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HISTOPATHOLOGIC STUDY OF THE HEPATIC AND RENAL LESIONS INDUCED BY EXPERIMENTAL TOXIC DIETARY APPLICATION OF AFLATOXIN B_1 IN BROILER CHICKS.

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ABSTRACT

The present study investigated the toxicologic histopathologic effect of aflatoxin B_1, 60 broiler chicks of one-day aged devided randomly and equally into three groups, for dietary study, using one group as untreated control, while the other groups were given aflatoxin B_1 mixed with diet in concentration of 0.5 and 1.5 ppm respectively, for 30 days, the study showed fatty degeneration of liver associated with bile duct proliferation, accompanied by infiltration of mononuclear cells and lymphocytes. Also there was degeneration in proximal convoluted tubules mostly as hyaline degeneration especially at 0.5 ppm dose level. Those at 1.5 ppm dose level, there was focal liver cell necrosis associated with fibrosis and enlarged proliferated bile ducts, sever vacuolation and necrosis of epithelial of renal proximal convoluted tubules with thickening of the glomerular basement membrane.

INTRODUCTION

Aflatoxins considered the most dangerous mycotoxin which contaminate food and animal diet, they have carcinogenic effects in man and animals, in addition to the histopathological changes which they induced (1).

Those include aflatoxin B_1, B_2, G_1, G_2 and the second generation metabolites M_1 and M_2 according to their light reflection of ultra violet light 365 NM (2). Aflatoxin B_1 considered one of the most effective mycotoxin as carcinogenic and was put in grade 2 between 300 toxic carcinogenic substances world wide with concentration of part of billion (3). Because of the dangerous contamination of food and animal diet , some restrictions were put by world organization for the permitted percentages of aflatoxin in food materials as (5-20) ppb (4). Histologic evidence of continued low –level aflatoxin consumption in poultry is seen in 2 immunologically active organs. Typical changes in the liver include fat accumulation in the hepatocytes and bile duct proliferation (5), also with portal fibrosis which is a common lesion associated with chronic poisoning by aflatoxin in the chicken (6). The present study intended to lerink to light the macroscopic and microscopic histopathological changes, which were caused by those toxins in the livers and kidneys of broiler chicken in particular, they were more prominent as the birds become more susceptible as they grow older.

Material and Methods

1-Chicks of meat broiler of fawpro of one-day , supplied by the Hatchery of Al-Saqqer company.

2-Chick commercial feed as starter supplied from Al-Saqqer company.

3-Chick feed containing aflatoxin B_1 with 2 concentrations of 0.5 and 1.5 ppm (part per million) was supplied from Agriculture College/ Preventive Depatment .The concentration of the aflatoxin was checked by High Performance Liquid Chromatography (HPLC) . Also recording the quantity of aflatoxin by HPLC method in Veterinary Central Laboratories.

4-10% Neutral buffered formalin for fixation of histopathological specimens.
5-Hematoxylin and Eosin stain with periodic acid schiffe (P.A.S.) stain.

Experiment design

For the present study, 60 chicks were used. Those were randomly divided equally into three groups, 20 chicks for every group. The first group of (20) chicks (the control) were given chick feed without any aflatoxins, the control chick feed was also checked as clean, by taking sample, and examined by High Preformonce Liquid Chromatography to make sure that it is negative from any aflatoxin.

While group 2 and 3 were given two concentration of aflatoxin as 0.5 and 1.5 ppm respectively. The experimental study project started on day one old chicks continued for thirty days. Chicks from each groyp were killed by cervical dislocation when they were 30 days aged.

During the treatment period, clinical signs, and macroscopic pathological changes were recorded .Sections of livers and kidneys were taken from all treated birds, fixed in 10% neutral buffered formalin and histopathlogical sections were prepared.

RESULTS

Clinical Signs

Clinical signs were recorded during the 30 days of treatment with two concentration of aflatoxin and compaired with control birds. Chicks of second group (feed diet with 0.5 ppm) showed rough feathers, poor appetite, reduced food consumption in comparison with the control untreated birds. While the third group (feed diet with 1.5 ppm) appeared with poor condition, slow growth with the same main festation of second group but with more in severity, 4 chicks of this group died a bout 3 weeks after treatment. The dead birds were characterized by loss of body weight and retarded growth in comparison with the untreated controls.

Macroscopical findings

Chicks treated with 0.5 ppm concentration of aflatoxins B_1,after 30 days of treatment, there was congestion of internal organs in general, farther more, there was enlarged pale livers, with evidence of small focal haemorrhages on its surface, some birds showed reduced size of liver with firm consistency, also enlarged gall bladder and glandular proventiculas with petechial haemorrhages on their external surface . kidneys were enlarged, pale with petechial haemorrhage on their cortex. While astriking necropsy finding of third group (treated with 1.5 ppm) have been observed, those were restricted to the liver, which appeared friable with petechial hemorrhages, some chicks showed moderate nodularity of their liver surface with fatty change. Others demonstrated presence of small foci of necrosis and liver enlargement. Changes in the kidneys of this group were the same as those mentioned in previous one, but they were higher in severity.

Microscopical observations

The hepatic histopathological changes of the second group (0.5ppm concentration), were characterized by fatty degeneration of hepatocytes in liver parenchyma (fig 1), associated with congestion of portal and central veins, in addition to sinusoidal congestion (which were filled with RBC) (Fig- 2).

Some chicks showed presence of infiltration of mononuclear cells, specially, lymphocytes and plasma cells in liver parenchyma and periportal regions, accompanied by bile duct proliferation (Fig-3), associated with liver cell atrophy in the adjacent areas.

Renal histopathological changes showed degeneration of proximal convoluted tubules characterized by swelling epithelial lining of some tubules and vacuolar degeneration of the other tubules associated with infiltration of mononuclear cells and lymphocyte between tubules (fig 4 and 5) also some tubules appeared with hyaline degeneration (fig-6) Further more some of the proximal convoluted tubules were dilated.

Samples taken from the liver of third treated group (1.5 ppm), showed sever fatty degeneration particularly in periportal area (fig 7) also there was loss of hepatic parenchyma, accompanied by focal liver cell necrosis (fig 8).

Also there was pyknotic nuclei and deep acidophilic cytoplasm (fig-9) associated with marked bile duct hyperplasia and portal fibrosis (fig-10).

The changes in the kidneys were characterized by presence of degeneration of proximal convoluted tubules, associated with necrosis and exfoliation of epithelial lining in some of those tubules, disturbance and irregularities of the epithelium (fig-11) and evidence of tubular regeneration supported by presence of cortical tubular basophilia (fig-12).

Further more, there was thickening of the glomerular basement membrane accompanied by increase glomerular cellularity and presence of periodic acid shift P.A.S. positive homogenous substances (fig-13) 30 days after experimental intoxication with after to in B_1.

Figure-1- Microscopic section of the liver in one of the aflatoxin treated (0.5ppm) chicks, showing focal parenchymal cell hyperplasia and fatty degeneration with congestion of sinusoids, after 30 days of intoxication (40X. Hα E stain).

Figure-2- Microscopic section of the liver in one of the aflatoxin treated (0.5ppm) chicks, showing congestion of portal vein and sinusoids with slight infiltration of mononuclear cells between hepatocytes, after 30 days of intoxication (10X. Hα E stain).

Figure-3- Microscopic section of the liver in one of the aflatoxin treated (0.5ppm) chicks, showing marked infiltration of mononuclear cells and bile duct proliferation in peri-portal zone, after 30 days of intoxication (40X. Hα E stain).

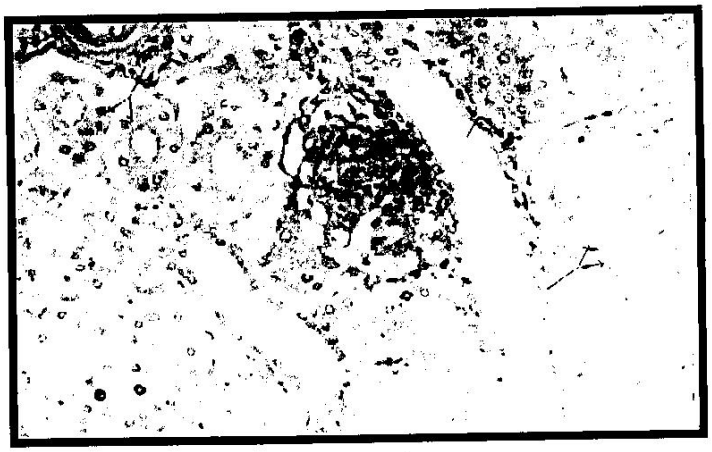

Figure-4- Microscopic section of the kidney in one of the aflatoxin treated (0.5ppm) chicks, showing vacuolar degenerating and cellular swelling of epithelial tubular lining, after 30 days of intoxication (20X. Hα E stain).

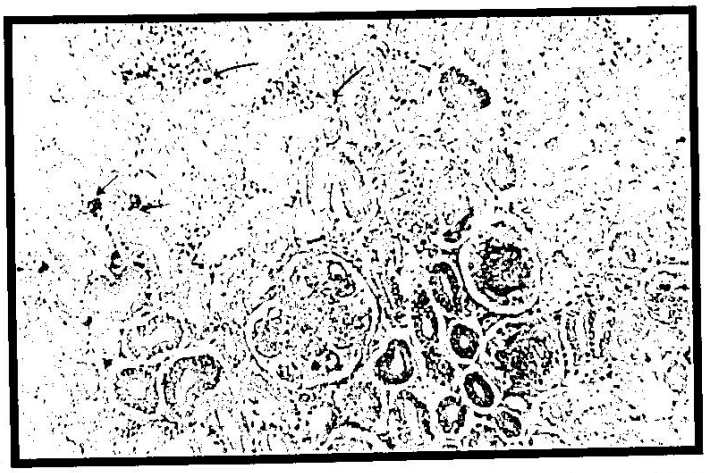

Figure-5- Microscopic section of the kidney in one of the aflatoxin treated (0.5ppm) chicks, showing infiltration of mononuclear cells and congestion between renal tubles, after 30 days of intoxication (10X. Hα E stain).

Figure-6- Microscopic section of the kidney in one of the aflatoxin treated (0.5ppm) chicks, showing hyaline degeneration and vaccuolation of epithelial lining tubules with loss of renal parenchyma, after 30 days of intoxication (20X. Hα E stain).

Figure-7- Microscopic section of the liver in one of the aflatoxin treated (1.5ppm) chicks, showing marked fatty degeneration in peri-portal zone, after 30 days of intoxication (10X. Hα E stain).

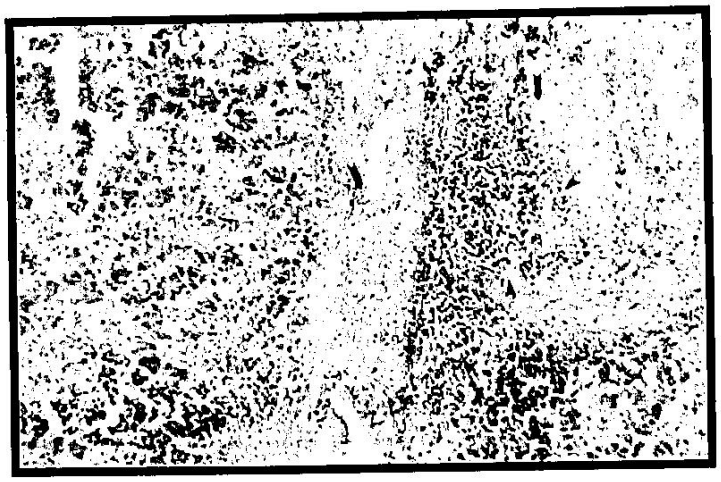

Figure-8- Microscopic section of the liver in one of the aflatoxin treated (1.5ppm) chicks, showing focal liver cell necrosis associated with infiltration of mononuclear cells, after 30 days of intoxication (10X. Hα E stain).

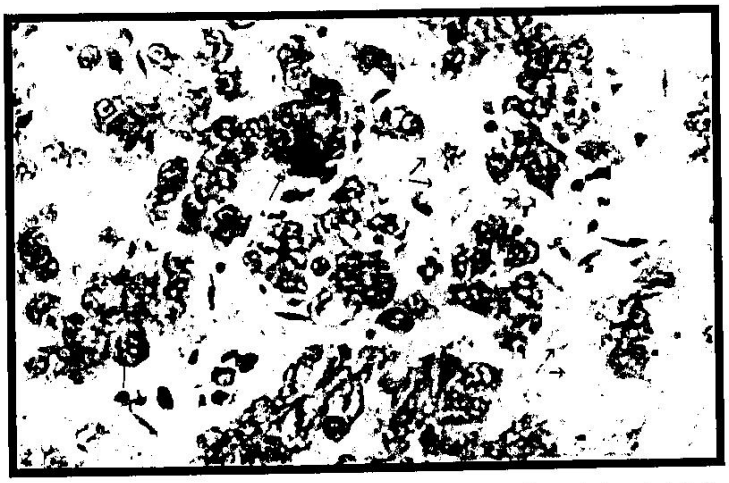

Figure-9- Microscopic section of the liver in one of the aflatoxin treated (1.5ppm) chicks, showing loss of hepatic parenenchyma associated with pyknotic nuclei and deep acidophilic cytoplasm of necrotic cell parenchyma, after 30 days of intoxication (40X. Hα E stain).

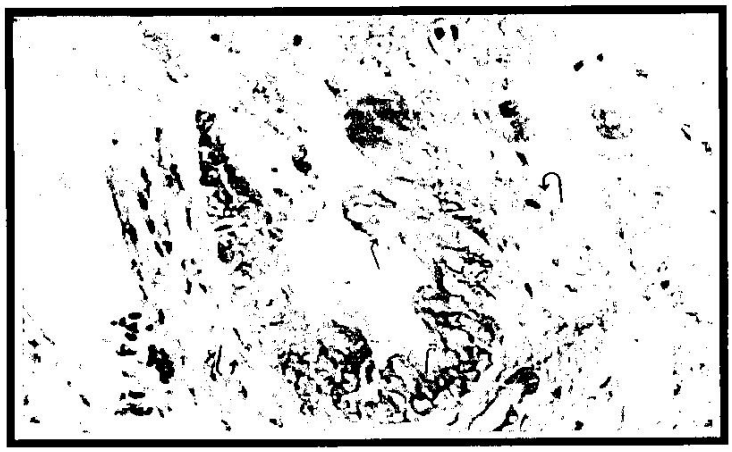

Figure-10- Microscopic section of the liver in one of the aflatoxin treated (1.5ppm) chicks, showing marked bile duct hyperplasia enclosed by fibrosis, after 30 days of intoxication (40X. Hα E stain).

Figure-11- Microscopic section of the kidney in one of the aflatoxin treated (1.5ppm) chicks, showing advanced degenerated tubules associated with necrosis and exfoliation of epithelial lining in some of the tubules, with diffuse mononuclear cell infiltration, after 30 days of intoxication (40. Hα E stain).

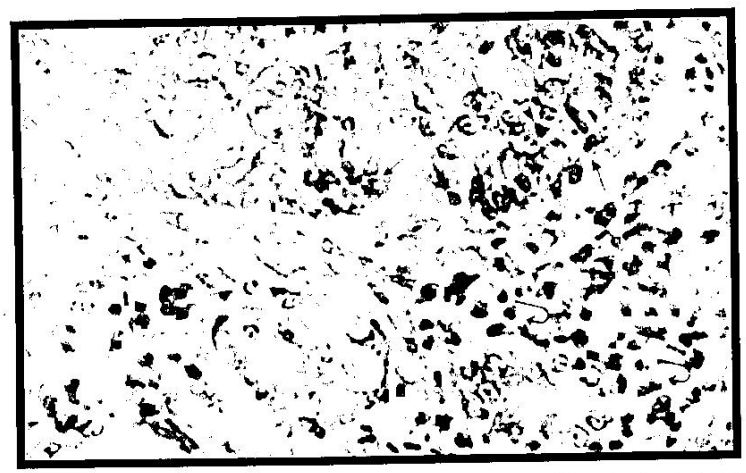

Figure-12- Microscopic section of the kidney in one of the aflatoxin treated (1.5ppm) chicks, showing basophilic cortical tubules associated with infiltration of lymphocytes, after 30 days of intoxication (40X. Hα E stain).

Figure-13- Microscopic section of the kidney in one of the aflatoxin treated (1.5ppm) chicks, showing presence of PAS positive homogenous acidophilic substance in glomerular tuft, after 30 days of intoxication (20X. PAS stain).

DISCUSSION

Many those reporting contamination of food, after reper to presence of aflatoxin, as the main toxic agent, but the fact, it was only one of several mycotoxins, which reached up to 280 and still the numbers is raising, as it was 1975, it was less than 100 (7). Aflatoxins were varied in their toxicity, the less toxic one was aflatoxin B_1, while the most toxic one was B_2, therefore, the effect of aflatoxin differ according to the varying types and their levels in food, animal species, age, sex, during of feeding and formula of food. The concentrated research on aflatoxin showed that, they induced three types of hereditary damage, and those were mutagenicity, teratogenicity and carcinogenicity (8). But we ought to remember, as the liver the main site of aflatoxin in the body and it was the goverming organ in enzymes, hormones and food metabolism, as it was considered the target organ for aflatoxins and their effects manifested as fatty degeneration, hepatitis, liver cell necrosis, hepatic cirrhosis and cancer (9), and that agreed with the results of the present research project specially during treatment of chicks with 0.5 ppm concentration, in addition to hemorrhage and congestion of internal visceral organs, especially liver and kidneys and that was agreed with previous research (10.11) as aflatoxins effects on liver and kidneys and that they cause haemorrhage in digestive tract besider their stimulatory effects on enzymes of blood, blood contents and blood coagulation. Aflatoxin may cause degeneration of rough endoplasmic reticulum and inhibit total protein formation (12).

The present finding in this study, vacuolation of heptocytes, hyperplasia of bile ducts and liver cell necrosis were in consistent with previous finding of other authors (12), especially chicks, which were treated with 1.5 ppm concentration The results of the present research demonstrated that chicks treated with aflatoxin appeared swollen pale kidney with focal cortical petechial haemorrhage, and that agreed with what was reported by (13), as the histopathological lesions of kidneys, which were characterized by degeneration of the proximal convoluted tubules especially as hyaline degeneration, which was considered as specific change cause by aflatoxin (14), further more, some studies, observed an increased susceptibility of chickens exposed to aflatoxicosis to other nephrotoxic agents such as high concentration sodium chloride , which caused sever degeneration of proximal convoluted tubules. (15.16).

In conclusion, the present study proved that the causes of destruction of livers and kidneys in poultry, could be due to food contamination with aflatoxin B_1, which was considered the most wide spread mycotoxin contaminating grains, which were stored in poor condition and which were treated wrongly from the drying point of view.

دراسة مرضية نسجية للآفات الكبدية والكلوية الناجمة عن التسمم التجريبي بذيفان الافلاتوكسين B_1 في أفراخ اللحم

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الخلاصة

لمعرفة التغيرات المرضية النسجية الناجمة عن التسمم ببذيفان الافلا B_1 ، استخدم 60 فرخ بعمر يوم واحد قسمت عشوائياً وبالتساوي الى ثلاث مجموعات ، غذيت الاولى (سيطرة) عليقة خالية من ذيفان الافـلا B_1 بينمـا غذيت المجموعتين الثانية والثالثة عليقة حاوية ذيفان الافـلاB_1 بتركيـز (0.5) ppm و (1.5) ppm علـى التوالي ولمدة 30 يوماً ، حيث لوحظ حدوث تغيرات تنكسية دهنية مع فرط تنسج قناة الصفراء وارتـشاح خلايـا

وحيدة النواة ولاسيما اللمفية ، فضلاً عن حدوث التنكس الفجوي داخل هبولي الخلايا المبطنة للنبيبات الملفوفة الدانية الكلوية ولاسيما التنكس الزجاجي وذلك في المجموعة الثانية ، بينما ادى التسمم التجريبي بتركيـز ppm (1.5) الى حدوث آفات نخرية بؤرية في متن الكبد مصحوباً بحدوث التليف البابي وتضخم قناة الصفراء بينما تميزت الكلى بحدوث التنكس الفجوي الشديد والنخر لظهارة النبيبات الملفوفة الدانية المصحوب بزيادة سـمك الغـشاء القاعـدي للكبيبة.

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