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CHRONIC AFLATOXINS :HISTOPATHOLOGIC EFFECT ON SOME ORGANS OF LAYING HENS

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ABSTRACT

A total number of 48 laying hens of Sliver Montazah strain at 6 months of age were used in the present experiment. The birds were randomly divided into four groups (12 layers in each) and allocated in an individual laying cages. Hens fed standard diet contaminated with 0 (control group), 50, 100 and 200 (treated group) ug Aflatoxin B1/kg diet for 3 months. At the end of the experiment (3 months), five birds from each group were randomly chosen and slaughtered for postmortem examination and collection of tissue specimens from the liver, ovaries, adrenal and thyroid glands. On conclusion, the results of the present work were indicative for that the liver appeared to be one of the main target organs for the effect Aflatoxin. The histopathologic changes were in the form of dose dependent changes of hepatotoxicosis (hepatocytic vacuolar and hydropic degenerations, fatty change, necrosis and mononuclear infiltrations and aggregations). The histopathologic changes in the other organs (ovaries, adrenal and thyroid gland) were also reveals presence of similar changes of Aflatoxicosis with dose dependent changes. The detected lesions of the ovarian affections were suggested to be of a serious effect of Aflatoxicosis on the quality and egg production. On other hand, the observed lesions in the adrenal and thyroid gland were also suggested for another effects of Aflatoxicosis on the hormonal functions of these gland and other subsequent effects or disturbances in the other body organs that depends on the functions of these glands.

INTRODUCTION

Aflatoxins are naturally occurring mycotoxins that are produced by many species of Aspergillus, a fungus, most notably *Aspergillus flavus* and Aspergillus *parasiticus*. After entering the body, Aflatoxins are metabolized by the liver to a reactive intermediate, Aflatoxins M1, an epoxide (Charles, 2006). Aflatoxins are known to be toxic and carcinogenic to animals and humans. They have been implicated in cancer (hepatocellular carcinoma) and other diseases in humans (Williams *et al*., 2004 and Charles, 2006). In children, chronic exposure to Aflatoxins leads to a high risk of developing liver cancer, as the metabolite Aflatoxins M1 can intercalate into DNA and alkylate the bases through its epoxide moiety. Aflatoxin B1 (AFB1) is the most prevalent toxin in cereals and grains used in feeds and present the greatest toxigenic threat, so it is considered as one of the most potent and naturally occurring animal carcinogens (Leeson *et al*., 1995)...

The primary effects of toxicity with Aflatoxins (Aflatoxicosis) in birds may be used for the clinical and histopathologic diagnosis of the disease. The commonly detected changes in the internal organs were manifested by increase in the size of the liver, spleen and kidneys (Osweiler, 1990) as well as decrease in the size of the bursa of Fabricius and thymus (Sur and Celik, 2003). The present study was performed in order to investigate the histopathologic effect of chronic exposure to Aflatoxin B1, on some organs (liver, ovaries, adrenal and thyroid gland) of laying hens (Sliver Montazah).

MATERIAL AND METHODS

The present study was carried out at the Animal and Poultry Department, Faculty of Agriculture (Dmanhour), Alexandria University.

Birds: A total number of 48 laying hens of Sliver Montazah strain at 6 months of age were used in the present experiment.

Aflatoxins: Aflatoxin was produced through the fermentation of rice by *Aspergillus parasiticus* NRRL 2999 as described by Shotwell et al (1966). The fermented rice was steamed and ground to a powder. The

Aflatoxin content was determined by spectrophotometric analysis given by Nabney and Nasbitt (1965). The rice powder was then incorporated into corn-soybean meal basal diets to provide the Aflatoxin level desired.

Experimental Design: The used birds were randomly divided into four groups (12 layers in each) and allocated in an individual laying cages. Hens fed standard diet contaminated with 0 (control group), 50, 100 and 200 (treated group) ug Aflatoxin B1 / kg diet for 3 months. All hens were kept under similar environmental and hygienic conditions and maintained on 16 hours light per day. Water and feed were provided *ad libitum*. The birds of all groups were observed allover the experiment for recording any clinical signs or mortalities till sacrifice at the end of the experiment.

Histopathologic Studies: At the end of the experiment (3 months), five birds from each group were randomly chosen and slaughtered for postmortem examination and collection of tissue specimens from the liver, ovaries, adrenal and thyroid glands. These specimens were fixed in 10 % neutral buffered formalin solution, then after washing in tap water passed through the paraffin-embedding technique (dehydration, clearing, dealcoholization and embedding in paraffin wax). Paraffin blocks were prepared and then section of 3-5 microns thick were cut and picked on glass slides followed by staining with hematoxylin and eosin (Culling, 1983) and subjected for the light microscopy to record the histopathologic changes in the various examined organs .

RESULTS AND DISCUSTION

I- Histopathologic changes in the liver:

The detected histopathologic changes in the liver of various groups were recorded and compared in scores (Table, 1). The score of these changes was dose dependent where the highest score (13) was seen in case of high levels (200 ug/kg) of ***AFB1*** intoxication. The microscopic examination for the liver in the control group revealed presence of a normal histological structure ***(Fig. 1a)***. The liver of intoxicated chickens with the low doses of ***AFB1*** were affected with mild degree of hepatocytic vacuolations of fatty change in addition to some infiltrations and aggregations of mononuclear cells while in

case of the medium doses of intoxication some focal areas severe degrees of hepatocytic, vacuolations, rupture and necrosis with excess of mononuclear inflammatory cell aggregations ***(Fig. 1b)*** were detected. The histopathologic changes following administration with the high levels of ***AFB1*** were some what more extensive and diffuse allover the liver and in some area the necrotic tissue replaced with some fibrous tissue proliferations ***(Fig. 1c and 1d).*** Some hepatotoxic changes of congestion, degenerative changes, necrosis with lymphocytic infiltration, and hyperplasia of the epithelial cells of bile duct and newly formed ductules and fibrous connective tissues were similarly seen in the liver of Japanese quail fed ***AFB1*** contaminated ration (Mobarak *et al*., 1995). On the other hand, the liver of Ballade rabbits fed on dietary Aflatoxins (100 ppb) showed widened portal spaces with round cell infiltration, irregularities of hepatocytic plats and focal necrosis (Abd El-Hamid *et al*., 1990)

2- <u>Histopathologic changes in the adrenal glands:</u>

The detected histopathologic changes in the adrenal glands of various groups were recorded and compared in scores (Table, 2). The score of these changes was dose dependent where the highest score (12) was seen in case of high levels (200 ug/kg) of ***AFB1*** intoxication. The histopathologic examination for the adrenal glands of control group revealed presence of normal histologic parameters ***(Fig.2a).*** In case of low doses of ***AFB1*** the adrenal medullary tissue showed mild degree of granular type of degeneration with intercellular edema while no changes could be seen in the adrenal cortex ***(Fig .2b).*** The detected lesions following intoxication with the medium doses of ***AFB1*** were represented by a more severe degeneration and coagulative necrosis in the medullary tissue while the cortex showed some degrees of vacuolar degeneration in cortical cells ***(Fig. 2c).*** The cortical changes become more advanced after intoxication with the high levels of ***AFB1***, as severe degree of hydropic degeneration and necrosis of the cortical cells in addition to presence of an excess or large numbers of mononuclear cell infiltrations ***(Fig. 2d)*** were seen in the cortex. Ehebruster (1979) reported that there were no effect of intoxication with ***AFB1***, where there were no differences could be observed between the

adrenal of chickens fed on Aflatoxin and those fed on Aflatoxin plus adsorbent.

3- Histopathologic changes in the ovaries:

The detected histopathologic changes in the ovarian tissue of various groups were recorded and compared in scores (Table, 3). The score of these changes was dose dependent where the highest score (9) was seen in case of high levels (200 ug/kg) of ***AFB1*** intoxication. On microscopic examination for the ovarian tissue, normal histologic criteria of developing follicular structures and other ovarian tissue were seen in the ovaries of control group ***(Fig. 3a).*** On the other hand, some changes of vacuolar degeneration as well as depositions of eosinophilic hyalinized bodies were seen in the ovarian tissues ***(Fig.3b)*** following intoxication with the low levels of ***AFB1***. The lesions of cellular hydropic degeneration were progressed after intoxication with the medium levels of ***AFB1*** and became in form of excess cellular necrosis and formation of hyalinized necrotic materials ***(Fig .3c).*** The ovaries after intoxication with the high doses of ***AFB1*** were seen to be affected with diffuse form of hydropic degeneration, necrosis as well as formation and depositions of excess variable sized, hyalinized eosinophilic materials ***(Fig. 3d).*** Mobarak et *al*., 1995 described some toxic effect of ***AFB1*** on the ovaries. Degenerative changes of the granulosa and theca cells with shrinkage and misshape of the growing follicles were seen similarly to those described in the present work. These findings suggesting possible alterations in steroids synthesis and gonadotropin receptors. Since ovarian steroids, progesterone and estrogen, are known to stimulate the preovulatory secretion of luteinizing hormone (Wilson and Cunningham, 1981), reduction in luteinizing hormone secretion is possible and may explain the observed decline in egg production. In addition, the degenerative changes of the ovaries may affect ovarian gonadotropin receptors (follicle stimulating hormone and luteinizing hormone) resulted in reduction in maturing ova and egg weight.

4- Histopathologic changes in the thyroid gland:

The detected histopathologic changes in the thyroid glands of various groups were recorded and compared in scores (Table, 4). The score of these changes was dose dependent where the highest score (10) was seen in case of high levels (200 ug/kg) of ***AFB1*** intoxication. The histologic structure of the thyroid gland in control

group of the present work appeared normally with its main structure of secretory acini that appeared to contain variable amounts of the eosinophilic secretions ***(Fig. 4a).*** The lumina of the secretory acini, in treated chickens with the low kevels of Aflatoxins, appeared abnormally stained faintly ***(Fig. 4b)*** due to abnormally present inflammatory exudations. The affected secretory acini appeared, in some area, to be separated with edema and interstitial cellular reactions ***(Fig. 4c)*** after intoxication with the high levels. The last group of intoxication with the high levels of Aflatoxins showed degenerated secretory acini of the thyroid gland that appeared filled with inflammatory exudations and separated with an excess aggregation of mononuclear cells ***(Fig. 4d).***

On conclusion, the results of the present work were indicative for that the liver appeared to be one of the main target organs for the effect of intoxication with Aflatoxin. The histopathologic changes due to these effects were in form of dose dependent changes. These detected changes of hepatotoxicosis were in form of hepatocytic degenerations (vacuolar and hydropic), fatty changes, necrosis as well as mononuclear infiltrations and aggregations. The histopathologic examination for the other organs (ovaries, adrenal and thyroid gland) reveals presence of some other changes of Aflatoxicosis with dose dependent changes. The detected lesions of the ovarian affections reflecting and suggesting for a serious effect of Aflatoxicosis on the quality and egg production. On other hand, the observed lesions in the adrenal and thyroid gland, are also suggesting for other effects of Aflatoxicosis on the hormonal functions of these gland other subsequent effects or disturbances in the other body organs that depends on the functions of these glands.

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Table (1) Score of histopathologic changes in the liver due to various levels of *AFB1* intoxication.

Histopathologic changes	Levels of intoxication			
	0 (Control)	50	100	200
Hepatocytic vacuolation	0	0	2	3
Hepatocytic hydropic degeneration	0	0	2	3
Hepatocytic fatty change.	0	1	1	2
Hepatocytic necrosis	0	0	1	2
Mononuclear cell reaction	0	1	2	3
Total score	0	2	8	13

Table (2) Score of histopathologic changes in the Adrenal gland due to various levels of *AFB1* intoxication.

Histopathologic changes	Levels of intoxication			
	0 (Control)	50	100	200
Edema	0	2	1	1
Mononuclear cell reaction	0	0	1	3
Cortical cell degeneration	0	0	1	2
Cortical cell necrosis	0	0	0	2
Medullary cell degeneration	0	1	2	3
Medullary cell necrosis	0	0	0	1
Total score	0	3	5	12

Table (3) Score of histopathologic changes in the ovarian tissue due to various levels of *AFB1* intoxication.

Histopathologic changes	Levels of intoxication			
	0 (Control)	50	100	200
Vacuolar and hydropic degeneration	0	1	2	3
Hyaline degeneration	0	1	2	3
Necrosis	0	1	2	3
Total score	0	3	6	9

Table (4) Score of histopathologic changes in the thyroid gland due to various levels of *AFB1* intoxication.

Histopathologic changes	Levels of intoxication			
	0 (Control)	50	100	200
Degeneration of the secretory epithelium	0	1	2	3
Abnormal secretion with inflammatory exudates	0	1	2	3
Mononuclear cell reactions	0	0	1	2
Edema	0	0	1	2
Total score	0	2	6	10

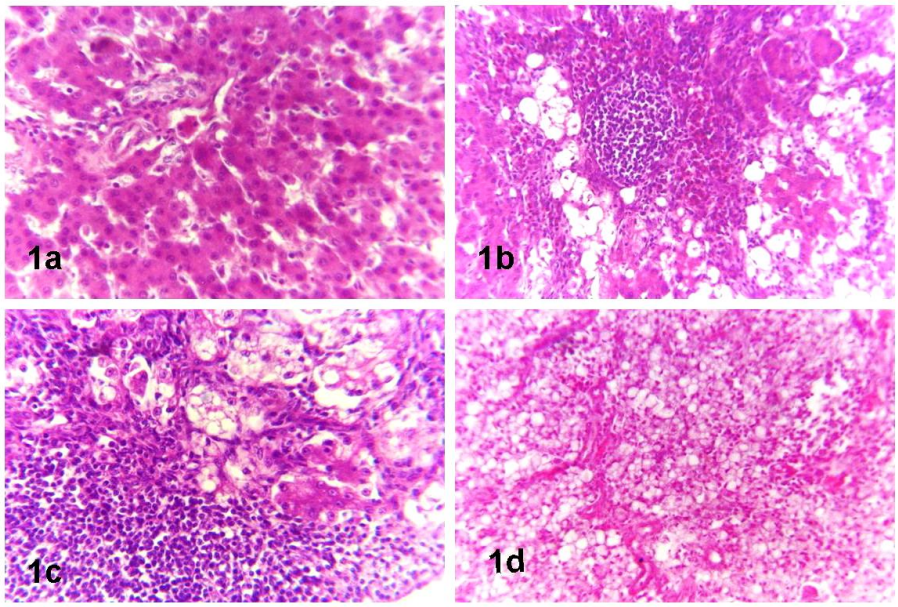

Fig .1a: Liver of control group is showing normal cells of the hepatic acini (H) H and E. x 400.

Fig. 1b: Liver of treated group with 100 ug **AFB1** is showing remnants of some hepatic acini (H); severely hydropic degenerated and ruptured hepatic cells (D) in addition to large focus of mononuclear cell aggregation (M). H and E. x 250.

Fig. 1c: Liver of treated group with 200 ug/kg **AFB1** is showing severely degenerated and necrotic hepatic cells (D) besides an area of extensive diffuse mononuclear all reaction (M). H and E. x 400.

Fig. 1d: Liver of treated group with 200 ug **AFB1** is showing severe diffuse hydropic degeneration and necrosis of most of all hepatic cells (D) separated with thin bands of fibrous tissue (arrow). H and E. x 250.

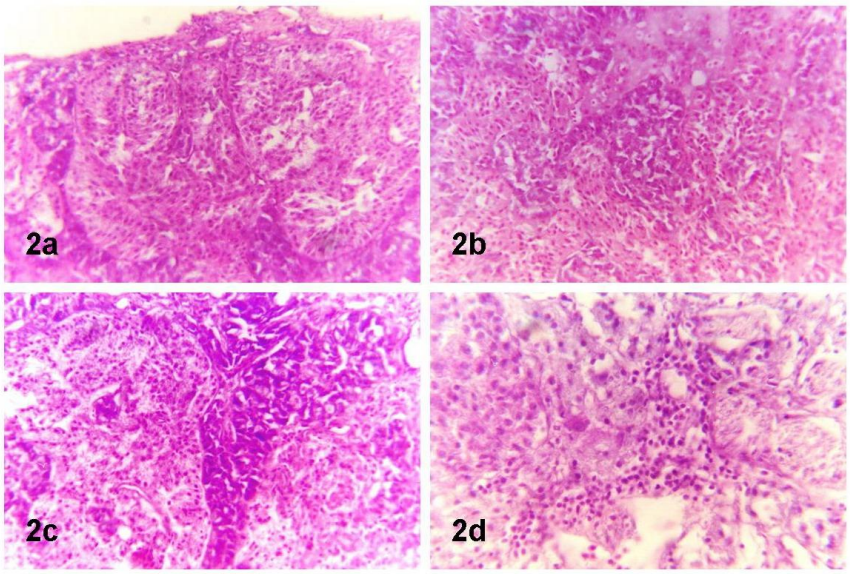

Fig. 2a: Adrenal gland of control group is showing **normal** cortical cells (asterisk) and bands of the medullary tissue (arrow). H and E. x 250.
Fig. 2b: Adrenal gland of treated group 50 ug **AFB1** is showing nearly normal cortical cells (thick arrow) with edema (asterisk) and granular degeneration of the medullary cells (thin arrow).
Fig. 2c: Adrenal gland of treated group with 100 ug **AFB1** is showing vacuolar degenerated cortical cells (asterisk) with severe degeneration and coagulative necrosis of the medullary tissue (arrow). H and E. x 250.
Fig. 2d: Adrenal gland of treated group with 200 ug **AFB1** is showing severe degree of hydropic degeneration and necrosis of the cortical cells (D) with an excess of mononuclear cell infiltration (M). H and E. x 400.

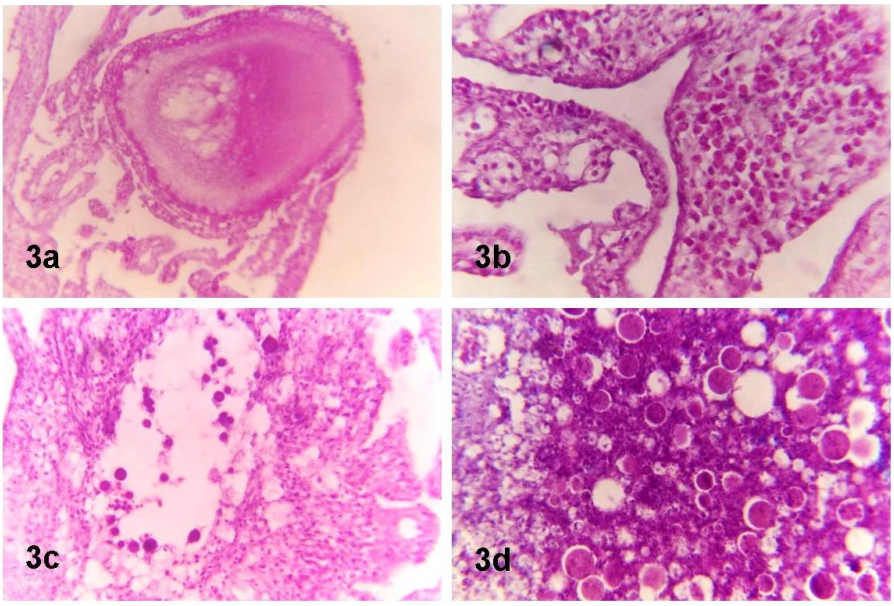

Fig. 3a: Ovarian tissue of control group is showing normal follicular structure (FO) with normal hyperplastic developing structures (asterisk). H and E. x 250.
Fig. 3b: Ovarian tissue of treated group with 50 ug **AFB1** is showing vacuolar degeneration of the ovarian tissue cells (D) with excess of eosinophilic hyalinized bodies (asterisk). H and E. x 250.
Fig. 3c: Ovarian tissue of treated group with 100 ug **AFB1** is showing hydropic degeneration, necrosis and excess of hyalinized necrotic materials (asterisk). H and E. x 250.
Fig. 3d: Ovarian tissue of treated group with 200 ug **AFB1** is showing diffuse hydropic degeneration and necrosis (D) with formation of the hyalinized eosinophilic materials of variable size (arrows). H and E. x 250.

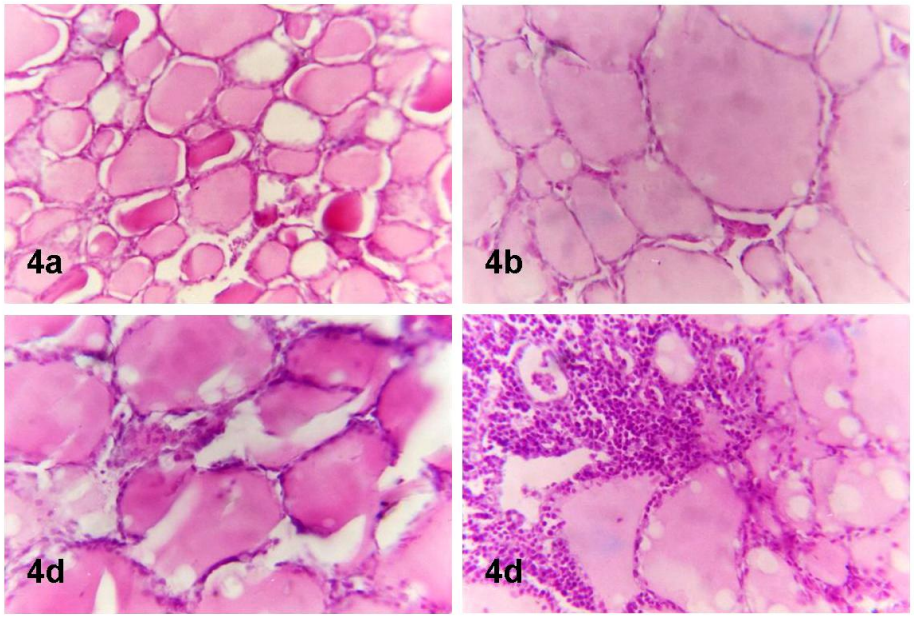

Fig. 4a: Thyroid gland of control group is showing normal secretary acini containing variable amounts of secretions (arrows). H and E. x 400.
Fig. 4b: Thyroid gland of treated group with 50 ug **AFB1** is showing mildly affected secretary acini with faintly stained abnormal secretion (asterisk). H and E. x 400.
Fig. 4c: Thyroid gland of treated group with 100 ug **AFB1** is showing edema with interstitial cellular reaction (arrow). H and E. x 400.
Fig. 4d: Thyroid gland of treated group with 200 ug **AFB1** is showing degenerated acini filled with inflammatory exudates (asterisk) and separated with an excess of mononuclear inflammatory cells (M). H and E. x 400.

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الملخص العربي

التغيرات التسممية المزمنة بالأفلاتوكسين:
التأثيرالهستوباثولوجى على بعض أعضاءالدجاج البياض

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تم اجراء تلك الدراسة على اجمالى عدد 48 دجاجة بياضة (عمر 6 أشهر) من سلالة المنتزة الفضى حيث قسمت الى 4 مجاميع عوملت بجرعات مختلفة من الأفلاتوكسين وهى: 0 (فى مجموعة الكنترول) و 50 ، 100 ، 200 ميكروجرام/ كج من العليقة (فى المجاميع المسممة) . وعند نهاية التجربة (ثلاثة شهور) تم ذبح الطيور لعمل الصفة التشريحية وأخذ عينات الأنسجة من الكبد والغدة الكظرية والمبايض وكذا الغدة الدرقية وذلك لتجهيزها لأجراء الفحص الهستوباثولوجى . ولقد اتضح من الفحص حدوث تغيرات تسممية أساسية بالكبد فى صورة استحالات فراغية ومائية وكذا تغيرات دهنية وتنكرز بخلايا الكبد بالأضافة الى التخللا ت والتجمعات من الخلايا أحادية النواه . ولقد لوحظ أيضا حدوث تغيرات تسممية مماثلة بباقى الأعضاء التى تم فحصها والتى كانت فى مجملها مرتبطه بمستوى الجرعات. ولقد خلصت تلك الدراسة الى توضيح مدى التأثير الهستوباثولوجى للأفلاتوكسين على الكبد وباقى الأعضاء مع توقع وتفسير حدوث ذلك على نوعية وانتاج البيض وكذا التأثير الخطير على الأضطراب الهرمونى الناتج عن التغيرات التسممية بكلاً من الغده الكظريه والغدة الدرقية