HISTOPATHOLOGICAL STUDY OF QUAILS LIVER EXPERIMENTALLY INDUCED BY AFLATOXIN

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(Received 22 January 2013, Accepted 15 May 2013)

Keywords: aflatoxin**,** quail **,** liver**.**

ABSTRACT

This study to evaluate the toxic effects of aflatoxin (AF) at a rate of 2.5 and 5 ppm on liver histopathological appearance of local quails.sixty quail chicks at one day old randomly divided into two pen replicates of ten chicks each were assigned to each of three dietary treatments. 1) basal diet containing no AF/kg diet (control); 2) basal diet supplemented with 2.5 ppm diet and 3) basal diet supplemented with 5 ppm diet. The chicks were individually weighed on a weekly basis, feed consumption and body weight gain were recorded weekly. The trial period was 5 weeks and quails were maintained on 24-h continuous light schedule and *ad libitum* access to diets and water. Feeding Aflatoxin to quails included liver fatty changes, necrosis, bile duct hyperplasia and aggregation of lymphocyte . The intensity of these changes were increased with increasing AF level in the diet. The results indicated that local quails are sensitive to AF.

INTRODUCTION

Aflatoxins, secondary metabolites of various Aspergillus spp., commonly contaminate a wide variety of tropical and subtropical food/feed stuffs. These mycotoxins are known to have strong hepatotoxic and carcinogenic effects and are

regulated by feed/food law in at least 100 countries (1). Chemically, aflatoxins are difuranocoumarin compounds and include B1, B2, G1, G2, M1, and M2 (2).

These mycotoxins contaminate a wide variety of agricultural commodities including oilseed meals, dried fruits, spices, and cereals (3. Aflatoxins are highly toxic and carcinogenic mycotoxins produced by *Aspergillus flavus* and *A. parasiticus* (1). Poultry feeds and ingredients are vulnerable to fungal growth and aflatoxin formation. Aflatoxins are relatively stable in feed products. Aflatoxin BI is the most toxic, and bepatotoxicity is the primary effect in nearly all animals. Aflatoxin producing fungi and aflatoxin contaminated animal feedstuffs are recognized worldwide (4; 5), usually with adverse implications for poultry production (6; 7). Aflatoxicosis occurs in poultry worldwide (8; 9).

Aflatoxicosis in chickens caused yellow, ocher discoloration of the liver, with multifocal hemorrhage and a reticulated pattern on the capsular surface. In time, the livers developed white foci as hepatic lipid content increased. Histological lesions occurred as fatty vacuolation of hepatocyte cytoplasm; karyomegaly and prominent nucleoli in hepatocytes; proliferation of bile ducts; and fibrosis. Basophilic, vacuolated, regenerative hepatocytes, and inflammation by heterophils and mononuclear cells occurred in the portal zones (10; 11).

The aim of the study was to describe the effect of feeding 2.5 ppm Aflatoxin to quails for 6 weeks on the histopathological changes in hepatic tissue.

MATERIALS AND METHODS

Animal care and experimental design:

This study was carried out on growing brown local quail, A total number of sixty one day old unsexed brown local quail chicks were randomly divided into 3

experimental groups containing 10 birds each (in two replicates). Birds were fed AF toxin at two dose, 2.5 ppm and 5 ppm. Quails were fed a commercial non-medicated, maize and soybean meal diet that met the nutrient requirements according to (12), with no added antibiotics, coccidiostats or growth promoters, antitoxin, up to 42 day. The ration based on yellow-corn soya bean contained 28% crude protein and 3015 Kcal metabolizable energy. Aflatoxin was produced, by the method described by (13). by growing *Aspergillus parasiticus* NRRL 2999 (Kindley provided from the college of agriculture and forestry) on rice. The rice culture was autoclaved, dried and ground to a fine powder. The aflatoxin content of the culture material was analyzed using Neogen ELISA kit (Neogen Corporation) with XL_{800} reader. The toxin was dissolved in acetone, added to experimental diets, and mixed to homogeneity by means of a twin –shell blender. The experimental ration was checked to contain no detectable levels of aflatoxins, Ochratoxins, Zearalenone, andT-2 toxin (obtained from santa cruz biotechnology, inc. california, USA) by the method reported by (14). The experimental treatments consisted of three groups: 1 st group: no AF toxin (negative control) 2 nd group : 2.5 ppm AF ; 3 id group : 5 ppm AF. Birds were vaccinated against Newcastle disease and infectious bronchitis by spray method at one day of age, Newcastle disease at 8 days and infectious bursal disease at 14 days of age.

Scarifying birds done at the end of the experiment, pieces of liver were put in 10% buffered formalin, fixed in paraffin, Staining sections by Hematoxylin and Eosin (15).

RESULTS

Histopathology of this study revealed degenerative reversible lesions were seen, from mildest to severest degree with various distributions in test groups of mild

parenchymatous degeneration characterized by granular appearance of the hepatocyte cytoplasm, observed, severe vacuolar degeneration (Fig. 1) . The vast majority of hepatocytes had significant cytoplasmic vacuolization; disseminated necrotic cells were observed in the experimental groups. The results of liver histopathology clearly demonstrated that severe histopathological lesions were observed in quails fed diets containing 2.5 and 5 ppm AF, although in the latter the lesions were more prominent. fatty change of hepatocytes was the most conspicuous, which appear at the portal area (Fig. 2). There was massive hepatocellular necrosis (Fig. 3). Clear hepatocellular swelling in both centrilobular and midzonal areas, with diltation of central vien (Fig. 4). The fat vacuoles formed within the hepatocyte cytoplasm were large and more numerous forming small fatty Lakers in group of quails received 5 ppm AF, while they were smaller in size and less numerous in groups which received 2.5 ppm AF (Fig. 2). dissociation of organ structure, periportal fibrosis with sever necrosis of hepatocyte (Fig.5).

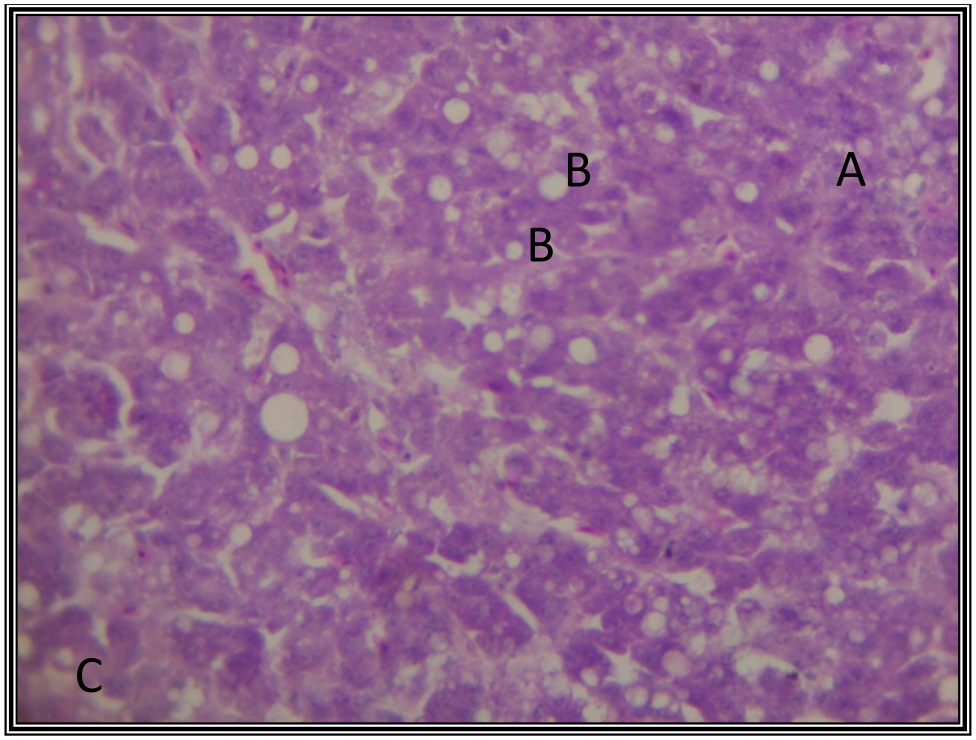

Figure 1. Liver from 2.5 ppm AF-treated group showing parechymatous degeneration characterized by granular appearance of hepatocyte cytoplasm (A), vacuolar degeneration (B) and necrosis (C). H & E × 420.

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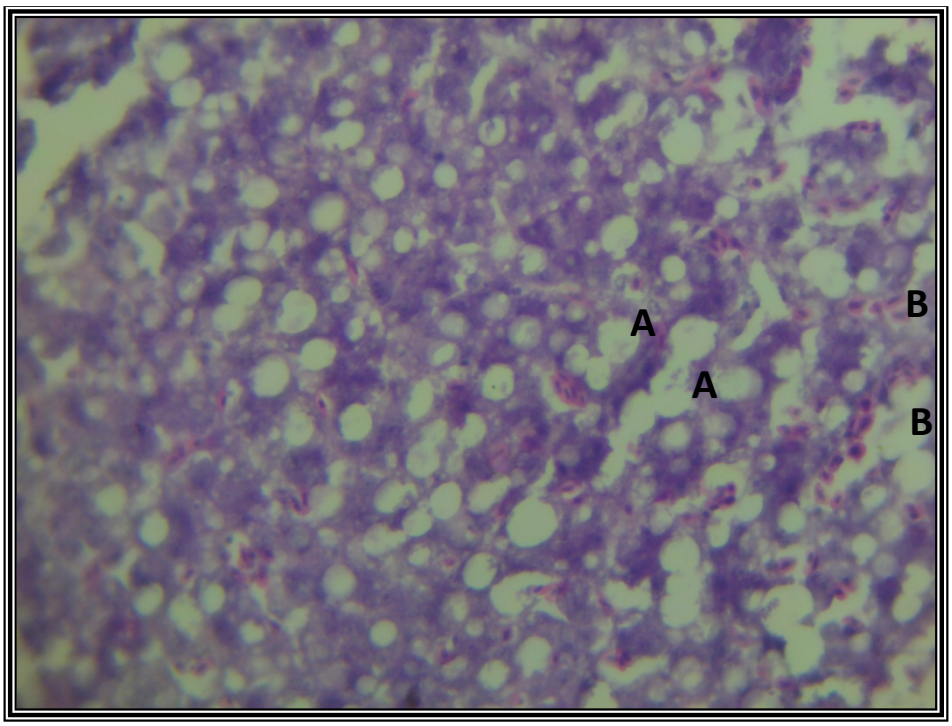

Figure 2. Liver from 5 ppm AF-treated group showing fatty change of hepatocytes (A) congestion of sinusoids (B). H & E × 420

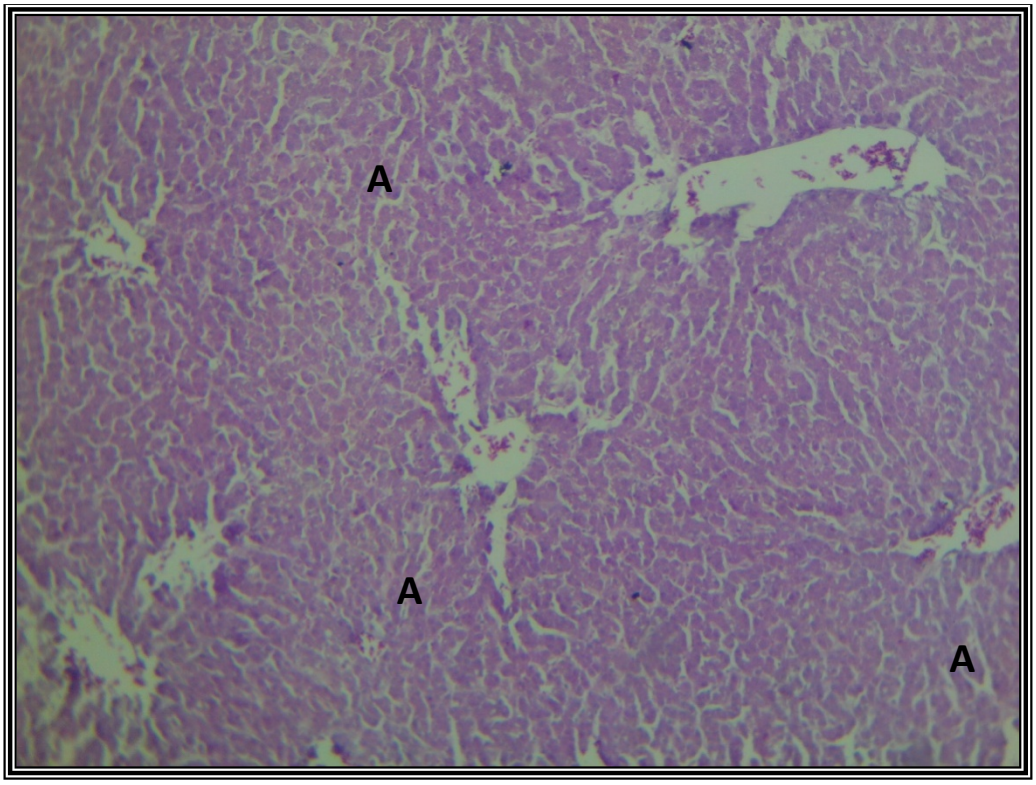

Figure 3. Liver from 5 ppm AF-treated group showing massive hepatocellur necrosis (A). H & E × 105

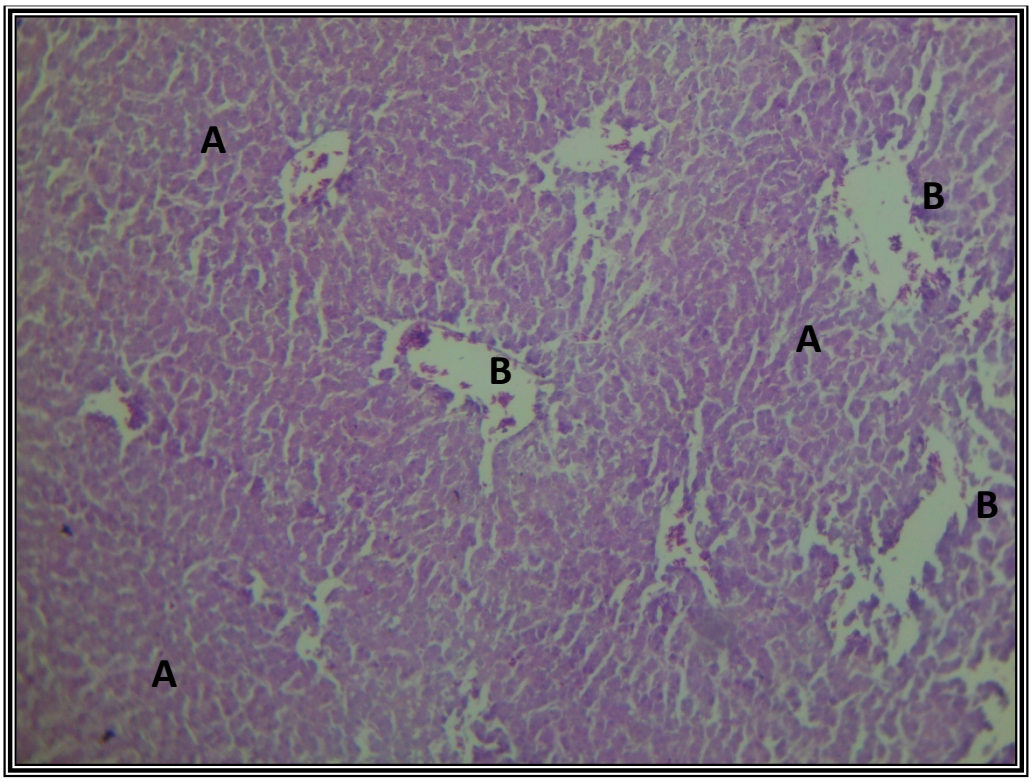

Figure 4. Liver from 5 ppm AF-treated group showing massive necrosis (A) and dilatation of central vein (B). H & E × 105

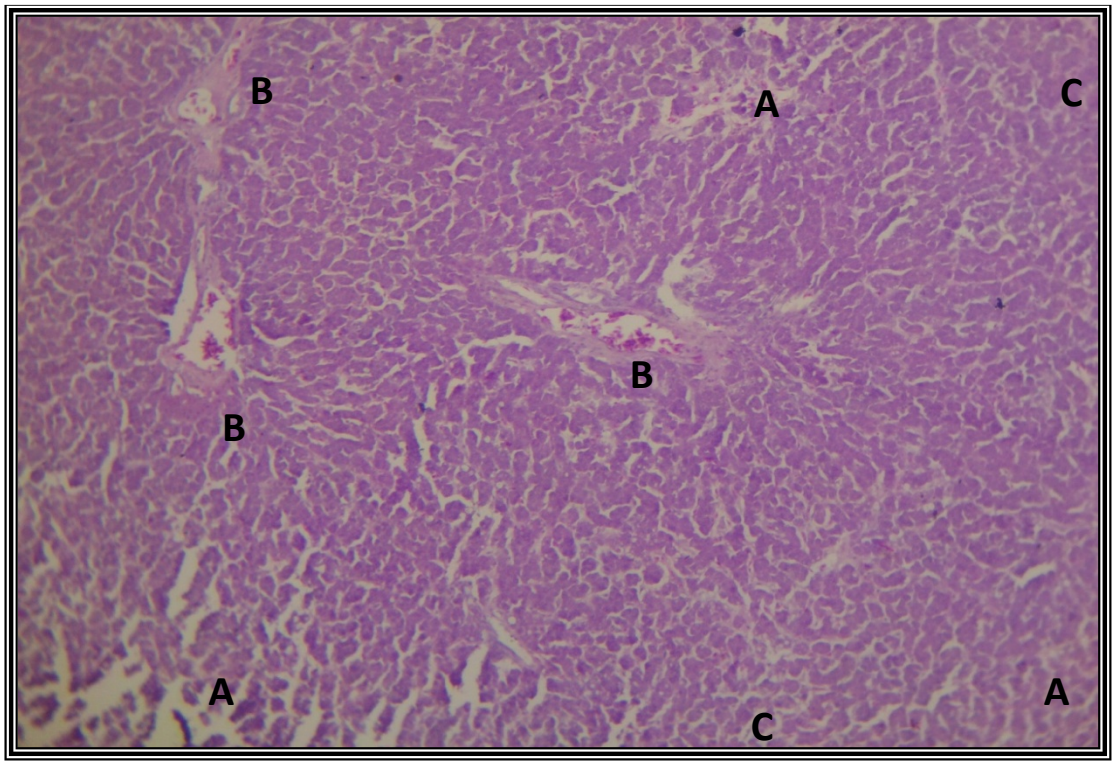

Figure 5. Liver from 5 ppm AF-treated group showing dissociation of organ structure (A),periportal fibrosis (B) with sever necrosis of hepatocyte (C). H & E × 105

DISCUSSION

Microscopic changes induced by feeding AF in liver of local quails reflect the effect of this toxin on these birds. This effect is directly correlated with the concentration of AF and the duration of the exposure (16). The liver is the principal target organ for aflatoxicosis, which has been described previously (17). In our study the microscopic appearance of the livers by feeding AF showed portal and parenchymatous degeneration, which is in the line of (18). Fatty changes, centro-lobular fatty cytoplasmic vacuolar degeneration and /or necrosis in quails and other types of poultry species reflect the chronic effect of this toxin. These findings coincide with findings of (19).

The toxic metabolites of AF bind to nucleic acids and nucleoproteins, essentials to cellular activity, and result in build –up of hepatic lipids with enlargement of the liver. With high- dose acute exposure , fat accumulate as a clear vacuoles in the cytoplasm

of hepatocytes in a dose – dependent and time Faison. Less severe liver histopathological changes were seen in quails of our experiment compared to those reported in broilers (20), fed the same AF dose of 5 ppm. although increasing liver droplets were seen in both species, but mononuclear cell filtration in portal areas reported by (20), was not seen by us in our experiment.

It is difficult to discuss the absence of bile duct proliferation in the livers of quails here in our study when quails fed 2500 ppb and 5000 ppb, in spite that (21), reported that even a dose of 100 ppb AF fed to Laying Japanese Quail was responsible for inducing bile duct proliferation. The unexpected absence of bile duct proliferation, may be due to the high local quail resistance in our experiment to AF comparing to broilers or layers (17). The resistance of local quails to feeding 2.5 and 5 ppm AF , may also could be discussed by the absence of mortality among fed quails to the both levels of AF. This observation are in the line of (22), who found that there were no mortalities in Japanese quails fed 1.25, 2.5 and 5 ppm AF. It is referred by (23), that japanese quails acquire genetic resistance to AF. This phenomenon of local quail resistance to histological changes induced by AF may aid in the selection of local quail lines with high resistance to AF . It is concluded from this study that AF caused a great liver damage and may require to a new approach to treat the AF contaminated feed stuff.

دراسة مرضية نسجية لكبد السمان المعرض تجريبيا لسموم الافلا

قيس قرياقوس ابراهيم

فرع علم الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

اجريت هذه الدراسة لتقييم الآثار السامة للأفلاتوكسين (AF) بمعدل 2.5 و 5 جزء في المليون على الصورة المرضية النسجية لكبد السمان المحلي. استخدم في التجربة ، السمان بعمر يوم واحد وقسم عشوائيا الى مكررين (عشرة فراخ لكل مكرر) في ثلاث معاملات غذائية . 1) مجموعة سيطرة التي لا تحتوي على سموم الافلا (السيطرة)، 2) العليقة الأساسية مضاف اليها 2.5 جزء في المليون سموم الافلا و 3) العليقة الأساسية مضاف اليها 5 جزء في المليون سموم الافلا. اسبوعيا تم وزن الافراخ بشكل فردي وسجل العلف المستهلك وزيادة وزن الجسم . استغرقت التجربة 5 اسابيع وعرض السمان لجدول اضاءة مستمر خلال 24 ساعة وكان استهلاك الوجبات الغذائية والماء بصورة حرة ومستمرة طيلة فترة التجربة.اظهرت التغيرات النسجية لكبد السمان المستهلكة لسموم الافلا وجود التغير الدهني، تنخر، فرط التنسج القناة الصفراوية وارتشاح الخلايا اللمفية. وزادت حدة هذه التغيرات مع زيادة مستوى سموم الافلا . أشارت النتائج إلى أن أفراخ السمان المحلية حساسة لسموم الافلا.

الكلمات الدالة: سموم الافلا، السمان،الكبد، التغيرات النسجية،

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