PATHOLOGICAL AND BIOCHEMICAL CHANGES INDUCED BY AFLATOXIN IN CHICKENS AND A TRIAL FOR TREATMENT USING LACTOBACILLUS ACIDOPHILUS

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ABSTRACT

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This study was conducted to follow up broiler chickens during aflatoxicosis by feeding them with ration contaminated with Aflatoxin B1 (AFB 1) for a period of six weeks from 0 to 42 days; and to evaluate the effectiveness of Nutritox (a commercial biological antimycotoxin) in alleviating aflatoxicosis. One hundred broiler chicks of one day old were divided into four groups; each of twenty five chicks; the 1st group received a standard ration and kept as a control group, the 2nd group received a ration contaminated with 1 mg AFB1/kg ration, the 3rd group received ration contaminated with1 mg AFB1/Kg ration plus Nutritox with a dose level 0.5gm/kg ration, the 4th group received a standard ration plus Nutritox with a dose level of 0.5 gm/kg ration. Chicks in all groups were weighed at the first and the end day of the experiment (42 days) and feed consumption for each group was calculated, blood and tissue samples were collected from each group at the end of the experiment. Results of the experiment revealed that AFB1 induced reduction in the body weight and feed consumption, while addition of Nutritox to the ration of chicks received AFB1 contaminated ration resulted in an improvement in the body weight and feed consumption compared with that group received contaminated ration with AFB1 which elicited a significant increase in the activity of liver and kidney enzymes with decrease in calcium and inorganic phosphorus levels. Addition of Nutritox to contaminated ration induced a significant improvement in enzymes, calcium and phosphorus levels. Regarding the histopathological results, examination of the internal organs sections revealed typical lesions of Aflatoxicosis, but addition of Nutritox to AFB1 contaminated ration decreased the severity of the pathological lesions. The body weight of chicks of the control group, feed consumption and most of the studied biochemical parameters were improved, these findings suggest that the AFB1, caused many alteration in the growth performance, and the biochemical parameters which are confirmed by many pathological changes in the internal oranges of the chicks. Addition of Nutritox to AFB1, contaminated ration was effective in alleviating the toxic effects associated with Aflatoxicosis.

التغيرات الباثولوجية والبيوكيميائية الناتجة عن الافلاتوكسين فى الدجاج ومحاولة العلاج باللاكتوباسيلاس اسيدوفيلاس

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أجريت هذه الدراسة على ١٠٠ كتكوت تسمين عمر يوم واحد قسمت الى اربع مجموعات تحتوي كل مجموعة على ٢٥ كتكوت. المجموعة الاولى (الضابطة) تم تغذيتها على علف بادي خالي من اي سموم فطرية ، المجموعة الثانية تم تغذيتها على علف بادي + سم الافلاتوكسين بمعدل (١ جم / كجم علف). والثالثه تم تغذيتها على علف بادي + سم الافلاتوكسين بمعدل (١جم / كجم علف) + مركب نتروتكس بمعدل ٠,٥ جرام / ١ كجم علف والمجموعة الرابعة تم تغذيتها على علف بادي خالي من اي سموم فطرية + نتروتكس بمعدل (٠,٥ جم / ١ كجم علف) استمرت التجربة حتى عمر ٤٢ يوم. تم وزن جميع الطيور في كل المجاميع فى بداية التجربة وفى آخرها كما تم حساب معدل استهلاك العلف ومعدل التحويل الغذائي والزيادة في الوزن كذلك تم جمع عينات عن الدم وانسجة الكبد والكلى من كل مجموعة. اظهرت النتائج ان الافلاتوكسين احدث نفوق وصل في نهاية التجربة الى ٣٢% مصحوبا بنقص معنوي في وزن الدجاج ومعدل استهلاك العلف وكان لاضافة نتروتكس اثر ايجابي في تحسين نسبة النفوق الى ٤% ايضا تحسن الوزن واستهلاك الغذاء كما ان اضافته الى العلف الخالي من السموم الفطرية ادى الى تحسين الوزن واستهلاك العليقة مقارنة بالمجموعة الضابطة. اظهر تحليل السيرم ان الافلاتوكسين ادى الى حدوث زيادة معنوية في نشاط انزيمات الكبد واضطرابات فى وظائف الكلى وقد احدث اضافة نتروتكس تحسناً ملحوظاً في قياسات هذه الانزيمات. وقد كانت التغيرات الكيميائية في الدم انعكاساً للتغيرات المرضية للاعضاء الداخلية حيث تسبب الافلاتوكسين في حدوث تغيرات تحلليه وتنخر في خلايا الكبد والكلى وكان لاضافة نتروتكس للعليقة اثر واضح فى تحسن ملحوظ في التغيرات الباثولوجية ومن هذه النتائج نستخلص ان التسمم بالافلاتوكسين تسبب في حدوث العديد من التغيرات البيوكيميائية والباثولوجية والتي انعكست كليا على الوزن ومعدل النمو في الدجاج كما ان اضافة نتروتكس كان له تاثير ايجابي فى التقليل من الاثار السمية المصاحبة للافلاتوكسين.

INTRODUCTION

Aflatoxin contamination occurs over large geographic regions and in much potential feed stuff, such as cotton seed, peanut, corn, rice, dried fish, shrimp, and meat meals.

Aflatoxin is a toxic product of fungal growth produced primarily by the moulds Aspergillus flavus, A. parasiticus and A. nomius in cereal grains particularly corn in which its spores germinate during storage. Refai M. (1988). Four types of AF are produced. AFB1, AFB2, AFG1 and AFG2, Avian species especially chickens; duckling and turkey poults are most susceptible to AFB1, toxicity. AFB1 is very hepatotoxic, carcinogenic and immunosuppressive. AFB1 does not affect only chickens but also affect other animals and human Williams *et al.* (2004).

AF produces severe economic losses and healthy problems in the poultry industry. The signs of aflatoxicosis in poultry include anemia, inhibition of immune function, hepatotoxicosis, mutagenesis, teratogensis, carcinogenesis, anorexia, hemorrhage, poor food utilization, decreased weight gain and increased the susceptibility to environmental and microbial stresses. Edds and Bortell (1983).

Many methods are used for antidoting Aflatoxin, a variety of physical, chemical and biological methods for detoxifying AF have been employed with limited success.

The present study was planned to study the ability of commercial product (Nutritox) to overcome the side effects aflatoxicosis in broiler chickens.

MATERIALS and METHODS

1-Drug

Nutritox®

Is a biological commercial product made in Agrarian Marketing Corporation Company (USA) imported by IFT Company.

Nutritox consists of four distinct groups of components

A- L. form bacteria: dried fermentation extract 370gm /kg.

B- Organic acids and their salts which function as acidifiers.

C- Activated sodium alumino silicate and silicon dioxide.

D- B. complex vitamins and minerals.

Dosage: According to the severity of mycotoxicosis; the appropriate dosage is recommended as it ranged from 250-500gm/ton of finished feed.

2- Experimental chickens

One hundred one-day-old commercial Hubbard broiler chicks were used. The chicks were reared under standard hygienic condition and fed a balanced commercial ration. All chicks were vaccinated against Newcastle disease at 7 and 21 day of age and Gumboro disease at 15 days old. These chicks were equally divided into 4 groups each of 25 chicks and housed in separate pens as follows:

Group I: fed on normal ration as a control group.

Group II: fed on ration mixed with Aflatoxin B1 (1mg/kg. diet daily)

Group III: fed on ration containing Nutritox (0.5kg/ton feed) and Aflatoxin B1 (1 mg/kg diet daily).

Group IV: fed on normal ration mixed with Nutritox (0.5kg/ton feed)

All chicks were kept under observation daily for detection of clinical symptoms and recording mortality rates caused by Aflatoxin. Chicks in all groups were weighed at the beginning and at the end of experiment (42day), feed consumption for each group were calculated. Five chicks from each group were sacrificed for histopathological study after collection of blood samples via heart puncture at the end of the experiment.

3-Aflatoxin:

Aflatoxin was produced by growing Aspergillus flavus (standard toxigenic strain) on crushed corn meal according to the method of Merwe *et al.* (1965) Identification and quantitative estimation of Aflatoxin present in crushed corn meal were done by thin layer chromatography. The prepared corn meal containing Aflatoxin was mixed with ration to provide a final concentration of 1 mgAFB1 /kg ration.

4- Body weight and feed consumption:-

Chicks in all groups were weighed at the beginning and at the end of the experiment, feed consumption for each group was calculated daily during the experimental period and mortality rate for each group was recorded.

5 – Blood sampling: -

Five ml of blood were collected from 5 birds from each group via heart puncture. The blood samples were collected into centrifuge tubes, left to clot at room temperature and then sera were subjected to biochemical analysis.

6- Biochemical studies:-

Separated sera were used for evaluation of Aspartate amionotransfrase (AST), Alanine amionotransfrase (ALT) according to Reitman and Francle (1957). Serum alkaline phosphatase (AP) was determined according to Rec (1972). Serum uric acid was measured according to the method described by Fossati (1980), creatinine was determined according to Husdan and Rapoport (1968), Serum calcium and inorganic phosphorous were determined according to Tietz (1970) and Yousef *et al.* (1975) respectively.

7 – Histopathological studies

The sacrificed chickens were subjected to post mortem examination; specimens were collected from the liver, and kidney, then mixed in 10% neutral formalin and embedded in paraffin wax. Sections of five microns thickness were prepared stained by haematoxylin and eosin and examined microscopically. Carlton and Mc Gavin (2001)

8 – Statistical analysis.

The data obtained from this investigation was statistically analyzed by student's "t" test according to Bland (1987).

RESULTS

Table 1: Mortality rates, mean body weight gain, feed consumption (F.C) and feed conversion rate (F.C.R) post administration of Aflatoxin (AF) and Nutritox (Nut) in broiler chickens (Mean values ± S.E)

Group	Mortality		Body weight gain	Feed consumption gm/bird	Feed conversion rate
	No.	%			
1- Control	0	0	1680.95 ± 14.95	3274.43 ± 10.65	1.95
2- AF	8	32%	1568.62 ±** 14.63	3310.86 ±* 9.87	2.11
3-AF.+nut	1	4%	1605.95 ±* 8.94	3325.09 ± 16.96	2.07
4-Healthy+nut	0	0	1820.95 ±** 18.64	3350.34 ± 6.95	1.79

* Significant at $p < 0.05$
** Significant at $p < 0.01$

Table 2: Effect of Aflatoxicosis and Nutritox on some serum biochemical parameters of broiler chickens (n = 5)

Group	AST Iu\L	ALT Iu\L	Alka.ph.	Creatinine Gm\dl	Uricacid g\dl	Calcium Mg\dl	Phosphorus Mg\ DL
G1 – Control	36 ± 3.36	9± 0.43	50.4 ± 2.8	0.85 ± 0.08	3.4 ± 0.07	9.28 ± 0.22	4.56 ± 0.09
G2 – AF.	56 ±** 2.2	16.2 ±*** 0.5	66.8 ±* 2.4	1.9 ±* 0.36	7.2 ±** 0.2	7.68 ±** 0.24	3.6 ±** 0.19
G2 – AF+Nutritox	46 ±* 1.6	11 ±** 0.14	51.2 ± 2.5	1.6 ±* 0.31	4.1 ±* 0.21	8.46 ± 0.44	4.12 ± 0.2
G1 – Nutritox	35 ± 1.7	8.7 ± 0.34	53.8 ± 1.28	1.1 ±* 0.04	3.2 ± 0.1	9.1 ± 0.17	4.52 ± 0.07

Significant at $p < 0.05$ *
Significant at $p < 0.01$ **

Table 3: Clarification the severity of the pathological lesions in different groups

Serial number	Group number	Category of lesions	Summary of lesions
0	G1 &G4	No lesions	----------
1	G3	Mild lesions	Represented by mild renal damage ,mild degenerative changes of hepatocytes after few days of the experiment
2	G3	Moderate lesions	Represented by congestion of renal blood vessels, degenerative changes of renal tissue and anemia the end of the experiment
3	G2	Severe lesions	Represented by hyperplasia of the bile duct and necrotic changes of hepatic parenchyma, Coagulative necrosis of renal parenchyma.

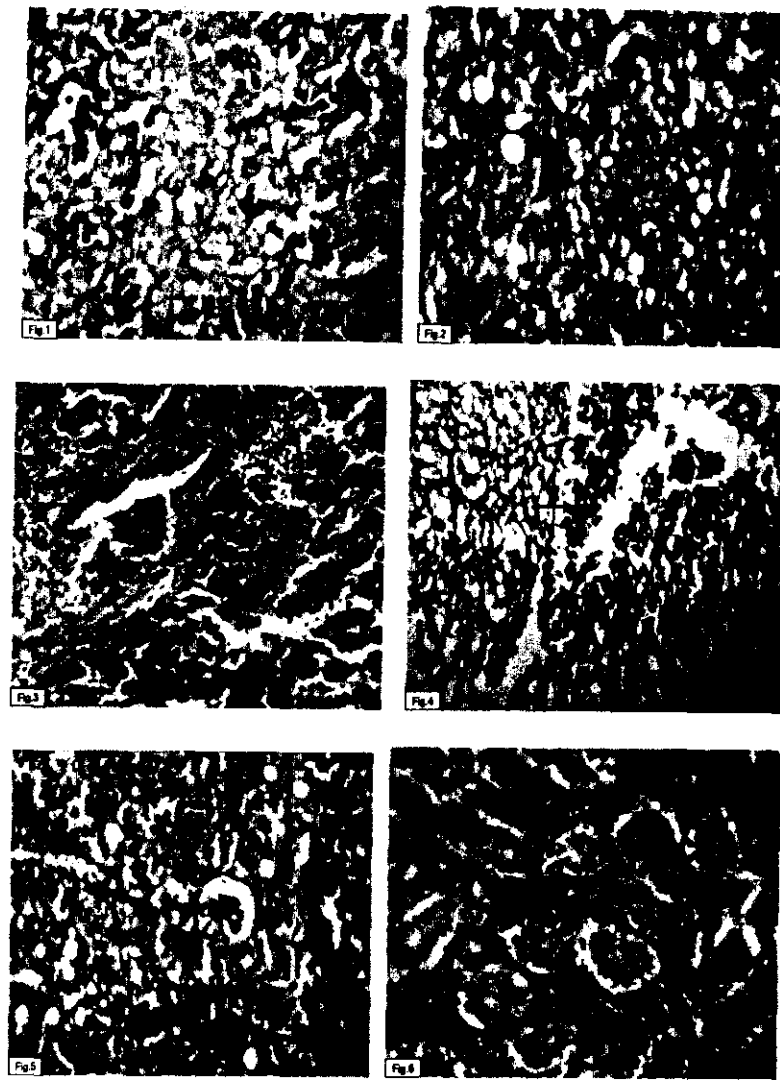

Fig. 1: Section of the liver of Chicken fed on ration mixed with Aflatoxin B1 (1 mg/kg diet daily) showing Coagulative necrosis scattered throughout the hepatic parenchyma, the nuclei of the affected cells showed karyolysis & Karyorrhexis. H&Ex300.

Fig. 2: Section of the liver of Chicken fed on ration mixed with Aflatoxin B1 (1 mg/Kg diet daily) showing that the portal areas are highly infiltrated with leukocytic cells and hyperplasia of the bile ducts H&Ex300.

Fig. 3: Section of the liver of Chicken fed on ration with Aflatoxin B1(1 mg/Kg diet daily) showing Extensive hyperplasia of the lining epithelium of the bile duct with numerous leukocytic aggregations H&Ex300.

Fig. 4: Section of kidney of Chicken fed on ration mixed with Aflatoxin B1(1 mg/Kg diet daily) showing severe congestion of the renal blood vessels and hemorrhage at the corticomedullary junction H&Ex300.

Fig. 5: Section of kidney of Chicken fed on ration mixed with Aflatoxin B1(1 mg/Kg diet daily) showing congestion and hypercellularity of the glomeruli in addition to degenerative changes of renal tubules H&Ex300.

Fig. 6: Section of kidney of Chicken fed on ration mixed with Aflatoxin B1(1 mg/Kg diet daily) Showing Coagulative necrosis of the renal parenchyma represented by Pyknosis and karyorrhexis of the nuclei of the affected cells in addition to Interstitial round cell infiltrations invading the renal parenchyma H&Ex300.

DISCUSSION

Mycotoxins, particularly AF have been reported to produce severe economic losses and health problems in the poultry industry. Moderate aflatoxicosis reduces the growth rate and increase mortality percent. El-Banna (2003)

The present results shown in Table (1) indicated that chicks fed ration contaminated with AFB1 mg/kg ration for 42days showed decrease in body weight gain, feed consumption with high feed conversion rate.

The same results were also reported by Dalvai nd MC Gowan (1989), Aravind *et al.* (2003) and Watts *et al.* (2003), the exact mechanism by which AF impairs growth is unknown , but it is probably multifactorial, involving disturbances in carbohydrate, lipid and protein metabolic interaction with the toxin and disturbance in hormones. Additionally poor appetite and reduced feed intake may partially account for reduced performance (Edrington *et al.*, 1994). Nasr – El Deen (2002); added that, the recorded losses in body weight may be a reflection of reduced feed intake or reduced utilization and metabolism of food stuff due to intestinal and hepatic lesions leading to impaired liver functions.

When Nutritox is included in the diet of chicken received 1mg AFB1 /kg ration, elicited a marked improvement in body weight than those received the same dose of AF alone and achieved body gains that were not significantly different from control group when examined over the experimental period.

Addition of Nutritox to ration free from AF revealed significant improvement in the body gain, feed consumption and decrease feed conversion rate compared with that of the control group (Table 1) this may be due to that Nutritox contain L. form of bacterial fermentation extract. Similar results were obtained by and El-Bauhy *et al.* (2011) in fish. Feeding chicks with ration contaminated with AFB1 1mg/kg ration without and with Nutritox throughout the experimental period, resulted in 32 % and 4% mortalities respectively as recorded in (Table 1). Addition of Nutritox lowered the mortalities to 4% compared to 32% in chicks fed the same dose of AFB1 alone these deaths may be attributed to impaired immunity, renal damage and anemia produced by Aflatoxin. Abdel-Khaleik (1985). The role of Nutritox maybe attributed to dried L. form of bacterial fermentation extract which are useful to chicks not only as food but also as biological controller of chickens diseases Yasuda and Taga (1980), another reason may be due to decreasing of the toxicity effect caused by AFB1 by adsorption mechanism Huff *et al.* (1992).

Groups fed ration contaminated with AFB1 showed significant increase in liver enzymes (AST, ALT and alkaline phosphatase), when antimycotoxin Nutritox is added to the ration, the levels of liver enzymes returned nearly to its normal levels (Table 2); this increase in liver enzymes was explained by the hepatotoxic effect of AFB, as reported by Williams *et at.* (2004). Addition of Nutritox significantly decreased the liver enzymes, this may be attributed to the beneficial effect of L. form of bacteria (dried fermentation extract) that can reduce Aflatoxin and so improve health status Misaghi (1994), Nasr-El-Deen (2002) and Youssef *et al.* (2003). Uric acid is the primary catabolic product of protein and non –protein nitrogen in birds. Hyperuicemia in birds occurs with starvation, gout, massive tissue destruction and renal diseases Coles (1986), in the present study, AFB induced significant increase in uric acid, similar findings were reported by Dawoud *et al.* (2002) who reported that the significant increase in uric acid suggests that kidney function was severely impaired.

The ability of Nutritox to reduce the biochemical alterations caused by aflatoxin was evaluated in our investigation. The results revealed that the addition of Nutritox to normal ration did not alter the biochemical parameters compared to control (Table2). The role of Nutritox in Aflatoxin contaminated ration elicited an improvement in the enzymatic activity (liver and kidney enzymes) and corrected the alteration in calcium and phosphorus among chicks received AFB1, 1mg/kg ration plus Nutritox, compared with chicken fed AFB1, alone. These may be due to Nutritox Contain components which can

adsorb all amount of Aflatoxin in ration Ramos and Hernandez (1997), C.L.xu *et al.* (2006) in layers and the beneficial effect of L. form of bacterial fermentation extract which inhibit the growth of Asp. flavus and also decrease the production of Aflatoxin. Reddy *et al.* (2010).

The biochemical changes occurred during aflatoxicosis in the present work was confirmed by the histopathological changes in the internal organs which were found after aflatoxicosis.

Macroscopically, the liver of broiler chicks at the end of experiment in group II , were yellowish, friable, and enlarged in size; moreover hemorrhage on skeletal muscles, hydro pericardium and enlargement of kidney were also reported.Such findings were also observed by Rosa *et al.* (2001).

Microscopically, the lesions were ranged from very severe lesions in second group which received Aflatoxin alone to very mild in third group which received Aflatoxin plus Nutritox. Biliary duct hyperplasia was detected in chicks exposed to1 mg AFB1/kg ration.

Livers of the second group showed diffuse retrogressive changes represented by vacuolar degeneration of most hepatic cells and fatty changes. Cellular necrobiosis scattered throughout the hepatic parenchyma. (Fig. 1)

Hyperplasia of the bile duct in the portal areas was seen. (Fig. 2), mild hyperplasia of the lining epithelium of the bile ducts and focally replaced the adjacent hepatic cells with numerous leukocytic aggregations (Fig. 3), in addition to thickened capsule. The detection of the newly formed bile ductules was similar to that reported by Kelly (1985) who suggested that the hyperplasia of the bile ducts is an attempt to regenerate hepatic parenchyma when the parenchymal cells have lost their capacity to regenerate themselves.

Kidneys of the second group showed edema between the renal tubules, severe congestion of the renal blood vessels and hemorrhage particularly at the corticomedullary junction (Fig. 4). Congestion and hypercellularity of the glomeruli and the renal tubules showed degenerative changes (Fig. 5).

Coagulative necrosis of the renal parenchyma represented by Pyknosis and karyorrhexis of the nuclei of the affected cells in addition to Interstitial round cell infiltrations invading the renal parenchyma (Fig. 6).

Our results were confirmed by Tag-el-Deen (1997) and Kim *et al.* (2003) who described the histopathological lesions of chickens during aflatoxicosis. They reported vacuolated hepatocytes, bile ducts hyperplasia with aggregation of inflammatory cells. The kidney showed degenerative changes and necrosis of renal tubules, with leukocytic infiltration.

Addition of Nutritox to AF contaminated ration in the third group moderately decreases the number of affected broilers, the incidence and the severity of the pathological lesions.

It could be concluded that AFB1, contaminated ration was marginally effective in alleviating some toxic effects associated with aflatoxicosis.

Nutritox contains organic acids and their salts which function as acidifiers also activated silicate has adsorbing capacity to the Mycotoxins molecules, rendering them un-absorbable and excreted with the droppings, in addition, Nutritox contains essential micro-nutrients for the growth and multiplication of the lactic acid-producing Gram positive bacteria in GIT.

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