HISTOPATHOLOGICAL STUDIES ON MYCOTOXICOSES IN BROLLERCHICKS

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Received for Puplicatian : 30/12/1990

INTRODUCTION

Mycotoxins have been suggested as a causative factor in a large number of diseases in animals and man. Many strict measures were taken to reduce the level of mycotoxins in food to guard against outbreaks of mycotoxicosis. Inspite of these measures, many important food stuffs especially milk, nuts, maize, grain and caffee beans still contain mycotoxins (Abdelhamid, 1989). Many studies were undertaken to asses the effect of mycotoxins especially in the liver. Most of them involved only the effect of one type of mycotoxins, (Smith et al., 1974 and Terao & Ueno, 1978). The aim of this study was to evaluate the possible histopathological changes caused by multimycotoxins contaminated feed in different organs in chickens.

MATERIAL AND METHODS

Brolier chicks of Lohmann breed were divided into five groups (6 birds / each group) and fed on one of the following rations :

1- Mycotoxin free commercial feed (control).

2- Control feed but contaminated with aflatoxin B_1 (ANB_1, 100 ppb) and sterigmatocystin (SN, 350 ppb).

3- Control feed contaminated with

aflatoxin B_1 (ANB_1, 100 ppb) and patulin (PN, 100 ppb).

4- Control feed contaminated with aflatoxin B_1 (ANB_1, 100 ppb) and penicillic acid (PAN, 850 ppb) .

5- Control feed contaminated with multi-aflatoxins, B_2a (0.9 ppb), G2 a (1.0 ppb), M_1 0.9 ppb and M_2 1.0 ppb.

The mycotoxins were in crystalline form, ANB_1 from Aldrich chem. Co.and the other types from Makor chemical LID.

The five tested rations were offered during the first four weeks of age, thereafter, the mycotoxin free feed was offered for the next 4 weeks as a recovery period.

After the period of treatment (at the end of the lst 4 weeks), 3 birds were sacrificed from each group and at the end of the recovery period the remaining 3 birds from each group were sacrificed. Parts of most of internal organs, skeletal muscles and bones were collected. The specimens were fixed in 10% neutral formaline, processed as paraffin blocks, 4 mu thick sections were cut and stained by Haematoxylin & Eosin.

RESULTS

Group I (Control group) :

This group had normal organs since all examined specimens reflected normal histological structures.

Group II, III, VI and V :

The most common and most severely affected organ was the liver, This was followed by kidney, heart, intestine and lung. The testis was affected in group V only. Specimens collected from muscles and bones showed no considerable changes, that of the endocrine glands were normal except for focal lymphoid collections in the thyroid gland.

(I) Liver :

In the group II (given aflatoxin B_1 and sterigmatocystin), the liver showed diffuse ballooning degeneration of hepatocytes (Fig.I).Central zonal congestion was seen in many

cases. Areas of liver cell necrosis which were infiltrated by polymorphs and mononuclear cells as well as swollen deeply eosinophilic hepatocytes. The portal tracts were infiltrated by mononuclear cells rich in eosinophils.

In the group III (given aflatoxin B_1 and patulin), the same previous changes were detected but with more severe ballooning degeneration (Fig. 2) as well as massive areas of cellular necrosis especially in central zone (Fig. 3).

In the group VI & V (given aflatoxin BI and penicillic acid) and (Multiaflatoxins, B_2 a , G_2a, M_1 and M_2) respectively revealed the same severe liver changes, After recovery the liver showed diminished cellular infiltrate with evidences of fibrosis in the form of variable degrees of portal, perivenular and perisinusoidal fibrosis (Fig. 4).

(2) Kidney :

In group II (given aflatoxin BI & sterigmatocystin) the renal tissue changes were mainly tubular in the form of cloudy swelling of the proximal convoluted tubules as well as minute foci of tubular necrosis (Fig. 5). The blood vessels were congested and some were thrombosed.

In group III (given aflatoxin BI & patulin) the same previous renal tissue changes were present but with superadded interstitial nephritis.

In group VI (given aflatoxin BI & Penicillic acid) and group V (given multiaflatoxins) the kidney lesions were more severe than in group II and III with considerable periglomerular fibrosis, tubular atrophy with focal detatchment of the tubular epithelial lining and prominent interstitial cellular infiltrate. (Flg.6).

Kidney specimen of the recovery birds revealed interstitial fibrosis.

(3) Intestine

This affected in groups of chickens fed mycotoxin contaminated diet.

The changes in group II & III were

in the form of plaques of fibrin and necrotic debris on the surface mucosa. Marked inflammatory cellular infiltrate was seen extending from mucosa to submucosa with necrosis of the glands . In group VI & V the intestine was more affected by cellular infiltrate , mucosal necrosis, villous atrophy and fibrosis (Fig. 7). These changes were not improved upon recovery.

(4) Heart :

The heart was affected only in the group II and III (given aflatoxin B_1 & steri gmatocystin and aflatoxin B_1 & patulin) respectively. The myocardium showed wide separation of the muscle fibres and round cells infiltrate (Fig. 8). On recovery the cellular infiltrate was nearly disappeared and bands of fibrosis were seen.

(5) Lung

This was affected only in the group II and III (given aflatoxin BI & sterigmatocystin and aflatoxin-BI & patulin). The prominent changes were marked congestion and thrombosis with deposition of brown black pigment (haemosedrin), (Fig.9). The vascular congestion was diminished after recovery.

(6) Testis :

This was affected in group IV, (given aflatoxin BI & penecillic acid) only. The testes showed slouphing of the cells lining the semineferous tubules. The interstitial tissaues showed marked fibroais. (Fig. 10).

DISCUSSION

The present study showed that mycotoxins affect various organs of chicks- fed mycotoxins contaminated diets. The liver was the most severely and frequently affected organ.

The most consistant higtopathological findings in liver after administration of aflatoxin B_1 was recorded to be hepatocellular necrosis, (Smith et al., 1974 & Colin, 1988) In the present study aflatoxin B_1 in combination with sterigmatocystin produced degenerative hepatocellular changes in the form of ballooning degeneration together with central zonal congestion, focal areas of necrosis as well as swollen deeply eosinophilic hepatocy-

tes, Our findings are in agreement with previous studies of (Nglndu et al. (1982) who reported single cell necrosis with administration of sterigmatocystin which increases in amount with the use of aflatoxin Bl, The detected liver cell changes may be attributed to either mitochondrial alterations with inhibition of electron transport, (Terao & Ueno, 1978) or to lysosomal damage produced by direct interaction between mycotoxins and lysosomal membranes leading to accelerated release of lysosomal enzymes, (Pitout et al., 1971 and Schabort & Pitaut, 1971). In addition, the present study revealed infiltration of the portal tracts with mononuclear cells that did not estend through the liver lobules. This is in contradistinction with serao& Ueno (1978) who observed absence of inflammatory reaction in the liver after ingestion of aflatoxin. The inflammatory reaction reported in this study may be a non specific reactive infiltrate.

In the group of chickens given aflatoxin B_1 and patulin, the degenerative and necrotic changes in the liver were severer than in group II (given aflatoxin B_1 and sterigmatocystin). This increased severity of hepatic injury may be attributed to a synergistic effect of aflatoxin B_1 and patulin. The latter was found to induce chromosomal breakage in salamander eggs during mitosis. (Umeda et al., 1972).

In groups IV-and V (given aflatoxin B_1 and penicillic acid) and (multiaflatoxins) respectively the liver changes were more or less the same as group III.

It is of interest to report that these changes diminished but never disappeared in chickens under recovery.

On the basis of the observations of the present study, it must be stressed that the liver is a commonly affected organ. This is because it is the first organ to be exposed to chemicals absorbed into the portal vein from the gastro-intestinal tract. Thus it is exposed to a higher concentrations of toxins. Also the liver is the major site of biotransformation and may generate toxic metabolites from chemicals taken into the liver cells.

The renal changes reported in this study was more or less the same in all groups, being severer in groups III (given aflatoxin BI and patulin), IV (given aflatoxin BI and penicillic acid and V (given multiaflatoxina) than those in group II (given aflatoxin BI and sterigmatocystin). The renal histopathological changes were mainly tubular in the form of cloudy swelling of prosimal convoluted tubuls with minute foci of tubular necrosis. Previous studies reported that sterigmatocystin induced renal tubular degeneration (Sreemannarayan et al., 1987). The renal tubular cell necrosis detected in the present study may be attributed to direct effect of aflatoxin BI on renal tubules.

The kidney is a frequent target for the toxic action of chemicals during fluid transfer associated with elimination of waste products (Glaister, 1986).

As regards the heart and lung, these organs were affected only in group II (given aflatoxin B_1 and sterigmatocystin) and group III (given aflatoxin BI and patulin). The heart revealed wide separation of the muscle fibers with round cells infiltration which were nearly disappeared on recovery. The prominent changes in the lung were marked congestion and thrombi with deposition of haemosiderin pigment. This vascular congestion was diminished after recovery.

The present study has shown that the testis was affected only in groupIV (given aflatoxin BI & penicillic acid). The testes showed slouphing of the cells living the semineferous tubules with marked interstitial fibrosis.

A finding which may be of interest is that the heart and lung were affected in groups II & 'II only while the testicular damage was present in chickens of group IV only with sparing of these organs in the remaining groups. This may indicate that different mycotoxins have a specific effect action on certain and not all organs.

In addition, the histopathological changes were diminished but never disappeared in the chickens under

recovery indicating that the recovery period of four weeks used in the present study was not enough to remove the mycotoxins induced abnormalities but only led to alleviation of the lesions.

In conclusion we could stress on the increased severity of multi-organ damage observed in the present study compared to those in the literature induced by monomycotoxicosis. A fact, that may reflect a synergistic action of multimycotoxicosis used in our study and which may represent a contaminant factor affecting many human foods.

SUMMARY

The histopathological examination of various organs of chicks fed mycotoxins contaminated food revealed the severe toxic effect of ingestion of different mycotoxins combination (aflatoxin BI 100 ppb + sterigmatocystin 350 ppb; aflatoxin B_1 100 ppb + patulin 100 ppb; aflatoxin B_1 100 ppb + penicillic acid 850 ppb; or aflatoxins B2 a 0.9 ppb + G2a 25 ppb + MI 0.9 ppb + M21.0 ppb), Even in low contamination levels of mycotoxins, remarkable pathological alterations were produced in different organs of birds. The most affected organs were the liver and kidney. The liver showed diffuse ballooning of hepatocytes, central zonal congestion, necrosis and cellular infiltration. The kidney was the seat of marked cloudy swelling of the proximal convoluted tubules and minute foci of tubular necrosis the severity of toxicity of the contaminats combinations of each of the last three diets was similar; but each of them was more toxic than the first contaminated diet (aflatoxin BI+sterigmatocytins. The recovery period of four weeks (during which the birds were fed on mycotoxins free diet) was not enough to remove the histopathological abnormalities but only led to alleviation of the lesions.

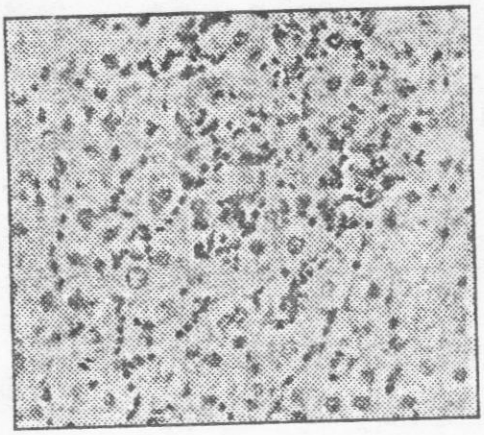

Fig. 1 : Section in the liver showing ballooning of the hepatocytes with foci of cellular infiltrate (Hx.& E.X 16).

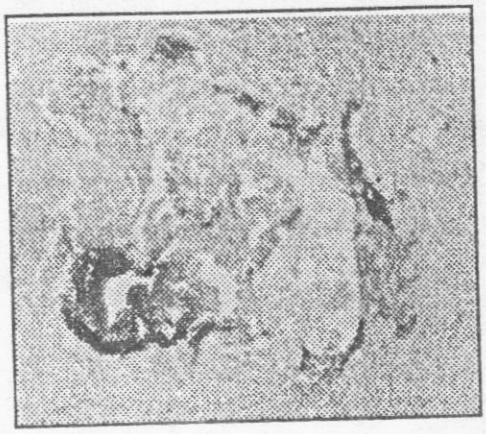

Fig. 3 : Section of the liver Showing area of eosinophilic heamogenous necrosis (Hx. & E. X 6).

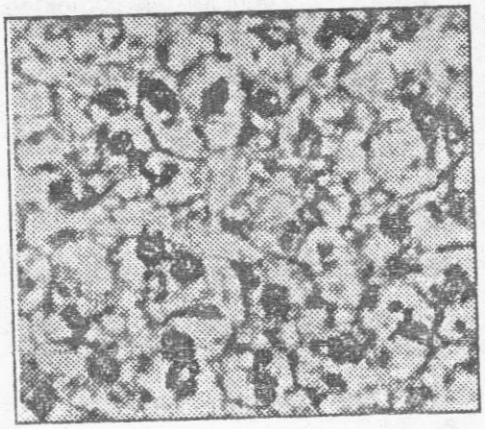

Fig. 2 : Section in the liver showing more severe degree of ballowing degeneration than the previous figure(Hx.&E.X 16).

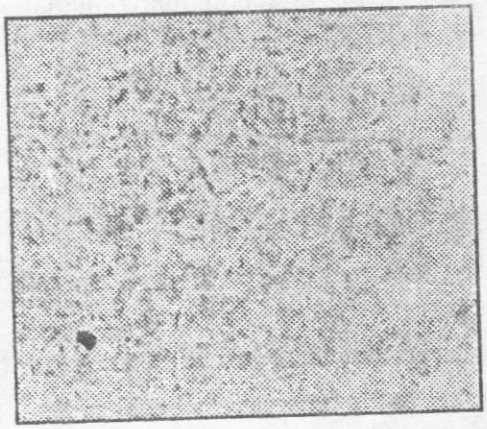

Fig. 4 : Section of the liver presenting perisinusoidal fibrosis (H; c. & E. X 10).

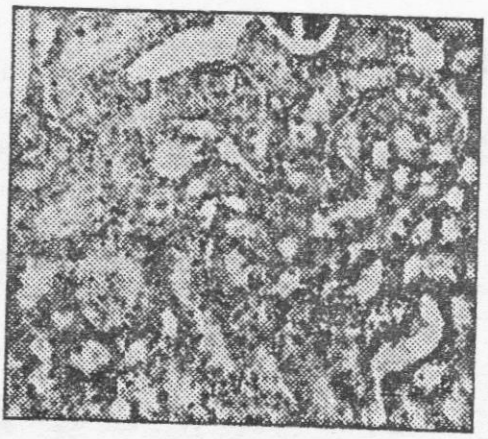

Fig. 5 : Section in the kidney presenting cloudy swelling oi the convuluted tubules (Hx. & E. X 10).

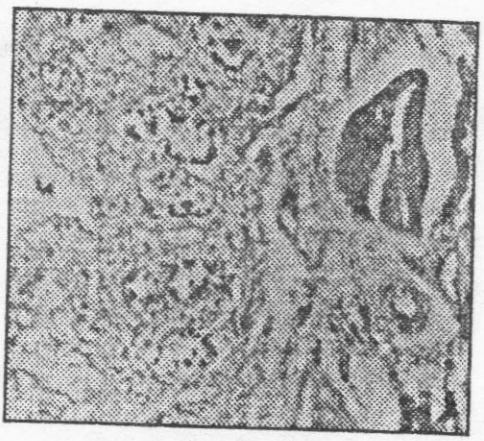

Fig. 7 : Section on small intestine showing villous atrophy , inflammatory reaction and glandular necrosis (Hx. & E. X 10).

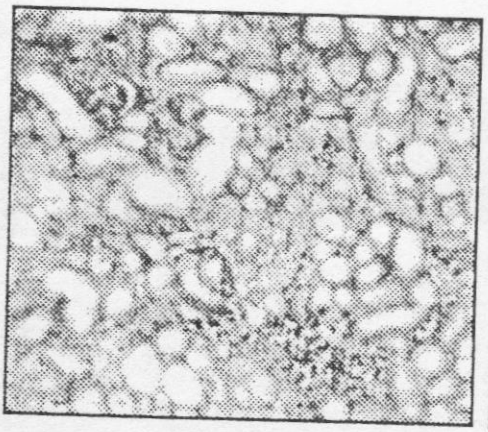

Fig. 6 : Section in the kidney showing tubular degeneration and necrosis with marked interstitial nephritis. (Hx. & E. X 10).

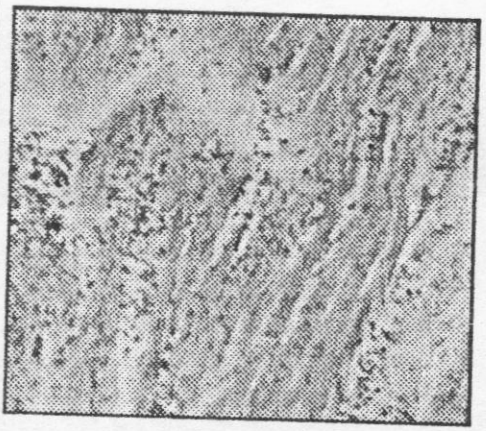

Fig. 8 : Section of the myocardium showing round cell infiltrate and separation of muscle fibres. (Hx. & E. X 10).

Fig. 9 : Section of the lung showing pulmonary thrombosis and congestion (Hx. & E. X 10).

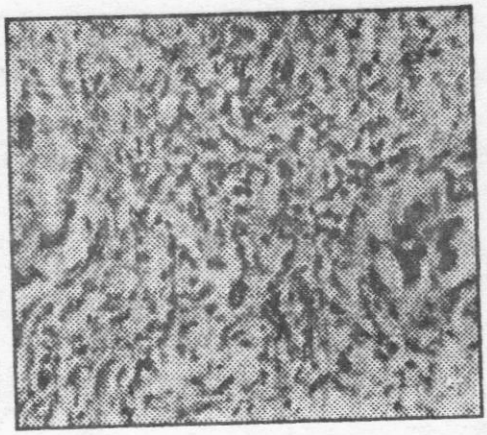

Fig. 10 : Section in testis presenting tubular atrophy with slouphing of their epithelium. The stroma ia fibrosed (Hx. & E. x 10).

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دراسة التغيرات الهستوباثولوجيه الناتجه عن تلوث العليقه فى دجاج اللحم بالسموم الفطريه (الميكوتوكسنات)

اجريت دراسة على ٣٠ من دجاج اللحم من النوع التجارى من عمر يوم واحد وحتى ٨ اسابيع (٤ اسابيع تلوث + ٤ اسابيع نقاهه) وقسمت الى خمس مجموعات كالاتى :

١ - المجموعة الضابطه تغذى على عليقه تجاريه خاليه من السموم .

٢ - المجموعة الثانيه تغذى على عليقه تجاريه + افلاتوكين ب ١ + سترجماتوستين .

٣ - المجموعة الثالثة تغذى على عليقة تجاريه + افلاتوكسين ب ١ + باتيولين .

٤ - المجموعة الرابعة تغذى على عليقه تجاريه + افلاتوكسين ب ١ + حمض البنيسيليك .

٥ - المجموعة الخامسة تغذى على عليقه تجاريه + افلاتوكسين ب ٢ ، جـ ٢ ، م ١ ، م ٢ .

واثبتت نتائج البحث وجود تغيرات مرضيه ملحوظه نتيجه لتعاطى هذه السموم فى كثير من الأعضاء الداخليه . وكان الكبد أكثر الاعضاء تأثرا وذلك بظهور علامات الضمور والانحلال فى خلاياه وما يعقبها من تليف . ويلى الكبد بنسبة الاصابة الكلى وذلك بظهور ضمور خلايا الانابيب والتهاب النسيج البينى .

لوحظ من الدراسة ان تأثير تلوث الطعام بأكثر من نوع السموم كان اكثر خطوره مما لوكان التلوث من نوع واحد . ويخلص البحث الى ان تلوث الطعام بالسموم الفطريه ذو تأثير خطير حتى بجرعات صغيره وبنفس النسب التى ثبت وجودها فى كثير من الاطعمه الادميه وخصوصا المستورده والمحفوظه . وبالاضافه الى ذلك ثبت ان فترة النقاهه لمدة ٤ اسابيع غير كافيه لازالة الآثار المرضيه السابقة .