Dept. of Pathology,
Fac. Vet. Med., Suez Canal Univ.

STUDIES ON RABBIT DOES AND THEIR PROGENY WHICH SUFFERED FROM HYDROCEPHALUS DUE TO NATURAL INTOXICATION WITH OCHRATOXIN A

(With 7 Tables and 11 Figures)

By

H. FETAIH, M. EL-HAMAMY and M. EL-BOUSHI*

* Dept. of Clinical Pathology, Faculty of Vet. Med., Mansoura Univ.

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دراسات على أمهات الأرانب وصغارها الذين يعانون من تجمع مائي في المخ نتيجة تسمم عارض بالأوكراتوكسين أ

حمدي عبد الوهاب فتيح ، محمود محمد الحمامي ، محمد السيد البوشي

أجريت هذه الدراسة على مزرعة بها ٥٠ من أمهات الأرانب و ٣٠٠ من نتاجها الصغار والتي كانت تتغذى على عليقه جافة ثبت أنها ملوثة بسم الأوكراتوكسين أ ، و الذى ينتج من فطر الأسبريجيلس و البنيسيليوم، و قد تم تحديد كمية هذا السم بواسطة جهاز الكروماتوجرفي (HPLC) بمستوى ٦٤٠-٧٦٠ ميكروجرام/ كيلوجرام من العليقه الجافة. وقد ظهرت الأعراض في هذه المزرعة في ٣٠ أم و كانت عبارة عن حالات إجهاض تبعها نقص في الخصوبة أو العقم، وكذلك أعراض عصبية متبوعة بالوفاة في ١٥ أم منهم ، و أيضا ظهرت أعراض في ٩٠ أرنب حديث الولادة حتى عمر شهر من نتاج هذه الأمهات وكانت عبارة عن تضخم في حجم الرأس وتشوهات خلقية بالعين مع أعراض عصبيه و عمى، ثم يتبع ذلك الوفاة. وقد أثبت تحليل السائل الموجود في المخ والنخاع الشوكي CSF في الصغار عن زيادة في البروتينات و الأنزيمات و نقص في الجلوكوز و بالنسبة لصورة الدم فأظهرت الدر سه وجود أنيميا في الأمهات و النتاج مع وجود ارتفاع في خلايا الدم البيضاء في الأسبوع الأول ، تبعها نقص واضح في خلايا الدم البيضاء بعد ذلك. وبالنسبة لوظائف الكبد للأمهات ونتاجها فقد وجد زيادة واضحة في إنزيمات الكبد AST, ALT وكذلك زيادة في اليوريا مع وجود نقص واضح في البروتين الكلى و الألبيومين والجلوكوز. وبالتشريح المرضى لصغار الأرانب وجد تجمع سائل مائي بالتجاويف الداخلية للمخ مع ترقق بعظام الجمجمة في حالات تضخم الرأس. أما بالفحص الميكروسكوبي لمعظم الأعضاء الداخلية لأمهات الأرانب وصغارها فقد وجدت تغيرات تنكسيه ونخر في خلايا المخ و أيضا نخر في الأنابيب الكلوية و في الكبد بصورة موضعية مع تنكس فجوى بالخلايا و تليف بسيط، بالإضافة إلى اضمحلال في التجمعات الليمفاوية بالطحال وبعض الالتهابات الرئوية. وخلاصة هذه الدراسة أن سم الأوكراتوكسين أ (OTA) يسبب تغيرات مرضية في الكلى و الكبد والمخ والرئتين في

أمهات الأرانب والصغار مع احتمالية نقله من خلال المشيمة إلى الجنين محدثا به تشوهات خلقيـــة أهمها تجمع سوائل بالمخ.

SUMMARY

This study was carried out on 50 rabbit does fed a ration contaminated with ochratoxin A (OTA) at a level of 640-760 microgram/Kg ration. Thirty does exhibited reproductive and nervous disorders and 90 of their progeny showed teratogenic lesions, including hydrocephalus. Analysis of cerebrospinal fluid (CSF) of the progeny revealed increase in TLC, AST, CK, total protein and LDH, while glucose was significantly decreased. The haematological parameters of the same cases showed normocytic normochromic anemia. Leukocytosis and lymphocytosis were found only in the 1st week of age. The haematological picture of rabbit does revealed leukocytosis and lymphocytosis till the end of 2nd week after giving a non-contaminated ration. The blood chemistry of progeny and does showed significant increase in ALT, AST, urea, total bilirubin lactate dehydrogenase and creatine, while total protein, albumin and glucose were significantly decreased. Pathological investigation of the rabbit does revealed degenerative changes and necrosis in the brain tissue. The kidneys had nephrotoxic changes and the liver suffered from vacuolar degeneration, focal necrosis and mild fibrosis. Atrophy of the lymphoid tissue was also seen in the spleen. The progeny suffered from severe hydrocephalus and brain necrosis. The kidneys and liver showed lesions like that of the does. The lungs revealed interstitial reaction and suppurative bronchopneumonia. It could be concluded that ochratoxin A has nephrotoxic, neurotoxic and hepatotoxic effects in both rabbit does and their progeny, with evidence of transplacental transmission and induction of teratogenic lesions.

Key words: Rabbit, hydrocephalus, ochratoxin A.

INTRODUCTION

Ochratoxins are secondary metabolites of the fungal genera *Penicillium* and *Asperigillus*. Although seven metabolites are included in the ochratoxins group, only ochratoxin A (OTA) has been found widespread as a natural contaminant (Rodricks et al., 1977). Like other mycotoxins, they contaminate various human and animal feeds, due to

the global occurrence of toxinogenic molds, and exert adverse health effects on human and animals (Fink, 1999).

Previous clinicopathological investigations revealed that the ingestion of mycotoxins in rabbits caused a significant reduction in packed cell volume, total leukocytic count, neutrophils count and depletion of lymphoid tissues. Also, the toxic effects of mycotoxins on serum biochemical parameters of rabbits have been documented and these effects include reduction in serum alkaline phosphtase and sorbitol dehydrogenase activities (Niyo et al., 1988). Renal failure and the resultant increase in blood urea and serum creatinine, and decrease in creatinine clearance, due to mycotoxicosis, was recorded by Hanika et al. (1984).

In pregnant mice and rats, I/P inoculation of ochratoxin A resulted in increased prenatal mortality, decreased fetal weight and various fetal malformations (Hayes et al., 1974 and More and Galtier, 1974). Also Mamdouh (1992) found that aflatoxicosis prevented ovum implantation or cause fetal resorption or abortion. Studies of Purchase and Theron (1968) and Albassam et al. (1987) showed that ochratoxicosis A, in rats, caused damage of the kidneys, liver, heart and intestine, in addition to disseminated intravascular coagulation. In addition, oral intoxication of rats with OTA was found to increase the activity of urine enzymes and this indicated early pathological changes in the kidneys (Pipeljenjak et al., 1991). Transplacental OTA intoxication, in mice, was found by Arora (1981) and Szczeck and Hood (1981) to induce teratogenic lesions and brain necrosis in the new born.

Tapia and Seawright (1985), Sharma (1993), Szczeck et al. (1974), Burns and Maxwell (1987), Cook et al. (1986) and Cventnic et al. (1992) had carried out several studies on ochratoxicosis in different animal species, including horses, pigs, guinea pigs, dogs and ducklings. They recorded many pathological lesions in these different species.

In broiler chicks, OTA was found to be nephrotoxic rather than hepatotoxic agent (Marz and kosutzky, 1992 and Dwivedi and Burns, 1984a). The kidneys in such cases revealed dystrophic calcification in the glomeruli, deposition of immunoglobulins in glomerular basement membrane, degenerative changes in the proximal convoluted tubules and interstitial reactions. The changes in the other organs were degenerative changes in the hepatocytes and depletion of the lymphoid tissues. The latter lesions were also described by Dwivedi and burns (1984 b) and Sandhu et al. (1995) and they considered OTA as an immunosuppressive substance affects the development and performance of the broiler chicks.

In rabbits, few studies had been carried out on mycotoxicosis. Hanika et al. (1983) studied citrinin mycotoxicosis in rabbits, and the observed pathological changes confined to the kidney where tubular nephrosis was detected. Serban et al. (1981) investigated the effect of natural mycotoxicosis by aflatoxins, including OTA, in rabbit breeding farms at a level of 1.1 ppm. They recorded the occurrence of small haemorrhages in many organs and severe changes in the lung exhibited as serohaemorrhagic edema, alveolitis and bronchopneumonia.

Although worldwide researches on mycotoxicosis in different animals have been performed, the natural ochratoxicosis in rabbits is not well documented. Therefore, the present study aimed to investigate the pathological and clinicopathological changes that occur in adult rabbits and their progeny due to natural accidental ohratoxicosis. This study was carried out on a rabbit farm in which the rabbits were fed on a stored ration contaminated with OTA.

MATERIAL and METHODS

Rabbits:

The investigated rabbits were obtained from a rabbit farm consisted of 50 mother does and their progeny. As shown in Table (1), the mothers in the farm were classified into 3 groups (gps. M1 - M3). Group (M1) which contianed 15 does and showed reproductive disorders and nervous manifestations ended by death. Gp. (M2) consisted of 15 does showed reproductive disorders only. The rabbits in this group either had aborted or gave stillbirths, and became infertile. Gp. M3 consisted of the 20 does that were apparently normal and taken as a control group. The progeny of the farm consisted of about 500 newly born rabbits. As shown in Table (2), they were classified into 3 main groups (gps. P1 - P3). Gp. (P1) consisted of 90 rabbits and showed teratogenic lesions and nervous symptoms. Gp. (P2) contained 200 stillborn rabbits. Gp. (P3) consisted of about 210 apparently healthy young rabbits, taken as a control group.

Table (1): Does:

Groups	No. of does	symptoms
M1	15	Reproductive and nervous disorders ended by death.
M2	15	Abortion, stillbirth and infertility.
M3	20	Apparently healthy (control).

Table (2):Progeny rabbits:

Groups	No. of rabbits	Symptoms
P1	90	Teratogenic lesions and nervous manifestations.
P2	200	Stillborn.
P3	210	Apparently healthy (control).

Ration:

***Analysis*:** Five representative samples from the given ration (commercially pelleted ration) had been taken for mycotoxins analysis. Quantitative determination of Ochratoxin A and Aphla toxins (B1, B2, G1 and G2) in rations samples were carried out by high performance liquid chromatography (HPLC) using fluorescent detector according to Awe and Schranz (1981)

***Replacement*:** The ration was replaced by another ration previously proved to be free from mycotoxins and the herd was closely observed for 4 weeks.

Blood and cerebrospinal fluid samples:

Blood samples were taken by heart puncture from the does of gp (M2) after 0, 1, 2 and 4 weeks of the outbreak and replacement of the ration. Those samples were grouped and labled as gps. (O), (1w.), (2Ws) and (4Ws.) Also, blood samples from the progeny which suffered from hydrocephalus (gr. P1), were taken during the outbreak, from rabbits at the ages 1, 2 and 4 weeks-old. The sample were labled as gps. (1W.-old), (2Ws-old) and (4Ws-old). Cerebrospinal fluid samples (CSF) have been taken from the same progeny groups, immediately after slaughter. The samples were drawn from cisterna magna at the atlanto-occipital articulation using sterile syringe (20 gauge and 1.5 inch) according to Rick et al. (1999). Blood samples and cerebrospinal fluids were also collected from the does and progeny of gps. (M2) and (P1) after 2 months of the replacement the ration, and these samples were considered as control. The hematological picture including erythrocytic count (RBCs), hemoglobin concentration (Hb) packed cell volume (PCV), blood indices (MCV, MCH and MCHC) and differential and total Leukocytic count (TLC) had been estimated according to Jain (1986). The cytology of cerebrospinal fluid (CSF) had been estimated according Coles (1986). Biochemical analysis of serum and CSF including activity of alanine amino transferase (ALT), aspartate amino transferase (AST) alkaline phosphatase (AP), total protein (TP), albumin (alb), glucose, blood urea nitrogen (BUN), creatinine, creatinine kinase (CK) and lactate

dehydrogenase (LDH) had been estimated. The diagnostic kits were obtained from "Human Company" (Germany).

Statistical analysis:

Five samples from each group were analyzed statistically using the program State View 4.01 (1993) to indicate the significant differences ($P< 0.05$) from the control.

Pathological examination:

Five rabbits from each mother group (gps M1, M2 and M3) were slaughtered and subjected to the routine postmortem examination. Ten rabbits from gp. (P1) and 5 rabbits from gp. (P3) were also slaughtered and examined.

For histopathological examination, specimens from the organs of all slaughtered rabbits were taken and immediately fixed in 10% neutral buffered formalin. Five microns-thick sections were prepared and stained with hematoxylin and eosin according to (Drury and Wallington 1980).

RESULTS

Toxins:

Ochratoxins A in the ration was detected by chromatography at the level 640-760 µgm/Kg ration, while the levels of aflatoxins B1, B2, G1 and G2 were non-detectable. Both ochratoxins and aflatoxins were non-detectable in the new replacing ration.

Symptoms:

The mother does of gp. (M1) exhibited nervous signs in form of extended limbs, torticollosis and circling, and suffered from general weakness ended by death. Also, some rabbits of this group suffered from abortion or gave stillbirths. The mothers does of gp. (M2) became infertile after giving stillbirths or abortion. After 2 months of replacement of the ration, these clinical symptoms were mostly disappeared from the still alive rabbits, specially the mother does. Thereafter, the rabbits of gp. (M2) showed marked improvement in health reproduction.

The progeny rabbits of gp. (P1) showed enlargement of the skull, blindness due to congenital bilateral closure of eyelids and nervous manifestations in the form of circling, lying down and inability to stand (Fig. 1). After 15 to 30 days of birth, the rabbits showed severe emaciation ended by death. The rabbits of gp. (P2) were stillborn and underweight.

Clinicopathological findings:

The cerebrospinal fluid of the taken samples had proved by physical examination to be colorless, transparent, non-coagulated and slightly alkaline. As shown in Table (3), the analysis of CSF of the progeny suffered from hydrocephalus, revealed increase in TLC, AST, CK and LDH in all examined cases. The total protein had significantly increased in gps (2Ws-old) and (4Ws-old), while glucose level had significantly decreased.

In Table (4), the hematological parameters showed normocytic normochromic anemia in the progeny rabbits of gps (2Ws-old) and (4Ws-old). Leukcytosis and lymphocytosis were reported in the gp. (1 w.-old), while lymphopenia was recorded in the gp. (4Ws-old).

In Table (5), the blood chemistry parameters of hydrocephalous progeny rabbits revealed significant increase in ALT, AST and urea in gps (2Ws-old) and (4Ws-old), while total protein, albumin and glucose had significantly decreased. Total bilirubin and creatine had significantly increased only in gp. (4Ws-old). In all examined groups, Lactate dehydrogenase had significantly increased, while AP and CK were non-significantly changed.

As shown in Table (6), the haematological picture of the intoxicated mother does revealed only leukocytosis and lymphocytosis in the sample groups (0), (1W.) and (2W.), i.e. during intoxication until to 2 weeks after replacement of the ration. Serum biochemical analysis during the same time, sample groups (0), (1W.) and (2W.), showed significant increase in AST and ALT (Table 7). Urea, creatinine and LDH had significantly increased in sample gps.(0) and (1W.). In the sample gp. (0), Alkaline phosphtase showed significantly increase while albumin showed significant decrease. In all groups, the total bilirubin, glucose and CK did not show any significant changes.

Pathological findings:

Does:

Gross lesions: Postmortem examination of rabbits in gps. (M1) and (M2) revealed congestion of the lungs, multiple white foci in the liver and presence of suppurative exudate in the uterine lumen of some of the aborted cases. Spleen was smaller than normal in comparison with control.

Histopathological findings: The microscopic picture was the same in gps. (1) and (2), but the brain lesions were seen only in gp. (1).

Brain: In the brain of rabbits in gp. (1), there was mild demyelination in both the cerebrum and cerebellum, in addition to neuronal degeneration, necrosis and neuronophagia. There was also marked pericellular edema around the Purkinje cells in the cerebellum.

Kidneys: The epithelium lining the renal tubules suffered from moderate nephrosis, indicated by fine granularity of the cytoplasm, with occasional vacuolation. Some of the tubules in the cortex showed necrosis of their epithelium. Hyaline casts were seen in some of the collecting tubules. The glomeruli showed proliferation of the mesangial cells (Fig. 4), thickening of the basement membrane, congestion of the glomerular capillaries and infiltration with few leukocytes.

Liver: There was centrilobular vacuolar degeneration of the hepatocytes and focal necrosis in some areas, in the vicinity of central veins. In portal areas, there were focal aggregations of lymphocytes with mild fibrosis (Fig. 5).

Spleen: The lymphoid follicles were mostly fewer and smaller in size than normal (Fig. 6).

Heart: Some cardiac muscle fibers showed degenerative changes in the form of clumping of the sarcoplasm with or without myocytolysis.

Progeny:

Gross lesions: The rabbits of gp. (P1) were highly emaciated. The heads of these rabbits showed marked enlargement of the skull, with thinning of parietal and frontal bones (Fig. 2). The cut section through the cerebral hemispheres showed internal hydrocephalus. The brain ventricles were severely distended with serous fluid and the cerebrum became like a sac filled with fluid (Fig. 3). The heart was pale in color and flabby. The lungs showed multiple foci of haemorrhages. The liver was pale and friable. The stillbirths of gp. (p2) were not necropsied.

Histopathological findings:

Sections from the affected rabbits of gp. (P2) were only investigated for microscopic lesions.

Brain: Both of the cerebrum and cerebellum showed pericellular and perivascular edema, status spongiosus in the white matter (Fig. 7), and loss of Purkinje cells. In addition, neuronal degeneration and necrosis with neuronophagia and gliosis were observed (Fig. 8).

Kidneys: There was moderate degree of nephrosis with formation of some renal casts. There was also focal interstitial haemorrhages. Mild acute glomerulonephritis could be detected. The glomeruli showed hyperplasia of mesangial cells, congestion of the glomerular tufts and mild thickening of the basement membrane (Fig. 9). There were few

leukocytes in the glomeruli, and the glomerular tufts were filling the Bowman's space.

Liver: There was vacuolation of the cytoplasm of most of the hepatocytes. The cytoplasm of other hepatocytes was highly granular (Fig. 10). Severe congestion of the blood vessels and sinusoids was also evident.

Lung: Haemorrhages in the alveoli and areas of collapse were noticed. There was mild acute interstitial pneumonia characterized by thickening of interalveolar septa with infiltration of neutrophils and increase in number of pneumocytes type II (Fig. 11). Peribronchial and perivascular aggregations of lymphocytes were also observed. The lungs of some cases showed suppurative broncho-pneumonia. There was infiltration of the alveoli and bronchioles with numerous neutrophils and mononuclear cells.

Heart and skeletal muscles: Some of the muscle fibers showed loss of striation, clumping of sarcoplasm and occasional vacuolation.

DISCUSSION

The problem in the farm under investigation started with the appearance of nervous signs and weakness followed by death in does. Some of these does showed reproductive disturbances such as stillbirths, abortion and infertility. These sings were similar to that occurred in pregnant rats and mice due to ochratoxicosis and reported by Hayes et al. (1974), More' and Galtier (1974) and Mamdouh (1992). Therefor, the contamination of the ration with mycotoxins was taken in consideration, especially after failure of many treatment programs. Ochratoxin A was found in the ration at levels of 640-760 ug/ kg ration. The most prominent symptoms and postmortem lesions in the progeny of intoxicated rabbits were enlargement of the skull, thinning of the skull bones and severe hydrocephalus. Such symptoms were not recorded before in rabbits due to ochratoxicosis. Benko (1991) and Dellepiane (1990) recorded hydrocephalus in rabbits due to encephalitozoonosis, toxoplasmosis and listeriosis. This study recorded OTA as an additional cause of hydrocephalus in rabbits. The lesions in the progeny could be due to transplacental transmission of the toxin. This suggestion is in accordance with that of Szczeck and Hood (1981) who suggested similar route in mouse fetuses. The teratogenic effect of OTA noticed in the

progeny appeared in the form of bilateral closure of the eyelids and hydrocephalus in gp (P1).

Cerebrospinal fluid analysis provides a general index of neurologic health and often provides evidence of the presence of the disease. CSF analysis has reasonable sensitivity but low specificity, so the entire picture of all finding of CSF analysis linked with the other clinical symptoms is a value in reaching the diagnosis (Kaneko et al., 1997). CSF normally has small number of nucleated leukocytes count. An increase in the number of CSF leukocyte in our study may be as a result of inflammatory lesion and toxic condition (Chrisman, 1992). Increase total protein in this study may be attributed to inflammatory reactions, neural degenerative or vascular lesion (Evans, 1995). The concentration of glucose in CSF is a proximately seventy percent of blood glucose level. The concentration of glucose in CSF is depended up on, the blood glucose level, the selective permeability of the blood-cerebrospinal fluid barrier and the presence or absence of glycolytic microorganisms (Coles, 1986). Decrease CSF glucose has been observed in this work may be as a result of hypoglycemia in the blood. Elevation level of non-specific enzyme AST and LDH in this study may be as a result of neural damage and or blood sources. While CK activity is more specific where plasma CK doesn't normally enter CSF, so increase CK activity in CSF is mainly from neural tissues damage (Munana, 1996). Degenerative and necrotic changes in cerebrum and cerebellum together with gliosis and pericellular edema were the main histopathological findings in both mothers and progeny. Such brain lesions were recorded to occur in mouse fetuses due to OTA (Szczeck and Hood, 1981), and in rabbits, but due to mycotoxin fumonisin B1 (Bucci, 1996). In this investigation the brain lesions were found accompanying the condition of hydrocephalus, which is not recorded before.

Elevations of serum urea and creatinine values in progeny and female groups have been recorded in this study. This elevation may be attributed to renal dysfunction as a result of ochratoxin (A). chratoxicosis nephropathy has been reported experimentally in pig and avian were feed crystalline ochratoxin (A) (Krogh et al., 1976 and Elling et al., 1975) respectively. Hypoglycemia has been reported in progeny rabbit groups only. This hypoglycemia may be due to ochratoxin (A) induced morphological and functional changes in renal tubule cells, in addition to the rabbits were off food. Where ochratoxin (A) reported in porcine mycotoxic nephropathy excretion of leucyl-amino-peptidase in proximal tubule resulted in impairment tubular transport and glucose reasbsorption

(Krogh et al., 1974). The microscopical examination of the kidney revealed tubular nephrosis in the proximal convoluted tubules and glomerular lesions suggestive of mild acute glomerulonephritis in both mothers and progeny. OTA was found to be nephrotoxic in pigs, rats, broiler chicks and guinea pigs Tapia and Seawright 1985, Albassam et al., 1987, Dwivedi and Burns 1984 a, Elling et al., 1975 and Cventnic et al., 1992, while Dwivedi and Burns 1984 a and Maxwell et al., 1987 observed acute glomerulonephritis in quails and broiler chicks only, due to ochratoxicosis. Bucci (1996) and Hanika et al. (1983) mentioned degenerative changes in the renal tubules of rabbits due to other mycotoxins, fumonisin and citrinin. No available data concerns the effect of OTA on the kidney of rabbits and this investigation highlighted the probable nephrotoxic effect of this type of mycotoxins.

Serum biochemical parameters show elevated levels of AST, ALT and AP in female and progeny rabbits. These results were in accordance with those reported by El-Mahdy et al. (1988). Total protein and albumin concentration declined in progeny group while albumin only decrease in female group. This hypoproteinaemia and hypoalbuminemia may have been due to renal excretion, impaired protein synthesis and or liver disorder caused by ochratoxins (Kaneko et al., 1997). Total bilirubin value shows significant increase in progeny rabbit only. This elevation may be as a result of resuming liver function and or destruction of erythrocyte (Coles, 1986). Lactate dehydrogenase one of the non-specific serum enzymes, where it is present in all body tissues (Duncan and Prasse, 1989). The elevation of serum LDH activity in both female and progeny rabbits may be as a result of damage in different body organs.

There was liver damage in the form of centrilobular degeneration with focal necrosis and mild signs of chronic cholangiohepatitis. Marked granularity in cytoplasm of the hepatocytes was also a marked finding. Maxwell et al., 1987, Cventnic et al., 1992, Albassam et al., 1987 and Purchase and Theron (1968) had previously reported the hepatotoxicity of OTA in quails, guinea pigs and rats. Such lesions in addition to that of the kidney, were considered by Marz and Kosutzky (1992) to be typical for ochratoxicosis, but in broiler chicks. This could be also typical for rabbits and their fetuses.

Regarding the hematological picture, there was normocytic normochromic anemia in progeny rabbits. This anemia may be due to inhibition of erythropoiesis as results of accumulation of metabolic waste products in the circulation and or accumulation of metabolic of the blood in the internal organs. Our results are in agreement with that of Gentry

and Cooper (1981) who reported anemia in rabbits had been injected a single dose I/V (0.5 mg/Kg) of T-2 mycotoxin. Leucocytosis and lymphocytosis in 1st week group of progeny rabbit groups, in our study may be as a result of body response to mycotoxicosis (Jain, 1986). The leucocyte showed that there was downward trend among the progeny rabbit group from 2nd week. Lymphopenia in the 4th week of progeny group may be attributed to inhibition lymphopoiesis as a result of accumulation of metabolic waste product in the blood (Duncan and Prasse 1989). This result is supported by Niyo et al. (1988) who also reported reduction in white blood cell count in rabbit daily-administrated mycotoxin T-2. Similarly Abd-El-Karim et al. (1991) recorded high reduction in total leucocytic count, heterophil and lymphocyte in one day old chickens received ochratoxins (A) directly per os in a dose of 30 microgram /chick. In contrast, Boorman et al. (1984) and Dwivedi and Burns (1984 a & b) found that there were non-significant differences in total and differential leucocytic count in mice and poultry respectively consuming ochratoxins (A) and those of the control group.

Spleen of the mothers showed decrease in size and number of the lymphoid follicles. This might indicate the immunosuppressive effect of OTA. This was in agreement with the opinions of Sharma (1993), Fink (1999), Dwivedi and Burns (1984a), and Dwivedi and Burns (1984 b) who found the same findings in broilers and some other animal species.

Concerning the pulmonary lesions, the detected inflammatory reaction was mostly interstitial, with edema, hemorrhages and occasional evidence of bronchopneumonia. This is in accordance with the findings of Serban et al. (1981) due to natural mycotoxicosis of rabbits by either aflatoxins or by ochratoxins.

It could be concluded that ochratoxin A has adverse effects on reproductivity of rabbits through inducing stillbirth, abortion and teratogenic lesions in the offspring, particularly hydrocephalus. Extreme nephrotoxic and hepatotoxic effects were also considered as important adverse effects on their health and production. The possibility of transplacental transmission of Ochratoxin A should be taken in consideration.

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FIGURES

Fig. 1: Progeny rabbits (gp. P1) one-month-old showing, torticollosis, congenital blindness, paralysis and inability to stand.

Fig. 2: The head of one a progeny rabbit (gp. P1) showing enlarged skull due to hydrocephalus.

Fig. 3: Hydrocephalus in the brain of a progeny rabbit (gp. P1) showing highly dilated fourth ventricle with thinning of the cerebral hemispheres that was filled by clear serous cerebrospinal fluid.

Fig. 4: Glomeruli of the kidney of a doe (gp. M2) showing proliferation of the mesangial cells. The renal tubules show also mild tubular nephrosis. H&E X320.

Fig. 5: Liver of a doe (gp. M2) showing, focal aggregation of lymphocytes in the portal area. H&E X200.

Fig. 6: Spleen of a doe (gp. M1) showing decrease in number and size of the lymphoid follicles. H&E X80.

Fig. 7: Vacuolation (status spongiosus) due to demyelination of the white matter in the cerebrum of a progeny rabbit (gp. P1). H&E X320.

Fig. 8: Neuronal degeneration and necrosis, with focal gliosis and neuronophagia in the brain of a progeny rabbit (gp. P1). H&E X320.

Fig. 9: Glomerular tufts in the kidney of a progeny rabbit (gp. P1) showing, mild thickening of the basement membrane, beside moderate tubular nephrosis. H&E X320.

Fig. 10: Liver of a progeny rabbit (gp. P1) showing characteristic granularity of the cytoplasm of the degenerating hepatocytes. H&E X320.

Fig. 11: Lung of a progeny rabbit (gp. P1) showing interstitial pneumonia with thickening of the interalveolar septa. H&E X200.

Table (3): Some CSF biochemical profiles (Mean ± S.E.) in progeny rabbits with congenital hydrocephalus (gp. P1) from parents naturally intoxicated with ochratoxin A:

Groups	TLC Thou/µL	Total Protein mg/dl	Glucose mg/dl	CK U/L	AST U/L	LDH U/L
1W.-old	25 ±1.56*	48 ±3.1	46 ±3.8	156 ±8.5*	31 ±3.2*	29 ±2.6*
2Ws-old	36 ±1.85*	105 ±5.8*	37 ±2.9*	198 ±10.5*	44 ±4.3*	36 ±3.1*
4Ws-old	43 ±2.1*	158 ±8.9*	31 ±2.8*	214 ±12.8*	52 ±4.9*	41 ±3.9*
Control	8 ±0.72	45 ±2.9	55 ±4.1	39 ±3.1	17 ±1.4	16 ±1.2

Significant at P< 0.05

Table (4): Some hematological parameters (Mean ± S.E.) in progeny rabbits with congenital hydrocephalus (gp. P1) from parents naturally intoxicated with ochratoxin A:

Group	RBCs Mill/µL	Hb Gm/dl	PCV %	MCV FL	MCH Pg	MCHC %	TLC Thou/µL	Neutro Thou/µL	Esinoph Thou/µL	Basoph Thou/µL	Lymph Thou/µL	Monocy Thou/µL
1W.-old	5.01 ± 0.17	10.4 ±0.96	33.1 ±1.49	66 ±2.87	20.7 ±1.32	31 ±1.22	14.94 ±0.84*	3.56 ±0.40	0.24 ±0.03	0.20 ± 0.02	10.25 ±0.51*	0.69 ±0.15
2Ws-old	4.34 ± 0.19*	9.4 ± 0.89*	28.4 ±1.12*	65.4 ±2.61	21.6 ±1.11	33 ±1.01	11.79 ± 0.64*	2.71 ±0.38	0.19 ± 0.02	0.017 ± 0.01	8.27 ± 0.44*	0.60 ±0.14
4Ws-old	3.48 ± 0.21*	7.6 ± 0.91*	21.3 ±1.25*	61.2 ±1.49	21.8 ±1.13	35 ±1.42	9.11 ± 0.72	2.41 ±0.36	0.20 ± 0.04	0.019 ± 0.02	5.83 ± 0.29	0.65 ±0.09
Control	5.86 ± 0.18	11.6 ± 0.86	35.4 ±1.05	60.4 ±1.92	19.7 ±1.28	32 ±1.34	9.74 ± 0.45	2.91 ±0.47	0.16 ± 0.04	0.017 ± 0.02	5.91 ± 0.31	0.74 ±0.11

* Significant at P< 0.05

Table (5): Some serum biochemical profiles (Mean ± S.E.) in progeny rabbits with congenital hydrocephalus (gp. P1) from parents naturally intoxicated with ochratoxin A:

Groups	AST U/L	ALT U/L	AP U/L	TP Gm/dl	Albumin gm/dl	TB mg/dl	Glucose mg/dl	Urea mg/dl	Creatine mg/dl	CK U/L	LDH U/L
1W.-old	39 ±5.3	35 ± 4.12	44 ± 5.7	6.25 ± 0.36	2.7 ± 0.19	0.94 ± 0.08	68 ±5.1	32 ± 2.8	0.84 ± 0.12	91 ±8.8	145 ± 9.8*
2Ws-old	49 ± 3.45*	48 ± 2.31*	43 ± 4.23	5.84 ± 0.39	2.01 ± 0.12*	0.95 ± 0.10	57 ±4.5*	45 ± 4.1*	1.32 ± 0.21	96 ±8.5	158 ±8.9*
4Ws-old	56 ±4.12*	54 ± 3.44*	55 ±5.86	5.11 ± 0.31	1.82 ± 0.15*	1.02 ± 0.09*	51 ±4.1*	51 ± 6.1	1.89 ± 0.19*	104 ±9.9	186 ± 10.7
Control	34 ±3.17	31 ± 2.91	48 ± 4.96	6.35 ± 0.38	2.9 ± 0.18	0.76 ± 0.07	75 ±6.2	28 ± 4.9	0.96 ± 0.18	98 ±7.8	85 ± 8.1

Significant at P< 0.05

Table (6): Some hematological parameters (Mean ± S.E.) in mother does (gp. M2) naturally intoxicated with ochratoxin A:

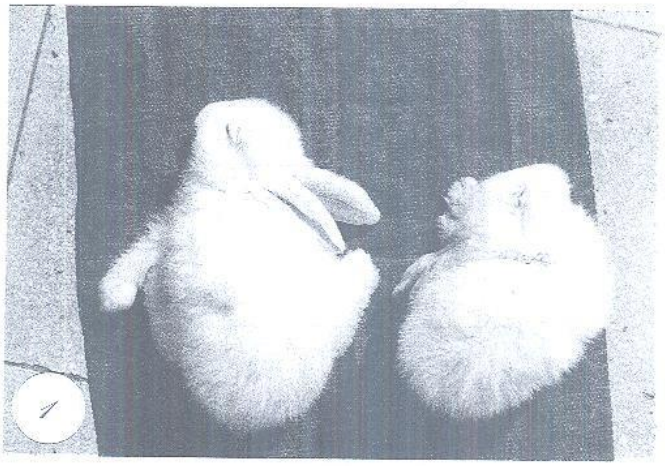
Groups	RBCs Mill/µL	Hb gm/dl	PCV %	MCV FL	MCH Pg	MCHC %	TLC Thou/µL	Neutro Thou/µL	Esinoph Thou/µL	Basoph Thou/µL	Lymph Thou/µL	Monocy Thou/µL
0	5.85 ± 0.19	11.02 ± 0.93	35.5 ± 1.42	60 ± 2.45	18.8 ± 1.32	31.00 ± 1.26	14.45 ± 0.64*	2.81 ± 0.35	0.16 ± 0.02	0.015 ± 0.02	10.84 ± 0.43*	0.63 ± 0.14
1W.	6.06 ± 0.18	11.8 ± 0.88	36.1 ± 1.24	59.6 ± 2.41	19.5 ± 1.01	32.6 ± 1.31	12.61 ± 0.55*	2.74 ± 0.32	0.14 ± 0.03	0.016 ± 0.02	9.10 ± 0.40*	0.61 ± 0.12
2Ws	6.11 0.15	11.56 0.81	36.9 1.31	60.4 1.89	18.9 0.95	31.3 ±0.98	11.52 0.42*	2.93 ±0.25	0.18 0.02	0.017 ±0.02	7.66 ±0.48*	0.73 ±0.08
4Ws	6.31 ± 0.17	12.2 ± 0.85	35.8 ± 1.12	56.7 ± 1.32	19.3 ± 1.33	34.00 ± 1.12	9.44 ± 0.51	2.99 ± 0.31	0.19 ± 0.04	0.018 ± 0.02	5.53 ± 0.39	0.71 ± 0.06
Control	6.21 ± 0.14	11.85 ± 0.79	35.2 ± 1.08	56.9 ± 1.72	19.1 ± 1.58	33.6 ± 1.24	8.69 ± 0.35	2.87 ± 0.37	0.15 ± 0.05	0.016 ± 0.02	4.96 ± 0.34	0.65 ± 0.09

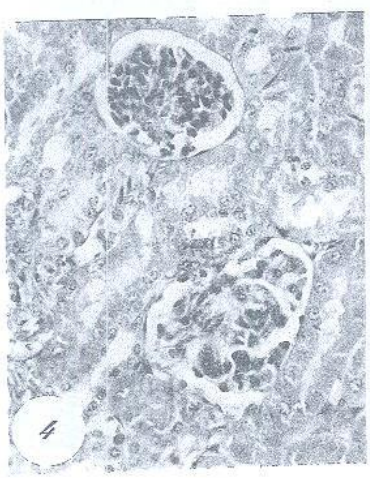
* Significant at P<0.05

Table (7): Some serum biochemical profiles (Mean ± S.E.) in mother does (gp. M2) suffered from natural ochratoxicosis A:

Groups	ALT U/L	AST U/L	AP U/L	TP Gm/dl	Albumin gm/dl	TB mg/dl	Glucose mg/dl	Urea mg/dl	creatine mg/dl	CK U/L	LD U/L
0	76 ±5.56*	72 ± 4.41*	74 ± 4.21*	6.32 ± 0.34	2.51 ± 0.11*	1.12 ± 0.14	92 ± 6.8	41 ± 5.1	1.12 ± 0.19	121 ±9.3	152 ± 10.4
1W.	64 ± 4.15*	61 ±2.83*	63 5.91	6.38 0.36	2.89 ±0.28	1.03 ±0.11	88 ±4.9	37 ±4.9	1.08 ±0.17	117 ±5.8	135 ±7.3
2Ws	59 ± 2.15*	57 ± 2.92*	61 ± 4.13	6.51 ± 0.21	3.25 ± 0.16	1.05 ± 0.09	85 ± 4.8	35 ± 4.2	0.98 ± 0.18	124 ±9.9	110 ± 9.3
4Ws	51 ±3.48	46 ± 2.88	57 ± 4.6	6.59 ± 0.24	3.41 ± 0.18	0.94 ± 0.10	91 ± 4.92	34 ± 4.4	0.89 ± 0.12	119 ±8.2	107 ± 10.2
Control	45 ±3.12	42 ± 2.57	58 ± 3.45	6.69 ± 0.31	3.2 ± 0.26	0.98 ± 0.08	82 ± 5.1	31 ± 4.6	0.95 ± 0.16	115 ±6.9	101 ± 8.9

Significant at P< 0.05





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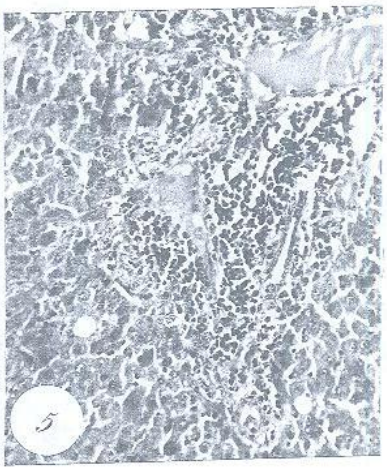

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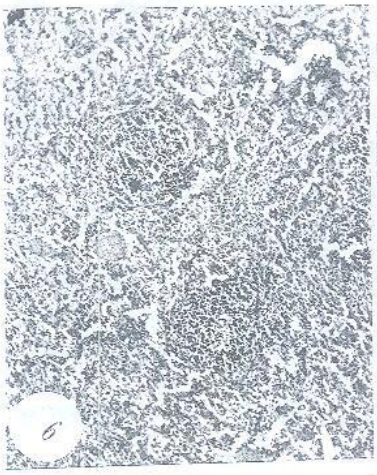

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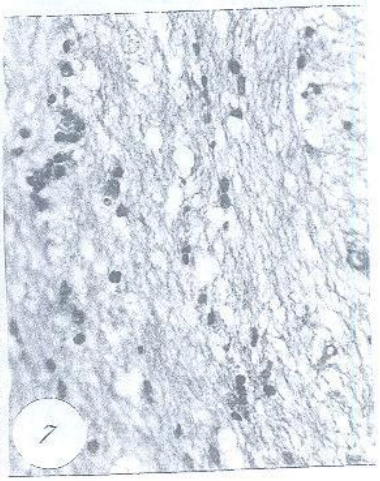

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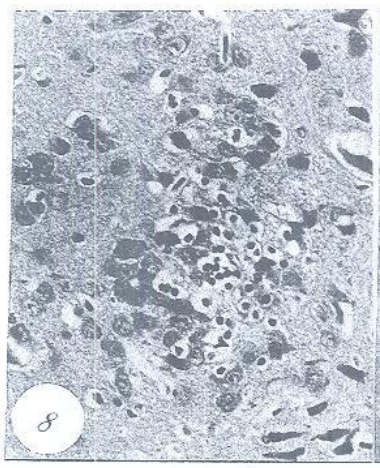
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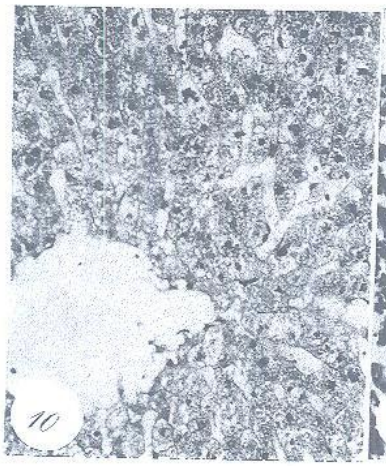
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