the liver is much more affected than the kidneys (Joo *et al.*, 2013).

The significant increase in uric acid and creatinine in the allantoic fluid of the OTA treated group reported in this study may be attributed to the damage in the embryonic excretory system during its development. Similar to this finding, Elaroussi *et al.* (2008) reported that serum levels of uric acid and creatinine increased in the chicks that were exposed to a diet contaminated with OTA indicating a possible severe damage to kidney functions. OTA produces acute proximal tubular epithelial necrosis in the kidneys and inhibits normal renal uric acid secretion. Therefore, the increase in the allantoic fluid uric acid and creatinine levels may be

due to a decrease in the glomerular filtration rate. The increase in the urea and creatinine levels may be due to nephrotoxic effects following the *in-ovo* inoculation of OTA (Zahoor-ul-Hassan *et al.*, 2012).

In the present study, T_4 was significantly higher in the OTA group than in the control group. A gradual rise was observed in both groups as the embryo advanced in age. The elevation of thyroid hormones during the incubation period may be necessary for the development and hatching of the embryos (Gregory *et al.*, 1998). Our results disagreed with the results of Elaroussi *et al.* (2006) who reported that the administration of 0.4 and 0.8 mg OTA / kg in chicken's diet significantly decreased serum T_4 concentration. This may be attributed to the difference in the thyroid gland response for the OTA between embryos and hatched growing chicks.

Histopathological examinations of hepatic tissues in the OTA group showed differences in the vacuolation and megalocytosis of hepatocytes and hyperplasia of the biliary epithelium. Similar hepatocyte vacuolation and megalocytosis with accompanying hyperplasia of the biliary epithelium was observed in the livers of birds fed OTA-contaminated diets compared to the control birds (Elaroussi *et al.*, 2008). An activation of capillary endothelium and Kupffer's cells, hyperemia of capillaries and pericapillary edema, in addition to granular or vacuolar degeneration, perivascular infiltration of mononuclear cells was observed (Koynarski *et al.*, 2007). They concluded that the degenerative changes in the liver probably are due to the route of elimination of OTA partially via the liver, due to enterohepatic recirculation and hepatobiliary way of excretion of OTA exerting the direct toxic effect of OTA on this organ (Koynarski *et al.*, 2007).

Development of nervous tissue appears to be very susceptible to the deleterious effects of OTA, which induce teratogenic effects in the chick embryos (Gilani *et al.*, 1978). Histopathological examination of the brain showed the degenerative structure in the OTA group. Jameel (2011) investigated the toxic effects of OTA in various internal organs of chicks. They reported that the histopathological sections of the brain especially cerebrum showed perivascular and perineuronal edema and shrinkage of many neurons due to degeneration of the central chromatolysis and massive sub meningeal hemorrhage. Cerebellum tissue section showed degeneration of purkinage cells of birds given orally crude OTA at 0.5 μ g /g of body weight, daily for 21 days (Jameel, 2011). The perivascular and perineuronal edema in the cerebrum



may be related to the increase in the permeability of the blood brain barrier leading to disturbances in the blood dynamics and escape of fluids to the nervous tissue. The edematous changes in the brain with hemorrhage might be due to the vascular damages induced by OTA.

The small molecular weight of OTA allows its passing through the blood-brain barrier, so it can quickly take up by the brain tissue. Similarity of OTA structure with the structure of the amino acids Phe, led to the inhibition of an enzyme that uses Phe such as Phe-tRNA synthetase. This leads to the inhibition of protein synthesis, as well as stimulates lipid peroxidation. OTA increases the incidence of reactive oxygen species (ROS) in the cells, this high level of ROS will cause the oxidation of lipid and protein, so it can change the cell structure (Ringot *et al.*, 2006). Therefore, the reported increase in TBRAS in the brain tissues that led to lipid peroxidation in the brain confirmed past findings. Furthermore, it postulated that this change leads to the increase of plasma membrane permeability to Ca_2^+ ions that interfere with cell calcium homeostasis. Increased Ca_2^+ ions entering the cell associated with the OTA toxicity due to the impaired Ca_2^+ regulatory mechanism is the beginning of cell injury (Zhang *et al.*, 2009). OTA also affects the activity of insulin-like growth factors (IGF-I) that regulate cell proliferation and survival. Although IGF-I receptor can still bind, its function for signaling is inhibited, and cell division does not occur. This indicates that the OTA can prevent the production of the normally central nervous system cells by affecting growth factor (Zhang *et al.*, 2009).

CONCLUSION

In conclusion, the present study reveals that the exposure of developing embryos to OTA induces extensive oxidative damage in studied organs; these oxidative effects may act as an important mediator in the toxicity of OTA to give rise to the impairment of the liver, brain, excretory and thyroid functions.

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