



The Hepatotoxic and Nephrotoxic Effects of Mycotoxin in Broiler Chickens

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ABSTRACT

This study has been carried out to highlight the effects of some mycotoxins (aflatoxin, ochratoxin and their combination) on pathological and clinicopathological picture in broiler chickens. Sixty Arber Acers broiler chicks (one day old) were classified into 4 groups each 15 chickens. The 1st group was kept as a control, the 2nd group was fed 2 ppm aflatoxin contaminated ration, the 3rd group was fed 4 ppm ochratoxin contaminated ration, and the 4th group was fed 2 ppm aflatoxin + 4 ppm ochratoxin (AF+OTA) contaminated ration from the first day of age till 6 weeks. At 2nd, 4th and 6th week of experiment, 5 birds of each group were sacrificed and samples of blood, serum and tissue specimens were collected for histopathological, hematological and biochemical investigations. Histopathological examination of liver from aflatoxin-treated group showed Kupffer cells activation, focal areas of sinusoidal dilatation and periacinar hepatic necrosis as well as hepatocellular vacuolations with mononuclear cells infiltration. As ochratoxins were mainly nephrotoxic, the most conspicuous changes in kidneys were tubular nephrosis, subcapsular hemorrhage, distension of some renal tubules with cellular casts with marked connective tissue proliferation and mononuclear cells infiltration, and atrophied renal tubules were noticed. Histopathological changes were more pronounced in group 4 (AF+OTA). The pathological changes increase in correlation with prolonged administration of toxins. Serum biochemical results confirmed hepatotoxic effects of these mycotoxins in chicken, which manifested by significant increases in ALT and AST values that associated with significant elevations in total leukocytes counting. Therefore, our results indicate that mycotoxins (aflatoxin, ochratoxin and their combination) have hepatotoxic and nephrotoxic effects on growing broilers.

KEY WORDS: Mycotoxin, Broiler, Hepatotoxicity, Nephrotoxicity.

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1- INTRODUCTION

Mycotoxins are secondary metabolites present worldwide in agricultural commodities and produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by animals (1). These mycotoxins are pharmacologically active substances that produced in a strain-specific way that elicit some complicated toxicological activities (2). Mycotoxin contamination of the feed and

food is a global problem because more than 25% of world grain production is contaminated by mycotoxins (3). Mycotoxins according to their chemical structure exert a broad variety of biological effects (4). Mycotoxins can be acutely or chronically toxic, or both, depending on the kind of toxin and the dose. Membrane-active properties of various mycotoxins determine their toxicity. Incorporation of mycotoxins into cell

membrane structures lead to alterations in membrane functions. In general, mycotoxins effects on DNA, RNA, protein synthesis and the pro-apoptotic action causing changes in physiological functions including growth, development and reproduction (5). Almost certainly, the main human and veterinary health burden of mycotoxin exposure is related to chronic exposure (e.g., cancer induction, kidney toxicity, immune suppression). However, the best-known mycotoxin episodes are manifestations of acute effects (e.g., turkey X syndrome, human ergotism, stachybotryotoxicosis) (2). Mycotoxicosis, which can occur in both industrialized and developing countries, arise when environmental, social and economic conditions combine with meteorological conditions (humidity, temperature), which favour the growth of moulds (6). Aflatoxins and ochratoxins (OTA) are natural contaminants of feed stuff that produced by *Aspergillus* and *Penicillium* species that can be present as contaminant in a variety of agricultural products. OTA was shown to cause a diversity of toxic effects in different animal species; it acts as a potent nephrotoxic and renal carcinogen and it was also reported to cause hepatotoxicity, immunosuppression and teratogenicity (7). Mycotoxin contamination can reduce the birds' ability to withstand stress by inhibiting the immune system (8,9). Therefore, this study was aimed to investigate pathological and clinicopathological effects of mycotoxins on liver, kidney and immune tissues of broiler chickens.

2- MATERIALS AND METHODS

2.1. Experimental animals:

One hundred one day-old Arbor Acher broiler chicks were used in this experiment. All chicks were maintained under continuous lighting for the experimental period (i.e. 6 weeks) and given a standard diet and clean

water *ad libitum* according to breed manual. All protocols were approved by the institutional review board for animal experiments of Cairo University.

2.2. Experimental design:

Chicks were randomly allocated into four groups (25 animals per group). The groups were as follows: (1) group 1; chicks were fed on a regular ration, mycotoxins free, and served as a control group, (2) group 2; chicks were fed 2 part per million (ppm) aflatoxin contaminated ration for 6 weeks, (3) group 3; chicks were fed 4 ppm ochratoxin contaminated ration for 6 weeks, and (4) group 4; chicks fed 2 ppm aflatoxin plus 4 ppm ochratoxin contaminated ration for 6 weeks.

2.3. Samples collections:

At the 2nd, 4th and 6th weeks of age, five chicks from each group were randomly collected and blood samples were collected from wing vein on EDTA as anticoagulant for hematological examinations and on plane tube for sera separation that used for biochemical investigations, then the birds were killed with cervical dislocation and examined for the gross lesions. Specimens of the liver, kidneys, spleen, thymus and bursa of Fabricius, were collected on formalin buffered saline (pH 7.2) for histopathological examination.

2.4. Preparation of mycotoxin contaminated ration:

Aflatoxin and/or ochratoxin contaminated diet was prepared by artificial contamination of the crushed yellow corn by *Aspergillus parasiticus* and/or *A. ochraceus*, respectively and left to grow on for 14 days at 28°C.

1. For induction of aflatoxicosis, group 2 chicks were fed on the toxicated diet, which prepared by mixing of broiler ration with

The Hepatotoxic and Nephrotoxic Effects of Mycotoxin in Broiler Chickens

artificially aflatoxicated corn to provide a final toxin concentration of 2 mg AF/kg feed.

2. For induction of ochratoxicosis, group 3 chicks were fed on toxicated ration prepared by mixing of broiler ration with artificially ochratoxicated corn to provide a final concentration of 4 mg OA/kg feed.

3. For induction of combined aflatoxicosis and ochratoxicosis, contaminated ration was prepared by mixing of broiler ration with artificially aflatoxicated and ochratoxicated corn to a final toxin concentration of 2 mg AF + 4 mg OA/kg feed.

2.5. Hematological and biochemical analysis

Red blood cell and total leukocytic count was carried out according to the method described previously (10). Differential leukocytic count, Hb concentration and PCV value were measured following the previous method (11). Serum biochemical analysis for measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST) and uric acid was carried out using diagnostic commercial kits containing all chemicals required as recommended by manufacturer.

2.6. Histopathological analysis:

To evaluate the histopathological changes, sections from each specimen (liver, kidney, spleen, bursa and thymus) were stained with hematoxylin and eosin following the standard method (12).

2.7. Statistical analysis:

Statistical analysis was performed with the statistical software package SPSS for Windows (version 18.0; SPSS Inc., Chicago, Ill.). The significance of differences between treated groups and control were evaluated by student *t* test. A *P* value of less than 0.05 was considered significant. Data were grouped and reported as means \pm standard errors of the means (SE).

3. RESULTS

3.1. Histopathological examination:

1- Aflatoxin administration:

- a. Liver: Two weeks after feeding aflatoxin, the liver of chicks showed focal areas of necrosis with lymphocytic cellular aggregation. There were vacuolar and hydropic degeneration of the hepatocytes with swollen, pale, vacuolated cytoplasm and inflammatory cellular infiltration in between. The portal areas revealed mononuclear cellular infiltration and hyperplasia of the biliary epithelium. Then changes progressed by the 4th week into multifocal areas of mononuclear cellular aggregation in between the hepatocytes. By the end of experiment (6th week), liver showed massive numbers of inflammatory cells infiltration surrounding the bile duct in the portal area. Focal coagulative necrosis of the hepatocytes accompanied by inflammatory cellular infiltration mainly lymphocytes, and macrophages were noticed (Figure 1.a).
- b. Kidney: Two weeks after feeding aflatoxin, there was congestion of the cortical blood vessels associated with hypercellularity of the glomerular tufts due to proliferation of the endothelial and mesangial cells of glomerular capillaries. The tubules showed coagulative necrosis of lining of epithelial cells with inflammatory cells infiltration in between. By the 4th week, focal aggregates of lymphocytes were observed in between the degenerated tubules. After 6 weeks of treatment, focal inflammatory cells infiltration was observed in between the glomeruli and tubules in association with focal areas of hemorrhage and congestion in blood vessels (Figure 1.b).

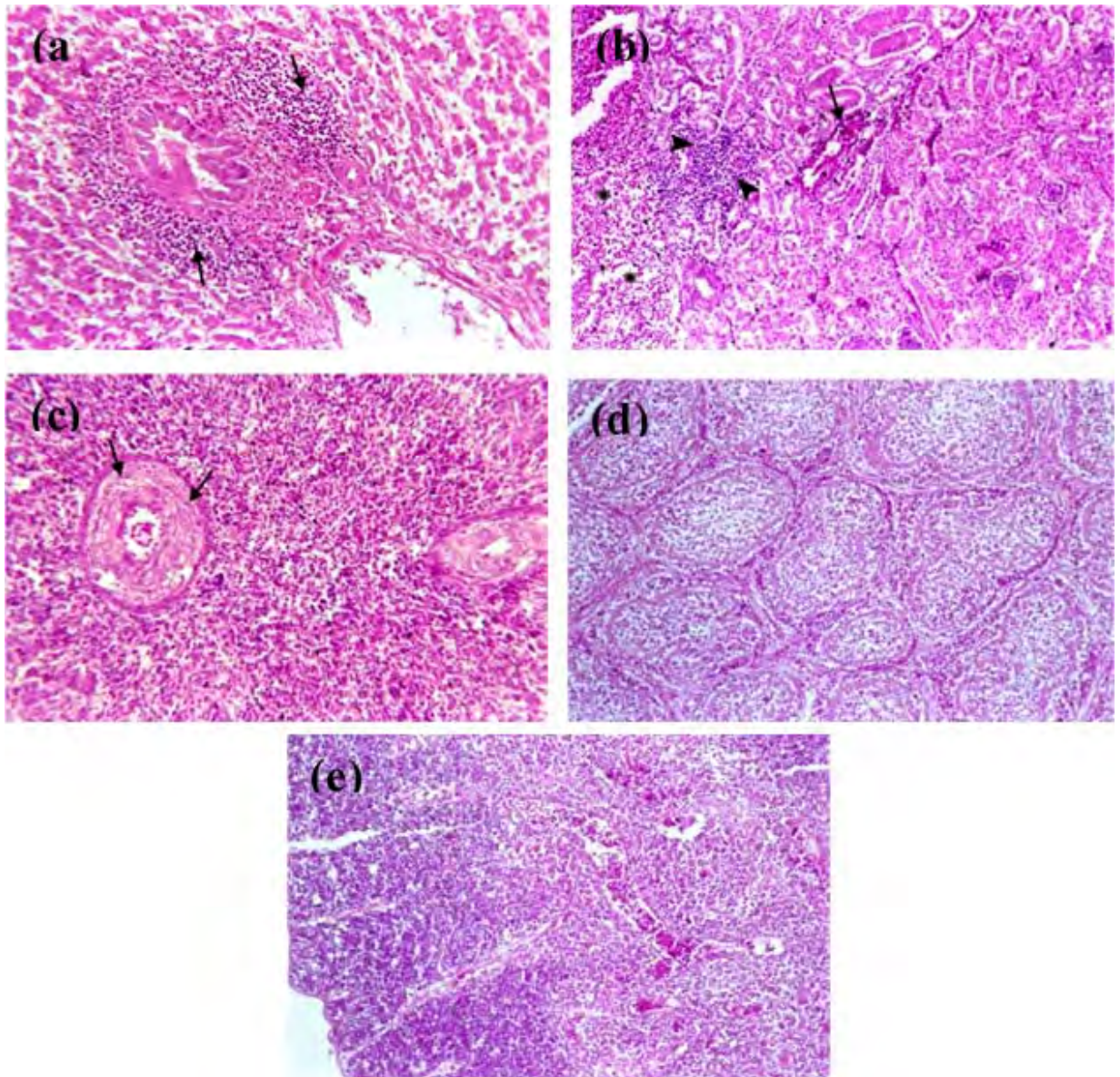


Figure (1). Histopathological changes in different organs 6 weeks after 2 ppm aflatoxin feeding. a. Liver of chicken showing inflammatory cells infiltration surrounding the bile duct in bile duct in portal area. (H&E x64). b. Kidneys of chicken (group2) at 6weeks showing focal inflammatory cells infiltration in between the tubules with congestion and hemorrhage. (H&E x40). c. Spleen of chicken showing swelling and oedema in the media of follicular blood vessels. (H&E x64). d. Bursa of Fabricius of chicken showing severe lymphoid depletion with fibrosis in between. (H&E x64). e. Thymus of chicken showing slight lymphoid depletion with congested blood vessels in the medullary portion. (H&E x40)

The Hepatotoxic and Nephrotoxic Effects of Mycotoxin in Broiler Chickens

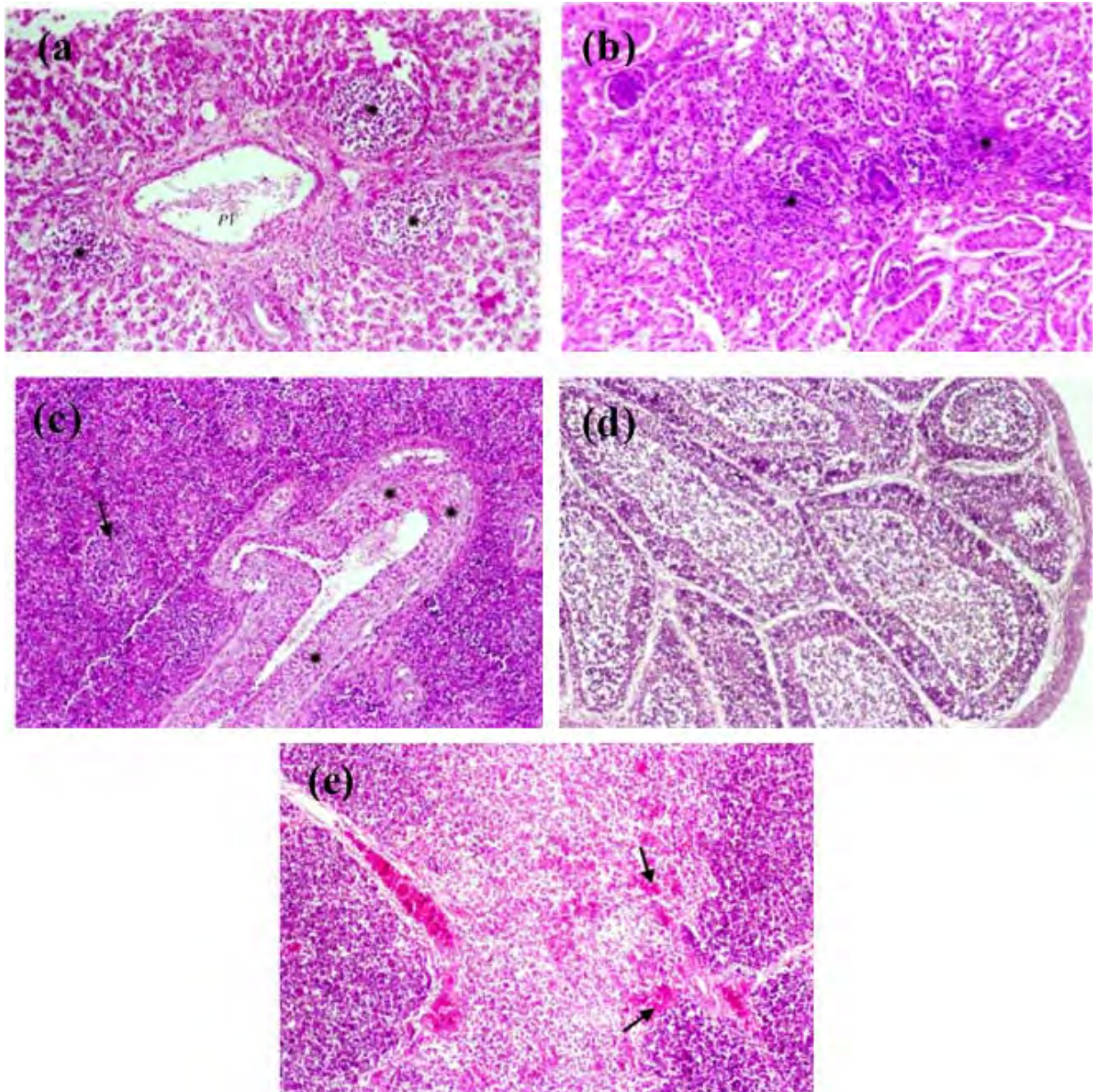


Figure (2). Histopathological changes in different organs 4 and 6 weeks after 4 ppm ochratoxin feeding. a. Liver of chicken at 6weeks showing focal circumscribed round aggregation of mononuclear leucocytes inflammatory cells infiltration in portal area with sever dilatation of portal vein with fibrous connective tissue proliferation and degeneration in hepatocytes . (H&E x40). b. Kidney of chicken at 6weeks showing focal area of fibrosis with inflammatory cells infiltration in between the necrosed renal tubules. (H&E x40). c. Spleen of chicken at 6weeks showing oedema with hypertrophy in the vascular wall of blood vessels with multiple focal microscopic nodules formation. (H&E x64). d. Bursa of Fabricius of chicken at 6weeks showing depletion in the central portion of the lymphoid follicles. (H&E x64). e. Thymus of chicken at 4weeks showing sever hemorrhages and congestion in the medullary portion. (H&E x40)

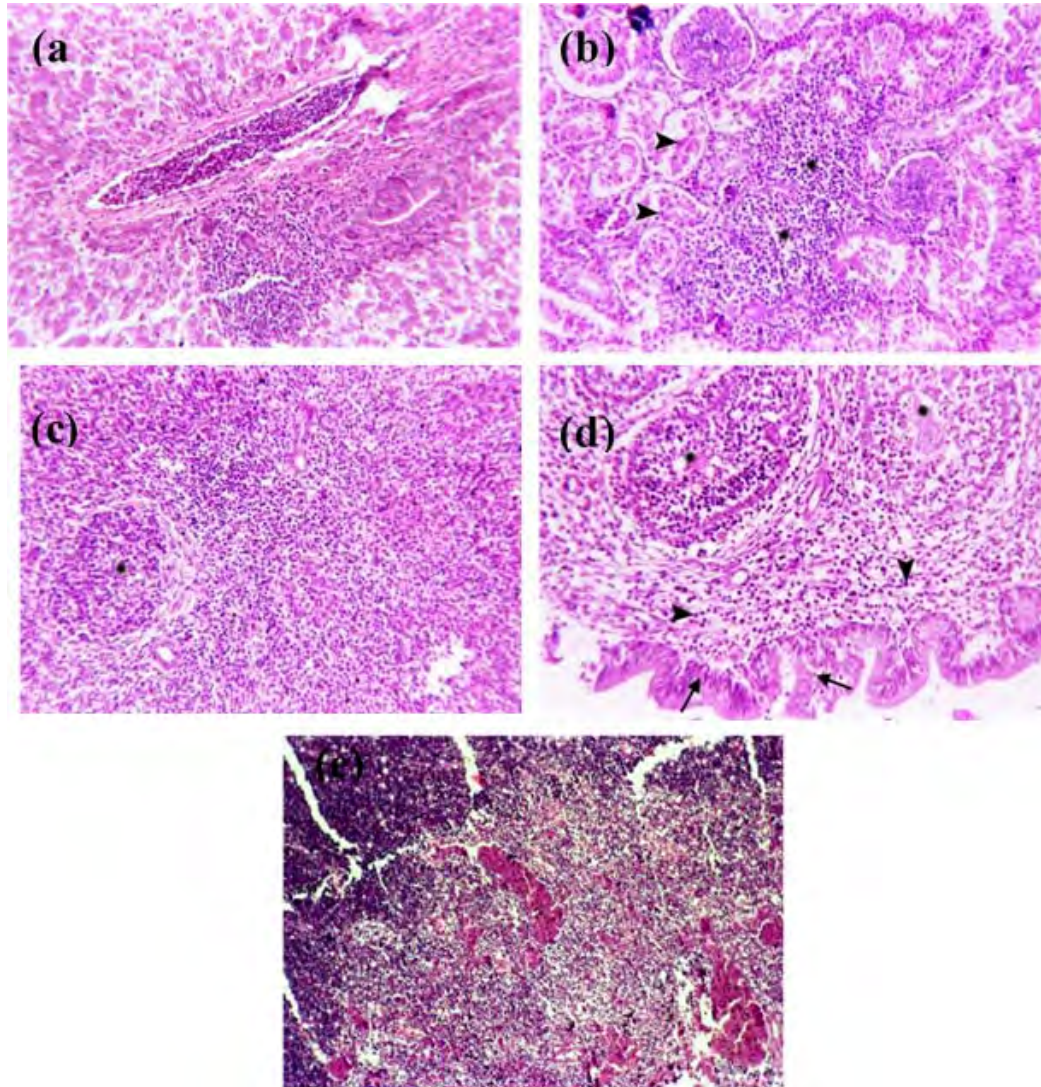


Figure (3). Histopathological changes in different organs 6 weeks after 2 ppm aflatoxin + 4 ppm ochratoxin combined feeding. a. Liver of chicken showing focal round aggregation of mononuclear leucocytic inflammatory cells in the portal area with severe congestion in the portal vein. (H&E x40). b. Kidney of chicken showing focal mononuclear leucocytic inflammatory cells infiltration in between the glomeruli and degenerated and necrosed tubules. (H&E x40). c. Spleen of chicken showing focal circumscribed round aggregations of mononuclear leucocytes replacing the splenic tissue. (H&E x40). d. Bursa of Fabricius of chicken showing mucosal epithelium hyperplasia with polyps formation and inflammatory cells infiltration in the medullary lamina propria with lymphoid depletion of the follicles with mild fibrosis. (H&E x64). e. Thymus of chicken showing congestion and hemorrhage in the medullary portion. (H&E x40)

The Hepatotoxic and Nephrotoxic Effects of Mycotoxin in Broiler Chickens

Table (1) Erytherogram parameters from control and mycotoxin-fed chickens. Data are presented as mean values \pm standard error of mean.

Group/Toxin & Dose	RBCs ($\times 10^6/\mu\text{l}$)			Hb (gm/dL)			PCV (%)			MCV (fL)			MCHC (%)		
	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks
Group 1 (Control)	2.93 \pm	1.60 \pm	1.40	7.86 \pm	5.23 \pm	8.56	19.33	17.67	15.00	65.83 \pm	116.66 \pm	108.48	40.88 \pm	30.27 \pm	59.88 \pm
	0.03	0.30	± 0.32	0.12	0.26	± 2.35	± 0.33	± 1.72	± 2.64	0.43	17.85	± 4.67	0.13	3.95	18.10
Group 2 (AF; 2 ppm)	3.13 \pm	1.93	2.26	7.66	4.70	8.36	20.33	18.66	20.66	65.51 \pm	104.04 \pm	92.60 \pm	37.63 \pm	23.64 \pm	40.49 \pm
	0.31	± 0.33	± 0.20	± 0.58	± 0.17	± 0.34	± 1.20	± 0.66	± 0.88	2.86	1.16	6.40	0.78*	1.55	2.62
Group 3 (OT; 4 ppm)	3.00 \pm	1.83	2.23	8.06	4.00 \pm	6.9	16.66	18.66	18.00	68.96 \pm	102.71 \pm	80.42 \pm	38.88 \pm	20.97 \pm	39.45 \pm
	0.25	± 0.03	± 0.23	± 0.90	1.74	± 0.55	± 0.88	± 0.88	± 2.08	0.87	1.38	2.48	1.24	5.24	6.50
Group 4 (AF; 2 ppm +OT; 4 ppm)	2.92 \pm	1.76 \pm	2.21 \pm	6.26 \pm	3.63 \pm	7.46 \pm	15.96	18.06	17.89	67.98 \pm	101.81 \pm	79.82 \pm	37.98 \pm	20.08 \pm	38.85 \pm
	0.18	0.28	0.21	0.08*	0.48	0.44	± 0.86	± 0.08	± 1.88	0.77	1.08	2.28	1.14	4.84	6.20

Table 2. Leukogram parameters from control and mycotoxin-fed chickens. Data are presented as mean values \pm standard error of mean.

Group/Toxin & Dose	WBCs ($\times 10^3/\mu\text{l}$)			Heterophils ($\times 10^3/\mu\text{l}$)			Lymphocytes ($\times 10^3/\mu\text{l}$)		
	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks
Group 1 (Control)	29.00 \pm 5.00	97.66 \pm 1.45	80.00 \pm 6.08	29.00 \pm 19.00	16.66 \pm 1.76	13.33 \pm 2.40	29.00 \pm 1.00	28.66 \pm 4.37	32.66 \pm 6.35
Group 2 (AF; 2 ppm)	55.00 \pm 2.51*	256.00 \pm 3.05*	67.00 \pm 12.42	17.33 \pm 6. 56	11.33 \pm 2.90	16.66 \pm 1.76	42.00 \pm 6. 00*	32.66 \pm 4.05	24.00 \pm 5.29
Group 3 (OT; 4 ppm)	44.33 \pm 4.70	199.33 \pm 1.20*	78.66 \pm 10.71	11.33 \pm 4.66	13.33 \pm 0.66	26.00 \pm 1.15*	23.33 \pm 7. 33	37.33 \pm 3.71*	8.00 \pm 1.15*
Group 4 (AF; 2 ppm+OT; 4 ppm)	34.66 \pm 3.17	163.33 \pm 1.76*	58.00 \pm 13.11	14.00 \pm 4.61	18.66 \pm 5.69	7.33 \pm 1.76	26.00 \pm 2.30	23.33 \pm 4.80	28.66 \pm 1.76

Table 3. Serum biochemical parameters from control and mycotoxin-fed chickens. Data are presented as mean values \pm standard error of mean.

Group/Toxin & Dose	ALT (U/ μl)			AST (U/ μl)			Uric acid (mg/dl)		
	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks	2 wks	4 wks	6 wks
Group 1 (Control)	10.1 ± 0.35	10.2 ± 0.65	10.3 ± 0.83	15.4 ± 0.53	16.1 ± 1.02	13.9 ± 1.12	6.24 \pm 0.63	6.13 \pm 1.51	6.03 \pm 1.49
Group 2 (AF; 2 ppm)	20.3 $\pm 2.11^*$	19.8 $\pm 0.91^*$	24.8 $\pm 1.15^*$	30.5 $\pm 3.17^*$	61.6 $\pm 2.82^*$	65 $\pm 3.00^*$	11.07 \pm 1.10	9.89 \pm 1.62	5.79 \pm 2.07
Group 3 (OT; 4 ppm)	25.9 $\pm 1.20^*$	28.9 $\pm 1.33^*$	32.8 $\pm 1.33^*$	40.1 $\pm 1.85^*$	70.8 $\pm 3.27^*$	85 $\pm 3.44^*$	8.03 \pm 0.66	5.87 \pm 1.30	13.39 \pm 5.03
Group 4 (AF; 2 ppm+OT; 4 ppm)	30.1 $\pm 1.91^*$	37.8 $\pm 1.75^*$	55.9 $\pm 2.90^*$	38.9 $\pm 2.47^*$	99.8 $\pm 4.61^*$	121.5 $\pm 4.31^*$	9.63 \pm 4.10	6.77 \pm 2.78	12.27 \pm 4.76

The Hepatotoxic and Nephrotoxic Effects of Mycotoxin in Broiler Chickens

- c. Spleen: There were thickening and hypertrophy in the tunica media, and swelling of lining endothelium of the intima of the follicular blood vessels. Circumscribed round areas of mononuclear cellular aggregations forming microscopic nodules and surrounded by fine connective tissue capsule with lymphoid depletion were noticed after 2 weeks of feeding. While after 4th week, there were oedema and hypertrophy in the tunica media of the follicular blood vessels. By the end of experiment, marked hypertrophy in the tunica media of some blood vessels and swelling of the lining endothelial cells were recorded (Figure 1.c)
- d. Bursa of Fabricius: Lymphoid depletion and necrobiosis in the lymphoid follicles with hypertrophy and hyperplasia in the lining mucosal epithelium were recorded after 2 weeks of aflatoxin feeding. Thickening of the interstitial tissue due to oedema and fibrous tissue proliferation were detected after 4 weeks of aflatoxin feeding. While by the 6th week, there was marked lymphoid depletion in the follicles associated with interfollicular fine fibrous tissue proliferation (Figure 1.d).
- e. Thymus: Two weeks after feeding aflatoxin, there were congestion in the blood vessels and focal hemorrhages in the medulla. Lymphoid depletion of the medulla with congestion in blood vessels was noticed at 4 and 6 weeks post-aflatoxin administration (Figure 1.e).
2. *Ochratoxin administration:*
- f. Liver: by the 2nd and 4th weeks of feeding ochratoxin, the liver showed congestion of the central and portal veins with multifocal areas of mononuclear cellular aggregation as well as vascular and hydropic degeneration in the surrounding hepatocytes, while marked congestion of the central, portal veins and hepatic sinusoids associated with circumscribed areas of round mononuclear inflammatory cells aggregation were seen adjacent to the portal area in the degenerated hepatic parenchyma by the 6th week of feeding (Figure 2.a).
- g. Kidney: The examined kidneys revealed congestion of the cortical blood vessels and intertubular capillaries. The interstitium connective tissue revealed focal aggregates of lymphocytes and fewer plasma cells. The renal glomeruli were occasionally enlarged and exhibited hypercellularity of the glomerular tufts due to proliferation of the endothelial cells of glomerular capillaries by the end of 2nd week of feeding ochratoxin. While by the end of 4th week, the interstitium connective tissue revealed massive aggregates of inflammatory cells mainly lymphocytes. The renal glomeruli were enlarged and exhibited hypercellularity of the glomerular tufts and swelling as well as proliferation of the endothelial cells of glomeruli. Vacuolar and hydropic degeneration of the lining epithelial cells of some proximal and distal convoluted tubules were observed. Circumscribed areas of mononuclear cellular aggregations, focal areas of fibrous tissue proliferation were observed in between the necrotic tubules infiltrated by moderate numbers of inflammatory cells mainly lymphocytes and macrophages were seen by the end of experiment (Figure 2.b).
- h. Spleen: The spleen showed congestion of splenic blood vessels with thickening and hypertrophy in the tunica media after 2 weeks of experiment. While, after 4 weeks, there was marked hypertrophy in the tunica media of follicular blood vessels with swelling in the lining endothelial cells. By the end of

ochratoxin feeding (6th week), there were hypertrophy in the tunica media of some blood vessels and swelling of the lining endothelial cells associated with multifocal circumscribed areas of mononuclear cellular aggregations forming microscopic nodules and surrounded by fine connective tissue capsule, swelling and oedema were observed in splenic capsule (Figure 2.c).

- i. Bursa of Fabricius: After 2 weeks, there was lymphoid depletion of the lymphoid follicles with hyperplasia in the lining mucosal epithelium. Moreover, subepithelial and interstitial tissue was infiltrated by inflammatory cells with oedema. By 4th week, lymphoid depletion of the follicles with thickening of the interstitial tissue by fibrous tissue proliferation was noticed. While at the end of experiment, there was lymphoid depletion of the follicles with mucosal hyperplasia of the lining epithelium and thickening of the interstitial tissue by oedema (Fig.2,d).
- j. Thymus: After 2 weeks, congested blood vessels with focal hemorrhages were observed in the medulla. At the end of experiment, the thymus showed congested blood vessels and focal hemorrhages were observed in both cortex and medulla (Figure 2.e).

3. Combined administration of aflatoxin and ochratoxin:

- a. Liver: Two weeks after feeding, multifocal areas of mononuclear cellular aggregation were observed in between the hepatocytes. While after 4 weeks, Activation of Von Kupffer cells with the presence of few numbers of macrophages and lymphocytes were noticed in between the hepatic acini. The portal areas revealed hyperplasia of the biliary epithelium with formation of newly formed bile ductules, thickening of the wall of bile ducts due to fibrous tissue proliferation and massive inflammatory cellular infiltration mainly lymphocytes, plasma cells and fewer macrophages. At 6 weeks, congestion was observed in the portal vein with massive number of leukocytic inflammatory cells infiltration in the portal area. Multifocal areas of mononuclear cellular aggregation in between the hepatocytes were prominent (Fig.3.a).
- b. Kidney: After 2 weeks, there were congestion of the blood vessels with focal aggregates of lymphocytes and fewer plasma cells. Circumscribed areas of lymphoid cells aggregation were seen in between degenerated and necrotic tubules. While, after 4 weeks, the interstitium tissue revealed focal inflammatory cellular infiltration and extravasated erythrocytes. The renal glomeruli were enlarged and exhibited hypercellularity of the glomerular tufts due to swelling and proliferation of the endothelial cells of glomerular capillaries. At the end of experiment, the interstitial and glomerular changes were markedly observed. Multifocally, the lining epithelial cells of proximal and distal convoluted tubules were occasionally swollen with vacuolated cytoplasm (degeneration); or they were hyper eosinophilic with shrunken, pyknotic nuclei, loss of cellular detail and rarely karyorrhexis (necrosis). Inflammatory cells mainly lymphocytes, and macrophages were observed in between the degenerated and necrosed tubules (Figure 3.b).
- c. Spleen: 2 weeks after feeding, the red pulp was expanded by large numbers of erythrocytes while the white pulp showed lymphoid depletion. While after 4 weeks from start of combined aflatoxin and ochratoxin feeding, marked hypertrophy in the tunica media of some blood vessels

The Hepatotoxic and Nephrotoxic Effects of Mycotoxin in Broiler Chickens

was characteristic. Multifocal areas of hemorrhages in the red pulp and lymphoid depletion of the white pulp, characterized by low numbers of mature small lymphocytes was observed. The splenic architecture was distorted by focal circumscribed areas of inflammatory cells aggregation mainly lymphocytes and macrophages. At the end of experiment, multifocal circumscribed areas of mononuclear cellular aggregations forming microscopic nodules and surrounded by fine connective tissue capsule were observed (Figure 3.c).

- d. Bursa of Fabricius: After 2 weeks, there were lymphoid depletion and necrosis of the follicles with hypertrophy and hyperplasia in the lining mucosal epithelium. After 4 weeks, the changes were accompanied by proliferations of fibrous tissues in between necrotic areas. At the end of experiment, there were lymphoid depletion of the follicles with mucosal hyperplasia of the lining epithelium and inflammatory cellular infiltration of the lamina propria (Figure 3.d).
- e. Thymus: After 2 weeks, the thymus revealed mild lymphoid depletion at the medulla associated with focal hemorrhage and congestion in blood vessels, while after 4 weeks, hyalinization of Hassel's corpuscles in the medullary portion was seen. At the end of experiment, lymphoid depletion and focal hemorrhages were detected in medullary portion (Figure 3. e).

3.2. Clinicopathological findings:

Red blood cell count of chicken fed 2 ppm Aflatoxin (group 2), 4 ppm Ochratoxin (group 3) and 2 ppm AF+4 ppm OA (group 4) did not show significant changes in comparison with those of control (group 1) at 2, 4 and 6 weeks (Table 1).

Blood hemoglobin (Table 1) showed a significant lower value in chicken fed 2 ppm AF+4ppm OA (group 4) at 2 weeks after treatment compared with control, while packed cell values (Table 1) of chicken fed AF or OA or combination groups did not show significant changes than control group at 2, 4 and 6 weeks. Mean corpuscular volume in all treated groups did not show significant changes in comparison with those of control (group 1) at 2, 4 and 6 weeks (Table 1). Mean corpuscular hemoglobin concentration in blood of chicken fed 2 ppm AF+4ppm OA (group 4) (Table 1) were significantly lower than control at the 4th week. Other groups did not show significant changes in MCHC. Total leucocytes count in blood of chicken fed aflatoxin (Table 2) was significantly higher than the control at 2 and 4 weeks. The OA and combination groups resulted in a significant increase in TLC than the control at 4 weeks. Values of heterophils count in blood of chicken fed OA groups (Table 2) were significantly higher than the control group at the 6th week. Lymphocytes count in blood of chicken fed AF were significantly higher than the control group (Table 1) at 2 weeks, while lymphocytes of chicken fed OA showed significant increase at 4 weeks which changed to a significant decrease at 6 weeks. Values of ALT and AST (Table 3) in sera of chicken fed Aflatoxin or Ochratoxin were significantly higher than the control group at 2 to 6 weeks. Combination of AF+OA group resulted in higher values followed by OA and AF groups. Values of Uric acid (Table 3) shows non-significant changes than the control in all groups.

4. DISCUSSION

Mycotoxins are one of the major factors suppressing poultry productivity (13). Therefore, the present study was carried out to investigate the pathological and hematological changes in the different organs. Mycotoxicosis was induced in broiler

chicken by feeding aflatoxins (2ppm) and ochratoxins (4ppm) singly and in combination. In regard to aflatoxin effect, histopathologically, at two weeks post aflatoxin administration, liver revealed focal areas of necrosis, vacuolar and hydropic degeneration of hepatocytes, mononuclear cellular aggregation in between hepatocytes with leukocytic cellular infiltration in the portal area biliary hyperplasia. At four weeks, multifocal areas of moderate mononuclear cellular aggregation in between the hepatocytes were common. While at six weeks; periductal massive numbers of inflammatory cells infiltration and focal coagulative necrosis of the hepatocytes accompanied by inflammatory cellular infiltration were detected. These findings agreed with earlier findings (14,15), meanwhile, our results were in partial agreement with some other authors (16,17) where fibrosis, trabecular derangement of varying degree were also observed. The degenerative and necrotic changes observed in aflatoxicosis could be attributed to the damage of critical cellular macromolecules (lipids, DNA and proteins) through the oxidative stress of aflatoxins, which may result in the peroxidation of lipids and oxidative damage of DNA. Moreover, the accumulation of intracellular calcium in cases of aflatoxicosis causes mitochondrial dysfunction and reduces adenosine triphosphate (ATP) generation, hyperplasia of bile ducts, which is a characteristic or pathognomic lesion may be due to direct effect of aflatoxins on biliary cells or the production of prostaglandins during peroxidation of lipids (18). The Kidneys revealed congested blood vessels, glomerular hypercellularity and coagulative necrosis of renal tubules at two weeks post aflatoxin administration; while focal areas of lymphocytic aggregation and hemorrhage in between renal tubules were noticed at four and six weeks. These results were in

agreement with the results of previous studies (14,16,17). Meanwhile, these results were in partial agreement with other studies (19), where thickening of glomerular basement membrane and degenerative changes in severe degree characterized by desquamation of epithelial tubular cells. Our results revealed also thickening in the wall of splenic blood vessels and nodules of mononuclear cellular aggregations in spleen. Lymphoid depletion was observed in Bursa of Fabricius and thymus at all periods of the experiment. Moreover, focal areas of necrosis were detected in these organs at four weeks; while fibrous tissue proliferation was seen in Bursa of Fabricius, focal medullary hemorrhage in thymus, at six weeks. These results agreed with the results previous works (17,20). Our results revealed that there were non-significant changes in red blood cells (RBCs) counts at week 6, while total leukocytic count (TLC) counts significantly increased especially heterophils at week 4. These findings agreed with the earlier findings (16). A drastic increase in the total leukocytic count, heterophils and lymphocytes were observed during cases of aflatoxicosis. This increase suggests that the toxin elicited an inflammatory response (21). Our results revealed that the level of ALT significantly increased in aflatoxicated birds, while uric acid level remains within normal level. These results agreed with the previous results (15,22,23), and in partial agreement with other results (24,25). Increased AST activity during aflatoxicosis, increased serum activity of enzymes, mycotoxicosis is believed to be the sequel of hepatocyte degeneration and a subsequent leakage of enzymes into the circulation (26).

Concerning the effects of ochratoxin; grossly, at two weeks post ochratoxin administration, the kidneys and livers were swollen and pale which changed to tan color, occasionally with yellowish coloration, and have rounded borders. At four and six weeks,

The Hepatotoxic and Nephrotoxic Effects of Mycotoxin in Broiler Chickens

the kidneys and livers were more enlarged and pale in color with presence of minute grayish white foci on the surfaces and cut sections. The ureters were markedly swollen and distended with urates. Decrease in the size of bursa, thymus lobules and spleen were also observed. These lesions were in complete agreement with previous results (27), meanwhile, were in partial agreement with other works (28,29). Where there were secondary air sacculitis, enlargement of gizzard and proventriculus, pancreas and heart, enlarged gall bladder, hemorrhage on the thigh muscles, hyperemic meninges with oedematous changes in brain, Moreover, our results disagree with previous work (30), where both bursa of Fabricius and spleen were not affected. Histopathologically, the current study revealed that microscopical examination of renal lesions in kidneys, at two weeks post ochratoxin administration, were characterized by congested blood vessels, and capillaries, focal aggregation of lymphocytes, hypercellularity of glomerular tufts, vacuolar and hydropic degeneration of convoluted tubules. At four weeks, similar lesions were noticed, while at six weeks, sub-acute to chronic interstitial nephritis characterized by circumscribed areas of mononuclear cellular aggregations and focal areas of fibrous tissue proliferation in between the necrotic tubules. These results were in complete agreement with results of previous studies (31) and in partial agreement with others (32), where hemorrhage and fibrosis were detected, while no agreement with the results (33) found normal kidneys. Microscopical examination of examined livers in the present study, revealed similar lesions at all periods of the experiment (2, 4 and 6 weeks post ochratoxin administration). Congested blood vessels, focal areas of mononuclear aggregation, degenerated hepatocytes and inflammatory cells aggregation of portal area were the predominant lesions. These findings agreed

with the earlier findings (31,32), meanwhile, our results were in partial agreement with other findings (34) where fibrosis, hemorrhage, allergic reaction and defense of the organ against ochratoxin effect. Microscopical findings in immune system (spleen, thymus, and bursa of Fabricius) at two weeks post ochratoxin administration revealed that congested blood vessels, with hypertrophy of their tunica media in spleen; lymphoid depletion of lymphoid follicles, interstitial inflammatory oedema in bursa of Fabricius; focal medullary hemorrhage in thymus. At four weeks, similar lesions were detected in spleen and thymus; interstitial fibrous tissue proliferation was found in bursa of Fabricius. At six weeks, mononuclear cellular aggregations forming microscopic nodules and surrounded by fine connective tissue capsule were seen in spleen; lymphoid depletion of lymphoid follicles, focal hemorrhages and interstitial inflammatory oedema were observed in bursa of Fabricius and thymus. Our microscopical findings in immune system (Spleen and Bursa of Fabricius) agreed with the earlier results (30,35).

Our results revealed that PCV, Hb, MCV and MCHC showed non-significant changes, while TLC counts showed a significant increase after 4 weeks of feeding ochratoxin. This increment in TLC mainly is due to increased lymphocyte. These findings agreed with the earlier findings (23,28,35). Also, our results revealed that there is a significant increase in ALT level, and non-significant increase in AST level in ochratoxicated birds. These results agreed with the earlier results (23,30). Uric acid a non-significant increase in ochratoxicated birds. These results agreed with the results mentioned previously (36). In regard to combined effects of aflatoxin and ochratoxin, postmortem examination of broilers administrated with 2 ppm aflatoxins+4 ppm ochratoxin revealed enlarged pale swollen

kidneys which changed to a tan color, demarcated ureters and filled with urates, pale enlarged livers occasionally with yellowish coloration with focal hemorrhage, and rounded borders, at two weeks post administration. Similar lesions in addition to grayish white foci on the surfaces of liver and kidneys were seen at four weeks. Meanwhile, at six weeks, the livers became grayish yellow in color and firm in consistency. The ureters were markedly distended with urate while bursa, thymus and spleen were decreased in size. These lesions were in complete agreement with the results mentioned previously (37) in addition to hematomas along the borders of liver (38). Histopathologically, at 2 weeks post administration liver revealed multifocal areas of mononuclear cells aggregation in between hepatocytes; at 4 and 6 weeks, portal area revealed hyperplasia of biliary epithelium with fibrous tissue proliferation and inflammatory cells infiltration, these findings agreed with the earlier findings (38). Our results indicated that kidney lesions were characterized microscopically by congested blood vessels, focal areas of lymphoid cells aggregation in between degenerated and necrotic tubules at 2 weeks post administration. Similar lesions as well as hypercellularity of glomerular tuft, degeneration and necrosis of renal epithelium were seen at 4 and 6 weeks. These results were in agreement with previous results (39). Microscopical examination of lymphoid organs in the present study revealed hemorrhage of red pulb, and lymphoid depletion in white pulb with nodules of mononuclear cellular aggregation surrounded by fine connective tissue capsule in spleen at 2, 4, and 6 weeks post administration, bursa of Fabricius revealed lymphoid depletion and necrosis of follicles at 2 weeks, with hyperplasia of mucosal epithelium, and interstitial fibrous tissue proliferation at 4 and 6 week. Thymus revealed also lymphoid

depletion, focal medullary hemorrhage at 2 and 6 weeks and hyalinization of Hussels corpuscle at 4 weeks. Cecal tonsils showed marked lymphoid depletion of lymphoid follicles at 2, 4 and 6 weeks. These microscopical findings in immune system (spleen, bursa of Fabricius and thymus) agreed with the previous results (38). Our results revealed that the level of ALT significantly increased in aflatoxin+ochratoxin administered birds from 2 weeks of toxins administration then increase during the 4 and 6 weeks administration. These results agreed with the previous results (37). In addition to, there is no change in the level of uric acid after 6 weeks of administration.

In conclusion, the previous results indicate that mycotoxins (aflatoxin, ochratoxin or its combination) have hepatotoxic and nephrotoxic effects on grown chickens and every endeavor should be adopted to reduce feed contamination by these toxins.

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التأثير الكبدي والكلوي الضار للسموم الفطرية على دجاج التسمين

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الملخص العربي

استهدفت هذه الدراسة معرفة الآثار السمية الغرض لكل من سم أفلاتوكسين 2 جزء / المليون وسم الأوراتوكسين 4 جزء / المليون أو كلاهما على بدارى التسمين. اجريت التجربة علي 100 ككتوت عمر يوم تم تقسيمها إلي اربع مجموعات (25 ككتوت / مجموعة) وكانت المجموعات كالاتي: المجموعة الأولى (المجموعة الضابطة) لم تعالج بأي معالجات وتمت تغذيتها علي عليقة خالية من السموم الفطرية - المجموعة الثانية تم تغذيتها علي عليقة تحتوي علي 2 جزء / المليون أفلاتوكسين - المجموعة الثالثة تم تغذيتها علي عليقة تحتوي علي 4 جزء / المليون أوكراتوكسين - المجموعة الرابعة تم تغذيتها علي عليقة تحتوي علي 2 جزء / المليون أفلاتوكسين + 4 جزء / المليون أوكراتوكسين لمدة 6 أسابيع وتم ذبح 5 طائر من كل مجموعة في الأسبوع الثاني والرابع والسادس . ولقد تم تجميع عينات الدم مع اضافة مانع تجلط - عينات سيرم وكذلك عينات من مختلف الأنسجة مثبتة في محلول فورمالين وذلك لإجراء دراسات تحليل الدم والتحليلات السيرولوجية والباثولوجية وذلك في الأسابيع الثاني والرابع والسادس حتي نهاية التجربة. ولقد أظهر الفحص النسيجي للمجموعة الثانية حدوث تحلل في الخلايا الليمفاوية ونقص شديد في عددها في الطحال و غدة فابريشس والغدة الزعترية (الثايمس) والتجمعات الليمفاوية بالأمعاء، وجود ارتشاح بالخلايا الليمفاوية في المنطقة البابية للكبد مع وجود نخر لبعض الخلايا وكذلك وجود نموات جديدة للقنوات الصفراوية، اما الكلي فقد لوحظ وجود نخر لبعض خلايا الأنابيب الكلوية. عند الفحص النسيجي للطيور في المجموعة الثالثة لوحظ حدوث نخر في الخلايا الليمفاوية ونقص في عددها في الطحال و غدة فابريشس والغدة الزعترية (الثايمس) والتجمعات الليمفاوية بالأمعاء، وجود نخر شديد بخلايا الكبد، أما الكلي فقد لوحظ حدوث فراغات بالسيتوبلازم لبعض الخلايا المبطنة للأنابيب الكلوية العلوية مع وجود نخر في بعض الخلايا الأخرى بجميع مراحلها. عند الفحص النسيجي للطيور في المجموعة الرابعة لوحظ حدوث نخر في الخلايا الليمفاوية ونقص في عدد هذه الخلايا في الطحال و غدة فابريشس والغدة الزعترية (الثايمس) والتجمعات الليمفاوية بالأمعاء، وكذلك وجود ارتشاح بالخلايا الليمفاوية في المنطقة البابية بالكبد مع وجود نخر لبعض الخلايا وكذلك وجود نموات جديدة للقنوات الصفراوية، أما الكلي فقد لوحظ حدوث نخر لبعض خلايا الأنابيب الكلوية. وجد أيضاً أنه في المجموعات 2-3-4 نقص غير معنوي في العدد الكلي في كرات الدم الحمراء بالإضافة الي نقص ملحوظ في نسبة الهيموجلوبين مع وجود زيادة في العدد الكلي في كرات الدم البيضاء بالاضافة الي زيادة في الخلايا الليمفاوية وخلايا الهينيروفيل. كما وجدت زيادة غير معنوية في مستوي السيرم من حمض اليوريك، مع زيادة واضحة في نشاط انزيم (ALT) مع زيادة غير معنوية في نشاط انزيم (AST). مما سبق نستنتج أن السموم الفطرية لها تأثير ضار علي الأنسجة المختلفة بالجسم ويتمركز تأثير الافلاتوكسين علي الكبد كما يتمركز تأثير الأوكراتوكسين علي الكلي مع زيادة واضحة في نشاط انزيم (ALT) وزيادة غير معنوية في نشاط انزيم (AST) .

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(1): 29-45, سبتمبر 2013)