Pathological and Clinicopathological Studies on aflatoxicosis in Broilers

By

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ABSTRACT

This study has been carried out to highlight the effects of aflatoxin on pathological and clinicopathological picture in broiler chickens. The experiment was carried out on a number of 50 Arber Acers broiler chicks of one day old, classified into 2 groups each 25 chickens. The 1st group was kept as a control, the 2nd group was fed 2 ppm aflatoxin contaminated ration, from the first day of age till 6 weeks. At 2nd, 4th and 6th weeks of experiment, 8 birds of each group were sacrificed and samples of blood and serum were collected for hematological and biochemical investigations. Tissue specimens from different organs were collected for histopathological examinations. As aflatoxins is mainly hepatotoxic, histopathological examination of liver showed Kupffer cells activation, hepatocellular vacuolations, focal hepatic hemorrhages, multifocal hepatic necrosis associated with mononuclear cells infiltration, portal infiltration with heterophilis, portal connective tissue proliferations associated with hyper activation and hyperplasia of epithelial lining the bile ducts. Atrophied some renal tubules was noticed in examined kidneys. Bursa of Fabricius showed lymphocytic necrosis and depletion especially in the medullary portion of lymphoid follicles. Spleen revealed lymphocytic depletion in white pulp as well as reticular cells hyperplasia. Thymus of chickens showed lymphocytic necrosis and depletions as well as focal thymic hemorrhage. The pathological changes increase in correlation with prolonged administration of toxins. Serum biochemical results confirmed hepatotoxic
INTRODUCTION

Aflatoxins are secondary metabolites of certain strains of Aspergillus (flavus and parasiticus), Thompson and Henke (2000). Aflatoxin ingestion by chicken result in many different symptoms such as reduced growth, increased susceptibility to infectious agents. The growth of chicks was not affected by concentration of aflatoxin below 200 mg/kg, Robens and Richard (1992). Aflatoxin affects all poultry species, young poultry especially ducks and turkeys are very susceptible, Bartos (1997). The effects of mycotoxins on poultry are dependent on the age, physiological state, and nutritional status of the bird at the time of exposure, Thompson and Henke (2000). Aflatoxins 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. Mycotoxin contamination can reduce the birds' ability to withstand stress by inhibiting the immune system, Bray(1991), Thompson and Henke (2000). Liver tissue is considered the aflatoxins target organs due to the protein inhibition pathway of aflatoxin elicited in the hepatocytes. Javed et al., (1993). Feeding one day-old broiler chicks 1.0 ppm aflatoxin from 7 days to 7 weeks of age, the result revealed that pronounced clinical signs, and reductions in body weight gain, also decreased organ to body weight ratios especially bursa and spleen, marked depletion and degeneration of lymphocytes in thymus, bursa , spleen and cecal tonsils, Shivachandra et al., (2003), liver showed coagulative necrosis, hyperplasia of bile ducts in the portal areas, fatty changes, and extensive haemorrhage, Hashem and Mohamed(2009), sever liver damage was observed in chicks fed 2 mg/kg of aflatoxin B1 for 21 days in the form of fatty liver and vacuolar degeneration. Zhao et al., (2010) therefore, this study was aimed to investigate pathological and clinicopathological effects of aflatoxins on liver, kidneys and immune tissues of broiler chickens.

MATERIALS AND METHODS

1- Experimental animals: Fifty 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- 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 on 2 part per million (ppm) aflatoxin contaminated ration for 6 weeks,

3- Samples collections: At the 2nd, 4th and 6th weeks of age, eight chicks from each group were randomly collected and blood samples were collected from wing vein for hematological and biochemical investigations, then the birds were killed with cervical dislocation and examined for gross lesions. Samples of the liver, kidneys, spleen, thymus and bursa of Fabricius were collected and fixed on formalin buffered saline (pH 7.2) 10 % for histopathological examination.

4- Experimental induction of Aflatoxicosis: the used chicks were fed on toxicated diet which prepared by mixing of broiler ration with artificially aflatoxicated corn to provide a final toxin concentration of 2 mg AF/kg feed.

5- Hematological and biochemical analysis: Red blood cell and total leucocytic count was carried out according to the method described by Natt and Herrick (1952). Differential leucocytic count, Hb concentration and PCV value were measured following the methods described by Schalm et al., (1975). 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.

6- Histopathological analysis: Samples from liver, kidney, spleen, bursa of Fabricius, and thymus were taken from sacrificed chicks and were fixed in neutral buffered formalin (pH 7.2). To evaluate the histopathological changes, sections from each specimen were stained with hematoxylin and eosin following the method of Bancroft (1990).

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 ± standard errors of the means (SE).

RESULTS

1. **Histopathological examination:**

   **Liver:** Two weeks after feeding aflatoxin, the liver of chicken showed focal areas of necrosis with lymphocytic cellular aggregation were replaced the hepatocytes. 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 in to 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).

   **Kidneys:** 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 with inflammatory cells infiltration in between. By the 4th week, Focal aggregates of lymphocytes were observed in between the degenerated tubules. After 6 week 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.c).

   **Spleen:** There were thickening and hypertrophy in the tunica media, and swelling in 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 at 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.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. Lymphoid depletion and necrosis of the lymphoid follicles as well as thickening of the interstitial tissue due to oedema and fibrous tissue proliferation were detected after 4 weeks of starting aflatoxin feeding. While by the 6th week, there was lymphoid depletion in the follicles associated with interfollicular fine fibrous tissue proliferation (Figure 1.e).

**Thymus:** Two weeks after feeding aflatoxin, there were congestion in the blood vessels and focal hemorrhages in the medulla. The medullary portion showed congestion in the blood vessels and lymphoid depletion after 4 week of treatment. While by the end of experiment, lymphoid depletion of the medulla with congestion in blood vessels were noticed (Figure 1.f).

2. **Clinicopathological findings:**

Values of red blood cell (RBCs ×10^6/µl) of chicken fed 2 ppm Aflatoxin (group 2) did not show significant changes in comparison with those of control (group 1) at 2, 4 and 6 weeks (Table 1). Blood hemoglobin (Hb; gm/dl) (Table 1) did not show significant changes in comparison with those of control (group 1) also packed cell values (PCV %) (Table 1) of chicken fed AF did not show significant changes than control group at 2, 4 and 6 weeks. Mean corpuscular volume of (MCV, fl) 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 (MCHC %) in blood of chicken fed 2 ppm AF did not show significant changes in MCHC. Total leucocytes count (TLC×10^3/µl) in blood of chicken fed aflatoxin (Table 2) was significantly higher than the control at 2 and 4 weeks. Lymphocytes count (×10^3/µl) in blood of chicken fed AF were significantly higher than the control group (Table 1) at 2 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.

Values of Uric acid (Table 3) shows non-significant changes than the control in all groups.
FIGURES

Figure (1) Histopathological changes in different organs after 2ppm aflatoxin feeding.

a&b. liver of chicken showing inflammatory cells infiltration surrounding the bile duct in portal area (H&E x160), with hyperplasia in bile duct (H&E x40).

c. kidney of chicken showing proliferation and swelling in the endothelial cells lining the glomerular tuft. (H&E x160)

d. spleen of chicken showing hypertrophy in the media and swelling of the lining intimal endothelium. (H&E x160)

e. bursa of fabricius of chicken showing depletion necrosis in the lymphoid follicles with fibrosis in between. (H&E x40)

f. thymum of chicken showing depletion in medullary lymphoid portion with congested blood vessels. (H&E x40)
### TABLES

**Table 1.** Erytherogram parameters from control and mycotoxin-fed chickens. Data are presented as mean values ± standard error of mean.

<table>
<thead>
<tr>
<th>Group/Toxin &amp; Dose</th>
<th>RBCs (×10^6/µl)</th>
<th>Hb</th>
<th>PCV</th>
<th>MCV</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 wks</td>
<td>4 wks</td>
<td>6 wks</td>
<td>2 wks</td>
<td>4 wks</td>
</tr>
<tr>
<td>Group 1 (Control)</td>
<td>2.93± 0.03</td>
<td>1.60±0.30</td>
<td>1.40±0.32</td>
<td>7.86±0.26</td>
<td>5.23±0.26</td>
</tr>
<tr>
<td>Group 2 (AF; 2 ppm)</td>
<td>3.13± 0.31</td>
<td>1.93±0.20</td>
<td>2.26±0.20</td>
<td>7.66±0.17</td>
<td>4.70±0.17</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group/Toxin &amp; Dose</th>
<th>WBCs (×10^3/µl)</th>
<th>Heterophils (×10^3/µl)</th>
<th>Lymphocytes (×10^3/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 wks</td>
<td>4 wks</td>
<td>6 wks</td>
</tr>
<tr>
<td>Group 1 (Control)</td>
<td>29.00± 5.00</td>
<td>97.66±1.45</td>
<td>80.00±6.08</td>
</tr>
<tr>
<td>Group 2 (AF; 2 ppm)</td>
<td>55.00± 2.51</td>
<td>256.00±3.0</td>
<td>67.00±12.42</td>
</tr>
</tbody>
</table>
Table 3. Serum biochemical parameters from control and mycotoxin-fed chickens. Data are presented as mean values ± standard error of mean.

<table>
<thead>
<tr>
<th>Group/Toxin &amp; Dose</th>
<th>ALT (U/µl)</th>
<th>2 wks</th>
<th>4 wks</th>
<th>6 wks</th>
<th>AST (U/µl)</th>
<th>2 wks</th>
<th>4 wks</th>
<th>6 wks</th>
<th>Uric acid (mg/dl)</th>
<th>2 wks</th>
<th>4 wks</th>
<th>6 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>ALT</td>
<td>10.1 ±0.35</td>
<td>10.2 ±0.65</td>
<td>10.3 ±0.83</td>
<td>AST</td>
<td>15.4 ±0.53</td>
<td>16.1 ±1.02</td>
<td>13.9 ±1.12</td>
<td>Uric acid</td>
<td>6.24 ± 0.63</td>
<td>6.13 ± 1.51</td>
<td>6.03 ± 1.49</td>
</tr>
<tr>
<td>Group 2 (AF; 2 ppm)</td>
<td>ALT</td>
<td>20.3 ±2.11*</td>
<td>19.8 ±0.91*</td>
<td>24.8 ±1.15*</td>
<td>AST</td>
<td>30.5 ±3.17*</td>
<td>61.6 ±2.82*</td>
<td>65 ±3.00*</td>
<td>Uric acid</td>
<td>11.07 ± 1.10</td>
<td>9.89 ± 1.62</td>
<td>5.79 ± 2.07</td>
</tr>
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</table>
DISCUSSION

Mycotoxins are one of the major factors suppressing poultry productivity, Yunus et al., 2011. 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. In regard to aflatoxin effect, grossly, at two and four weeks post aflatoxin administration, pale enlarged livers occasionally with yellowish coloration and focal hemorrhage; kidneys were swollen enlarged, pale in color. At six weeks post aflatoxin administration, the liver was enlarged, pale yellow in color, firm in consistency and the cut section revealed minute grayish white foci. Kidneys were swollen, pale; with granular surface. Intermuscular hemorrhage and decrease in size of bursa, thymus, and spleen were constant lesions. These lesions were in complete agreement with Karaman et al., (2010), meanwhile were in partial agreement with Zhao et al., (2010) where severe liver attributed the hepatic damage in aflatoxicosis to the effect of toxic metabolites formed in the liver through the effect of toxin on hepatic cytochrome enzymes; also it suppresses protein synthesis. 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 leucocytic 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 of Tessari et al., (2006), and Zhao et al., (2010) meanwhile, our results were in partial agreement with DelBianchi et al., (2005) and Karaman et al., (2010) 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 pathognomonic lesion may be due to direct effect of aflatoxins on biliary cells or the production of prostaglandins during peroxidation of lipids. (Hashem and Mohamed 2009). The Kidneys revealed congested blood vessels, glomerular hypercellularity and coagulative necrosis of renal tubules at two weeks postaflatoxin 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 Del Bianchi et al., (2005), Tessari et al., (2006), Karaman et al., (2010). Meanwhile were in partial agreement with Valdiva et al., (2001) 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 detected by Shivachandra, et al., (2003) and Karaman et al., (2010). Our results revealed that there were non-significant changes in red blood cells (RBCs) counts at week 6, while total leucocytic count (TLC) counts significantly increased especially heterophilis at week 4. These findings agreed with the earlier findings of Del Bianchi et al., (2005). A drastic increase in the total leukocytic count, heterophils and lymphocytes were observed during cases of aflatoxicosis. The increase in the total leukocytic count, heterophils and lymphocytes may be due to the toxic effect of mycotoxins on the circulating cells, sequestration of cells and/or effect of aflatoxins on the bone marrow and lymphoid tissues (Hashem and Mohamed 2009). 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 results by Raju and Devegowda (2000), Aravind et al., (2003), Zhao et al., (2010), and in partial agreement with Oguz et al., (2000), and Oguz et al., (2002). 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 (Gibson, et al., 1990). The elevation in hepatic enzyme activity ALT reflects hepatic damage and leakage of enzymes in blood stream (Hashem and Mohamed 2009). In conclusion, the previous results indicate that aflatoxins have hepatotoxic effects on grown chickens, and every endeavor should be adopted to reduce feed contamination by these toxins.

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