

HISTOLOGICAL AND BIOCHEMICAL STUDY IN MICE EXPERIMENTALLY INFECTED WITH *TOXOPLASMA GONDII* AND EXPOSED TO AFLATOXIN

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ABSTRACT

This study was conducted for examine the histological effects of aflatoxin and *Toxoplasma gondii* on some organs like liver, heart, kidney and brain in mice. Were , mice divided into six equal groups; group 1 (untreated control), group 2 were injected intraperitonealy with *T. gondii* tissue cysts, group 3 fed diets contaminated with 0.5 ppm of aflatoxin, group 4 were fed diets contaminated with 1 ppm aflatoxin, group 5 were fed 0.5 ppm of aflatoxin and injected with *T. gondii* cysts and group 6 were fed 1 ppm aflatoxin with injected *T. gondii* tissue cysts. The experiment maintained for 40 days.

At the end of the experiment, histological changes were increased specially at groups 5 and 6 including necrosis and lymphocytic infiltration, there are significantly increased in the level of MDA and significantly decrease of GSH level in liver tissue specially in groups (2) and (4) as compared with group (1) normal healthy control.

Keywords: Mycotoxins, Aflatoxin, *Toxoplasma gondii*, Histological changes, Glutathione, Malondialdehyde.

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INTRODUCTION

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii* (Ryan et al, 2004). The parasite infects most general of warm-blooded animals, including humans, cattle, sheep, goat, rabbit, mice but the primary host is the feline (cat) family. Animals are infected by eating infected meat, or by ingestion of feces of a cat that has itself recently been infected, or by transmission from dam to infants. Cats are the primary source of infection to Human and Animals hosts, although contact with raw meat, Fecal contamination of hands is a significant risk factor (Torda, 2001). *T. gondii* infections have the ability to change the behavior of rats and mice, making them drawn to rather than fearful of the scent of cats. This effect is advantageous to the parasite, which will be able to sexually reproduce if its host is eaten by a cat (Berdoy et al, 2000) The infection is highly precise, as it does not affect a rat's other fears such as the fear of open spaces or of unfamiliar-smelling food.

T. gondii infections have also cause behavioral changes in humans, including slower reaction times and a six fold increased risk of traffic accidents among infected males. The prevalence of human infection by *Toxoplasma* varies greatly between countries (Berdoy et al, 2000). Factors that influence infection rates include diet (prevalence is possibly higher where there is a preference for less-cooked meat) and proximity to cats (Flegr et al, 2009) ; (Meerburg et al, 2009)

Aflatoxins (AF) are among the most common mycotoxins to which humans are exposed. They are highly substituted coumarin derivatives containing a fused dihydrofurofuran moiety. Human beings and mammals are exposed to aflatoxin through food/feed-stuffs, milk, meat and eggs. Consumption of AF in many parts of the world varies between 0 ng-30, 000 ng/kg/day. Epidemiological and experimental

studies have shown that AF are hepatotoxic (Luyendyk, 2003), hepatocarcinogenic (yang et al, 2000), mutagenic (Catterall et al, 2002) and teratogenic (Vismara, 2007).

Toxoplasmosis can cause serious pathologies including hepatitis, pneumonia, blindness, and severe neurological disorders. These types of diseases are seen particularly in people and animal with weak immune systems (Yazar et al, 2003); (Nishikawa et al, 2007). Yet, the pathogenic mechanisms in healthy people could not be explained completely. It is assumed that the malondialdehyde (MDA) arising from the lipid peroxidation is an indicator of the oxidative stress in tissue and cells. Lipid peroxidase is a derivative of feeble unsaturated fatty acid which is produced as a result of decomposition of a set of complex components (Koltas, et al, 2006). Glutathione (GSH), an endogen originated peptide which can be synthesized in the liver without need for genetic data, is made up of glutamic acid, cysteine and glycine amino acids, and is an important antioxidant. It defends the cells against oxidative damage by undergoing reaction with free radicals and peroxidase (Uchiyama et al, 1978).

MATERIALS AND METHODS

Animals and treatments: Sixty young inbred Swiss female albino mice BALB/C weighting 20-25 g were obtained from animal house at the pharmacy department of Mosul technical institutes. All were negative for anti-toxoplasma antibodies in the latex agglutination test, animals were provided with feed and water ad libitum under laboratory conditions, These mice were randomly divided into six groups (10 mice per group) and caged separately:

1. Group 1 (untreated control).
2. Group 2 were injected with *T. gondii* tissue cysts.
3. Group 3 were fed diet contaminated with 0.5 ppm AF.
4. Group 4 were fed diet contaminated with 1 ppm AF.
5. Group 5 were fed diets contaminated with 0.5 ppm AF and injected with *T. gondii* tissue cysts.
6. Group 6 were fed diets contaminated with 1 ppm AF and injected with *T. gondii* tissue cysts. The experiment extended for 40 days.

Infection and autopsy: Mice were intraperitoneally inoculated with 5×10^5 / ml tachyzoites of *T. gondii*. Autopsies were performed for light microscopic examination to study the histological changes.

Light microscopy: The liver, heart, kidney and brain of each studied mouse were fixed in 10% formalin, dehydrated and embedded in wax for light histo - pathologic examination. Hematoxylin and Eosin (H&E) and Giemsa staining were used. Tachyzoites in the peritoneal fluid of all acutely infected mice were processed for microscopic study as previously described (Venturini et al, 1996). In brief, they were fixed in 4% gluteraldehyde in 0.1 M phosphate buffer, post-fixed in osmium tetroxide and treated with uranyl acetate prior to dehydration and embedded in Spurr's epoxy resin, before sectioning and staining for light microscopy.

***T. gondii* tissue cysts:** *T. gondii* tissue cysts were obtained from brain tissue of naturally infected local breed mice. Brain tissue was grind, suspended in phosphate buffer saline (PBS), at pH 7.2, and then filtered through gauze. One ml (containing 100 tissue cysts) was injected in Swiss mice intraperitoneally as described by (Shotwell et al, 1996). Mice were daily inspected for any febrile sign.

Aflatoxins: AF were prepared through inoculation of rice with *A. parasiticus* NRRL 2999 and were incorporated into the diet to provide the described level of 0.5 and 1

ppm_(obtained from Dr. Aqeel Mohammed Sharif in College of Veterinary medicine \ Mosul University\ Iraq) as described by (West et al, 1973) and modified by (Nabney et al, 1965). Fermented rice was then autoclaved and grind. The aflatoxin content were measured by spectrophotometer analysis (Wiseman et al, 1967) (Ferguson et al, 1999)

Determination of Glutathione and Malondialdehyde: Serum glutathione was measured by a modified procedure utilizing Ellman's reagent (D'Mello et al, 1997). while the level of malondialdehyde was carried out using the modified method of (Yazar et al, 2004).

Statistical analysis: The data were statistically analyzed using ANOVA as means \pm standard error of the mean, $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

In the present study many histological changes were observed in mice intestine infected with *T. gondii* parasite fig (1). Numerous perivascular and leptomeningeal infiltrations of inflammatory cells were observed, occasional occurrence of necrosis of the brain parenchyma was also noticed, It was reported that necrotic foci followed by mononuclear inflammatory reactions are the most prominent lesions of toxoplasmosis in organs heavily infected with tachyzoites and this appears to be directly related to the rapid intracellular replication of the parasite (Jubb et al, 1993). The hazards of Aflatoxins may be either those of acute toxicity or those of long-term chronic toxicity and carcinogenicity, These toxicity had relationship with the route of administration, exposure period and dosage (Baptista et al, 2008). Mycotoxicoses were induced in mice by feeding AF (0.5 ppm) to study the pathological changes, Degenerative and muscle fibers atrophy were observed in the myocardium of all toxin treated mice. However, in AF fed birds, haemorrhages were noticed in the epicardium and myocardium (Balachandran and Ramakrishnan, 1987). The liver is the target organ for AFB1. Ingestion of this mycotoxin, is known to be capable of inducing acute poisoning, aflatoxicosis, and is believed to be participated in the development of primary liver cancer (Neal, 1995). Moreover, under this pathological condition, the active process of cellular self-destruction, apoptosis, can occur (Meki et al, 2004). This may return to that AFs have induced oxidative damage and caused to generate free radicals which reacted with cellular component and led to pathological changes in liver and kidney functions (Clifford et al, 1967). This may referred to the action of cytochrome P450 which is secreted from liver cells, renal tubular cells and lung cells. This enzyme caused detoxification of toxin complex compound and this will lead to degeneration of organ cells and may cause cancer (Ankrah et al, 1993) coincide with (Choudhary and Verma, 2006) who carried out his experiment on AF by oral administration for 30 days and noticed significant increase in lipid peroxidation in the kidney, consequently, this explain increasing urea, uric acid and certain of blood serum level in AFs treated mice when compared with control animals. Histopathological changes observed in the livers of AF similar to (Colakoglu and Donmez, 2012). In experimental models of infection with *T. gondii* parasite, acute mortality can result either from a failure to control the parasite number or due to the excessive immunological response against the infection (Lawrence et al, 2003), It is very well known that hepatocytes are active metabolic cells and when such parasite invade the cell it can lead to disturbances in its metabolic activity which inturn leads to shape distortion, which could be due to edema and accumulation of fluid within the cells. Similar changes were also found by other

workers and some relate the disturbances in the hepatocyte function and with *T. gondii* (Hussein and Brasel, 2001; Eissa et al, 1990). Microscopic examination also revealed intense infiltration of the liver with mixed inflammatory cells, these cells leave the blood and enter the tissues as a part of the immunological response of the mice to the infection with *Toxoplasma Gondii* parasite fig (3, 6, 10), this change was also mentioned by other workers (Lawrence et al, 2003; Ferro et al, 1999). Also, this result agree with (Rana and Kassabbashi, 2007). So mechanisms of liver damage and the histological changes induced by *T. gondii* infection is either due to a direct effect of the parasite on the tissues leading to cell death and tissue damage or it could be related to indirect effect of infection due to the excessive immunological response to the parasite (Ferro et al, 1999). Reduction in protein content could also be due to increased hepatocellular necrosis. Thus, reduction in protein biosynthesis as well as increased necrosis could be responsible for a decrease in protein. Many other investigators reported a decrease in protein concentration in skeletal muscle (Raval and Verma 1992), heart (Neal, 1995), liver and kidney (Meki et al, 2004; Clifford et al, 1967; Ankrah et al, 1993) of AF-fed animals. the pathological changes in different organs fig (2-5). the lesions were more marked and consisted of both the lesions seen in AF and *T.gondii* suggest the cumulative interaction of these toxins in inducing severe changes in organs fig (7-13). Placenta, brain and heart are the most commonly affected organs in *Toxoplasma* induced abortion in sheep and goat (Dubey, 1988). Hence, *T. gondii* might affect the information processing within a wide variety of brain functional systems, provided that the parasite is able to alter brain function locally (Berenreiterova et al, 2011). At later stage of infection, it was comparatively easier to spot tachyzoites in the heart and diaphragm that reinforces tachyzoites proliferation in muscles, indicative of the subacute phase of *T. gondii* infection eventually leading to parasite encystations in muscles and brain (Frenkel, 1973).

Table (1) show that MDA level in liver tissue was significantly ($P < 0.05$) increased in group (2) , group (4) , group (5) and group (6) as compared with group (1) normal healthy control and table (1) also show that GSH concentration in liver was significantly decreased in group (2) and group (4), group (5) and group (6) as compared with group (1) normal healthy control. GSH is an important constituent of intracellular protective mechanisms against a number of noxious stimuli including oxidative stress. It plays a role in preventing the transformation of hemoglobin into met hemoglobin due to oxidation. Moreover, it maintains the sulfhydryl (-SH) groups in proteins in a reduced state and protects these groups against oxidation (Akkus, 1995).

The MDA product can cause the cross-linkage of membrane elements by affecting the ion exchange from cell membranes, which gives way to aftermaths including a change in ion permeability and enzyme activity. In a previous study, it was reported that MDA increases in *Toxoplasma* seropositive patients (Yazar, 2004). A decrease in the GSH activity in *Toxoplasma* seropositive patients indicates a decline in the response to oxidative stresses. The high level of MDA suggest in the patients infected with toxoplasmosis could be the decreased activity of the defense system protecting the tissues from free radical damage (Gilbert, 1984).

Table (1): Effect of Aflatoxin and *Toxoplasma gondii* on MDA and GSH level in liver tissue.

Animal \ group	MDA U mol/L	GSH U mol/L
Control (group 1)	17.24 ±0.10d	3.01 ±0.04a
Toxoplasma (group 2)	24.05 ±0.38b	1.80 ±0.11c
Aflatoxin (0.5 ppm) (group 3)	17.98±0.91d	3.09±0.12a
Aflatoxin (1 ppm) (group 4)	19.10 ±0.01c	2.12 ±0.20b
Aflatoxin 0.5 ppm and injected with <i>T. gondii</i> tissue cysts (group 5)	24.10±0.42b	1.89±0.62c
Aflatoxin 1 ppm and injected with <i>T. gondii</i> tissue cysts (group 6)	27.08±0.54a	1.02±0.12d

Different letters in column differ significantly at level of $P<0.05$, Mean \pm SE for 10 mice.

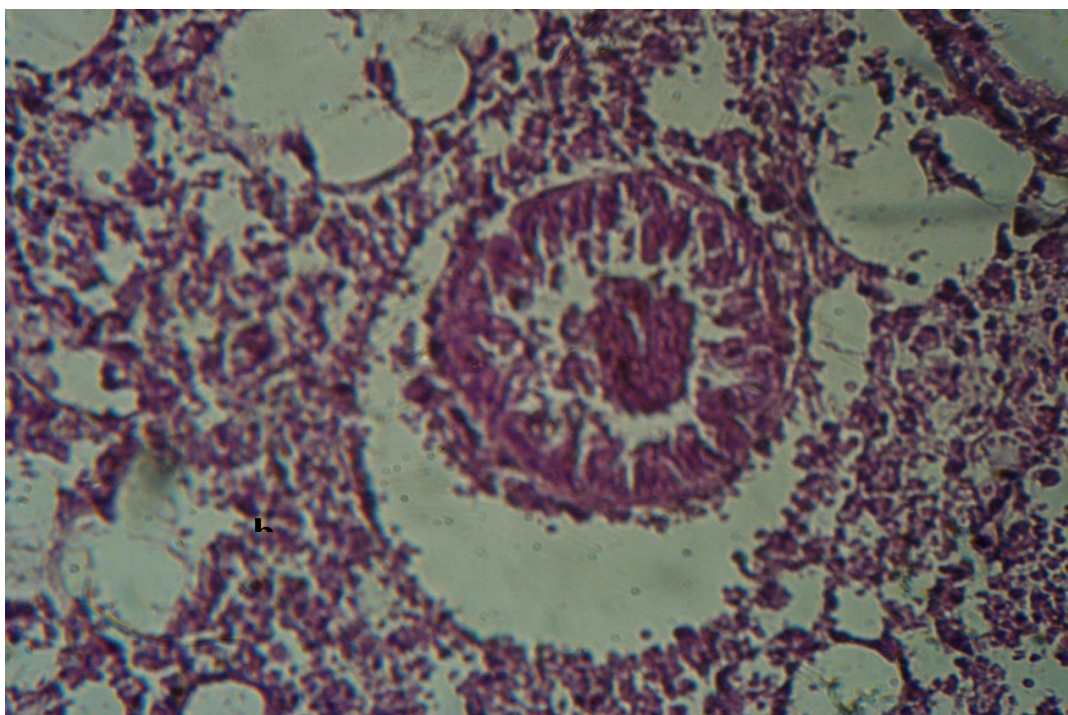


Fig. (1): Photomicrograph section of mice intestine, injected with toxoplasma tissue cysts, showed mucus cell proliferation (a), lymphocyte infiltration (b) tissue cyst (c). H&E, 165 X.

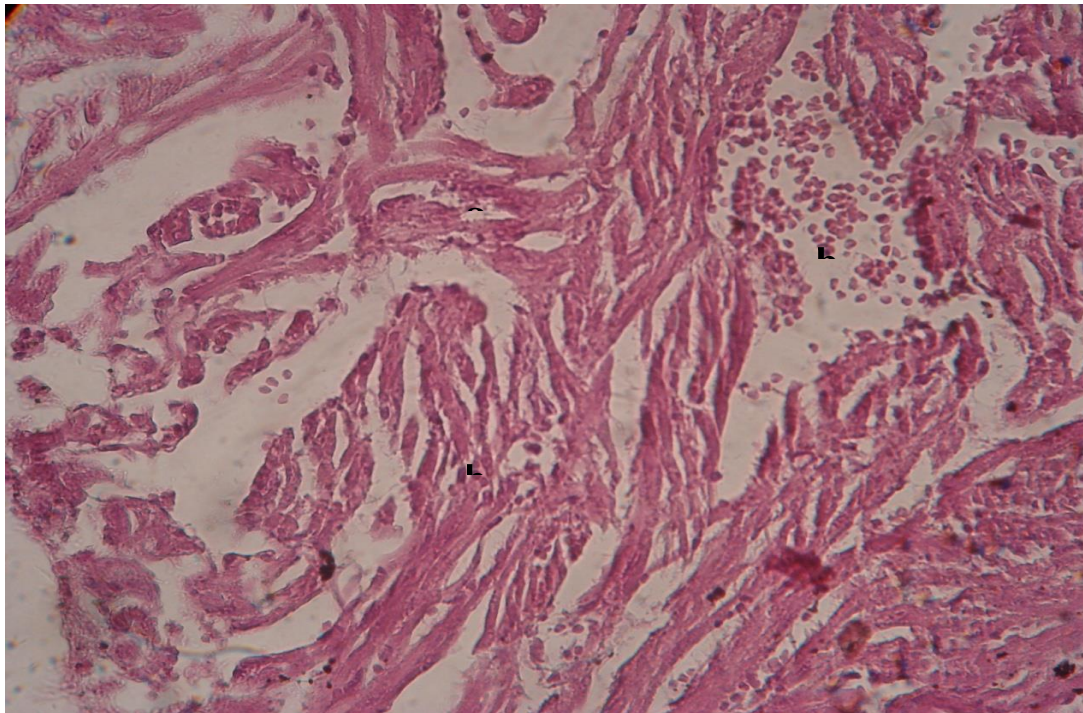


Fig. (2): Histological section of mice heart , fed diet contaminated with 0.5 ppm AF, showed muscle fibers atrophy (a) and tissue cyst (b). H&E, 145 X.

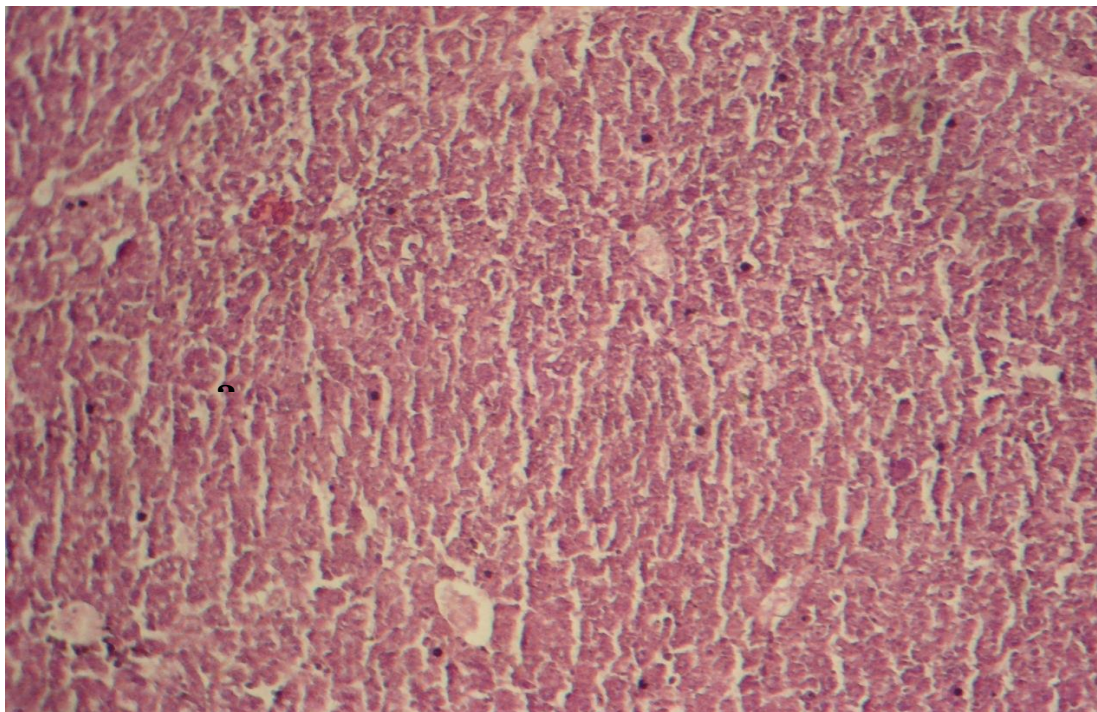


Fig. (3): Histological section of mice liver, fed diet contaminated with 1 ppm AF , showed loss of lobular architecture with moderate paranchymal cell necrosis (a).H&E, 115 X.

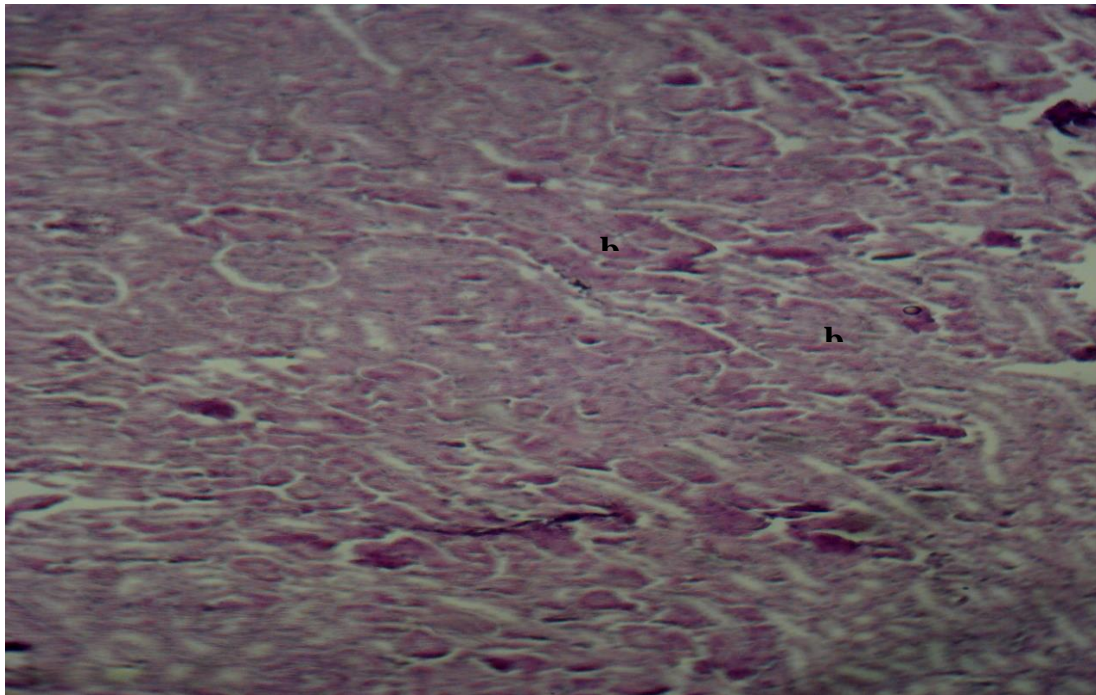


Fig. (4): Histological section of mice kidney, fed diet contaminated with 1 ppm AF, showed glomerular fibrosis (a) and sclerosis (b). H&E, 145 X.

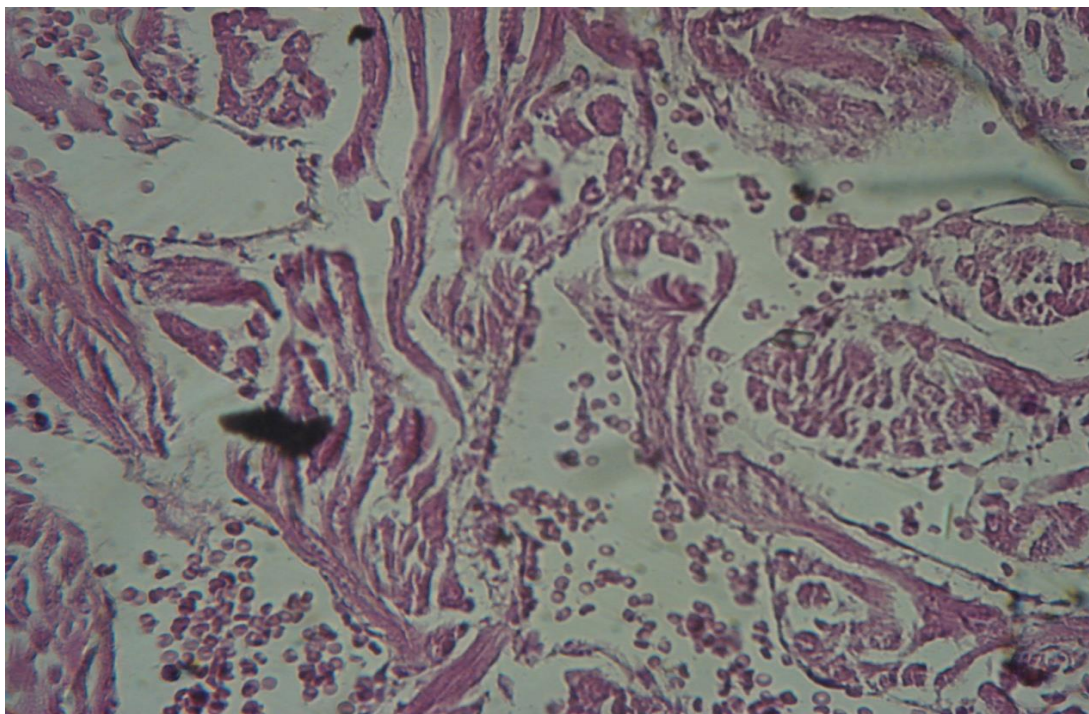


Fig. (5): Histological section of mice heart, fed diet contaminated with 1 ppm AF, showed atrophy of muscle fibers and shrinkage of fibers (a) H&E, 145 X.

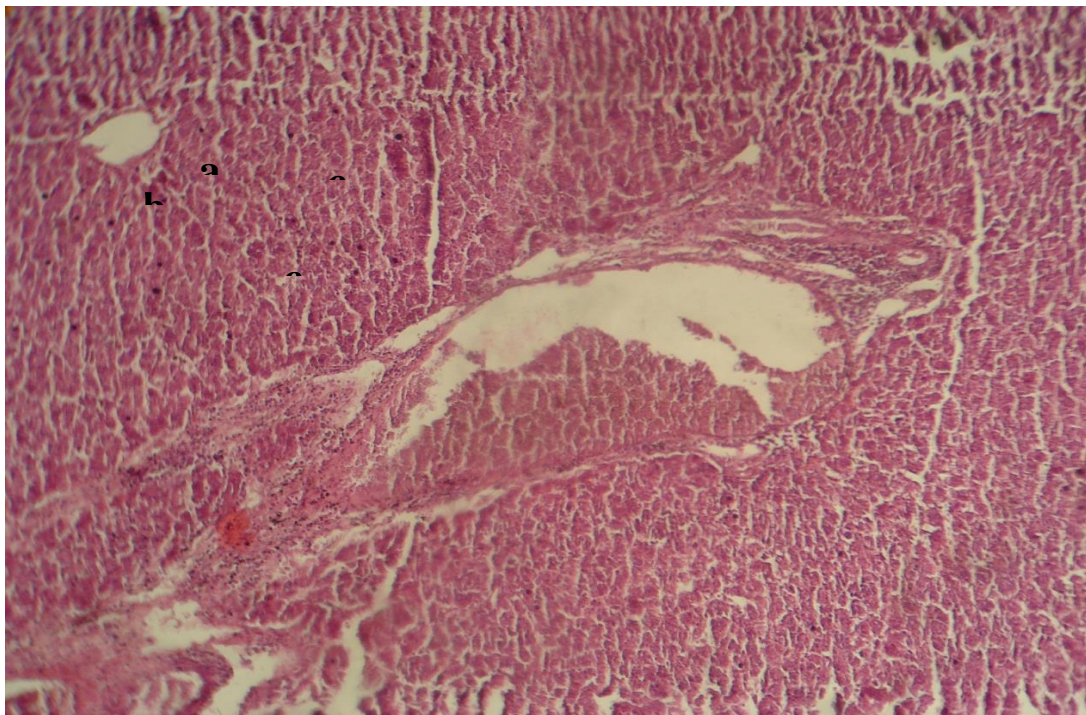


Fig. (6): Histological section of mice liver, fed diet contaminated with 0.5 ppm AF and injected with toxoplasma tissue cysts , showed loss of lobular architecture with paranchyma cell necrosis (a), lymphocytic hyperplasia (b) and tissue cyst (c). H&E, 115 X.

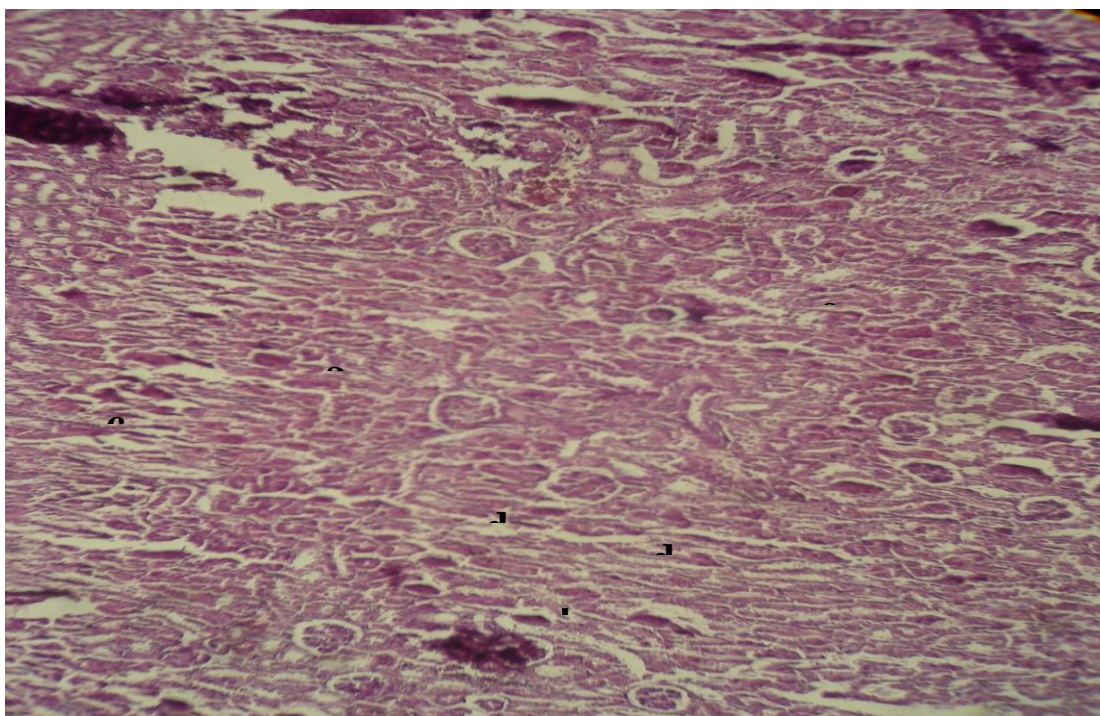


Fig. (7): Histological section of mice kidney , fed diet contaminated with 0.5 ppm AF and injected with toxoplasma tissue cysts, showed fibrosis (a) , sclerosis (b) , segmental necrosis (c) , glomerular capillary collapse (d) and tissue cyst (e). H&E, 115 X.

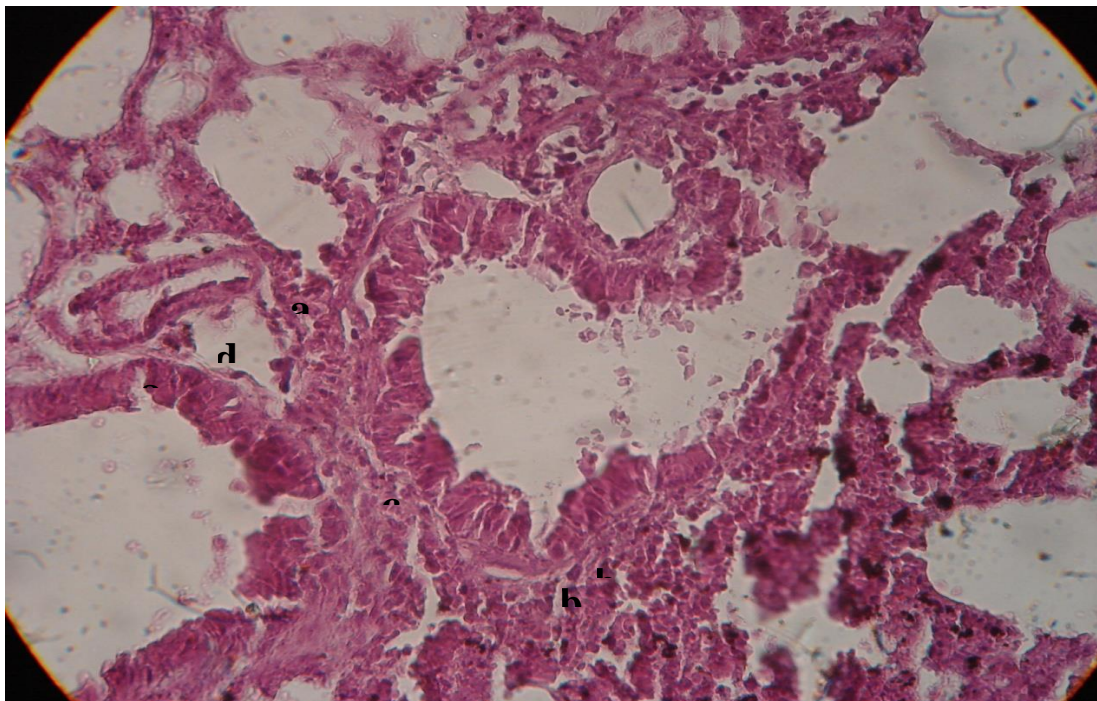


Fig. (8): Histological section of mice intestine, fed diet contaminated with 0.5ppm AF and injected with toxoplasma tissue cycts, showed glandular proliferation (a), with lymphocytic infiltration (b) and epithelial proliferation (c) tissue cyst(d). H&E, 165 X.

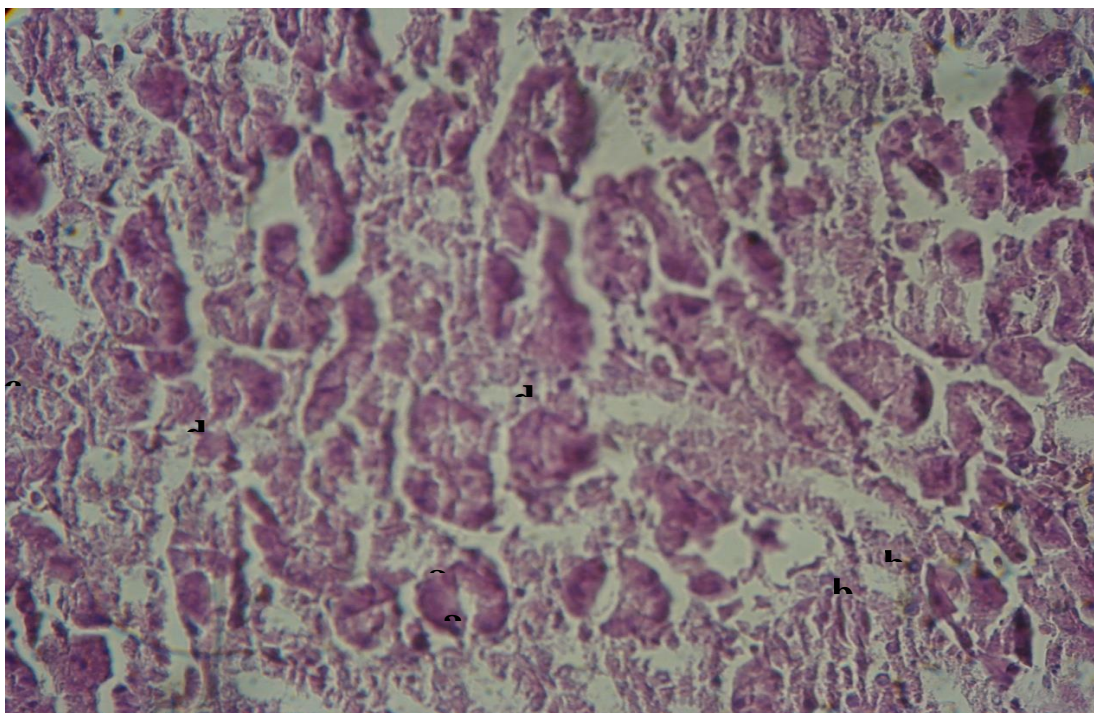


Fig. (9): Histological section of mice brain , fed diet contaminated with 1ppm AF and injected with toxoplasma tissue cysts , showed focal moderate necrosis (a), foci of gliosis (b) and lymphocytic infiltration (c) tissue cyst (c). H&E, 165 X

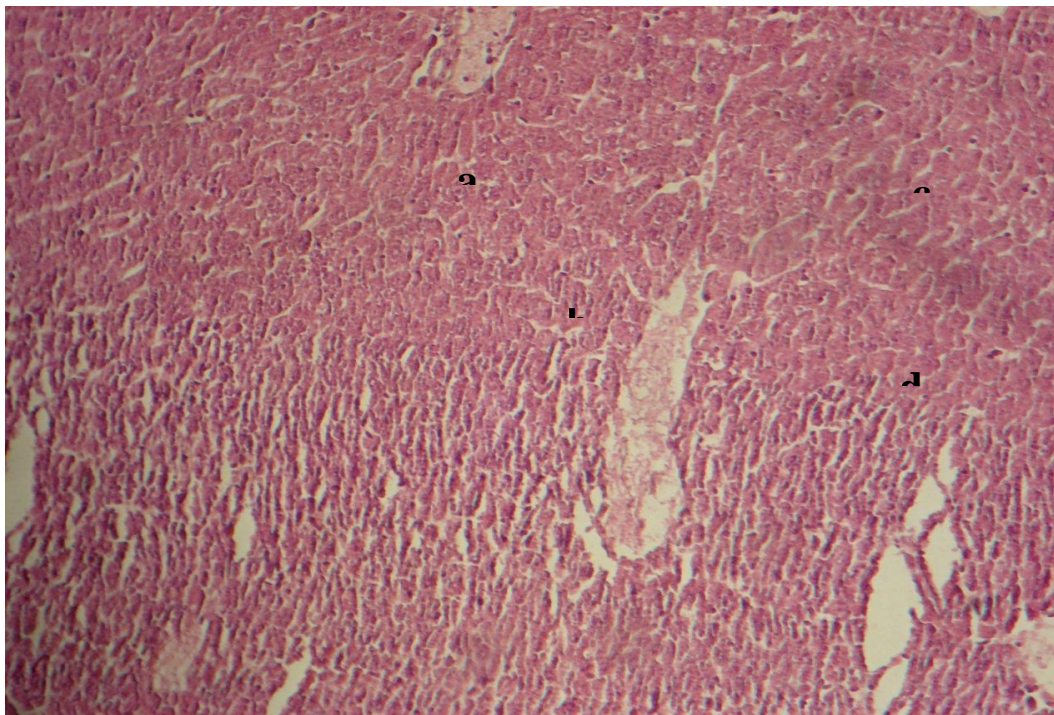


Fig. (10): Histological section of mice liver, fed diet contaminated with 1ppm AF and injected with toxoplasma tissue cysts , showed paranchymal cell proliferation (a), sever necrosis (b), mild lymphocytic infiltration (c) tissue cyst (d). H&E, 115 X.

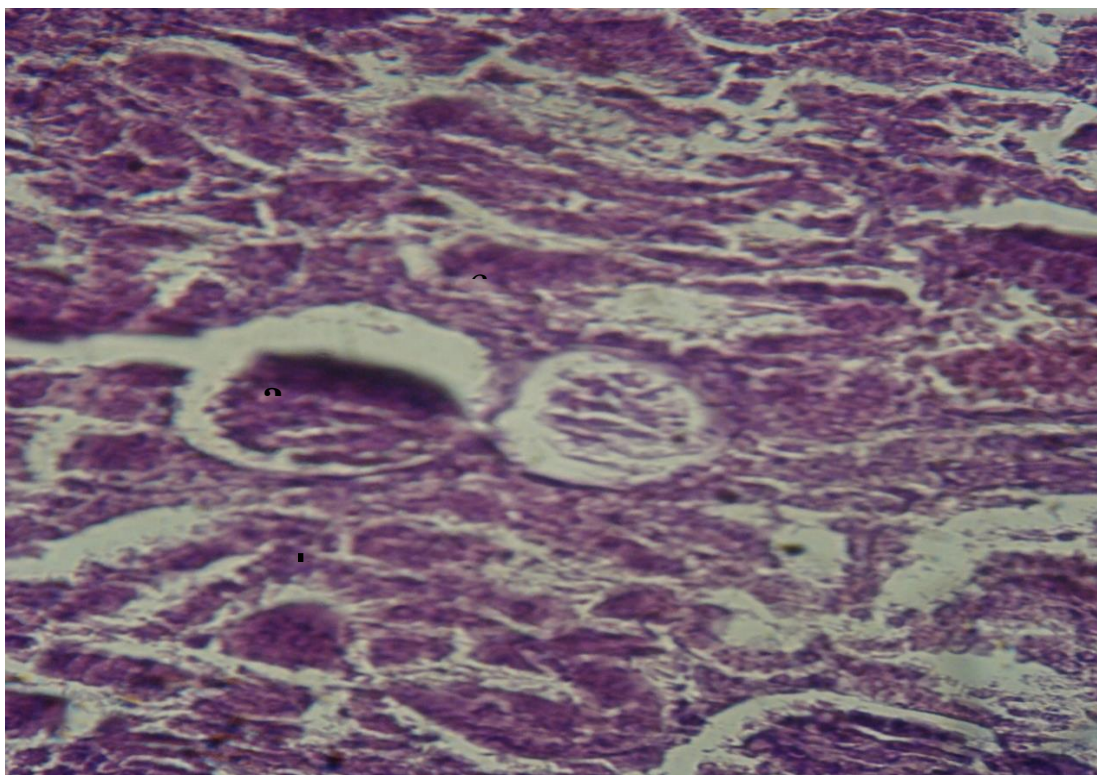


Fig. (11): Histological section of mice kidney, fed diet contaminated with 1ppm AF and injected with toxoplasma tissue cysts , showed glomerular sclerosis (a), focal necrosis (b), endothelial proliferation (c). H&E, 145 X.

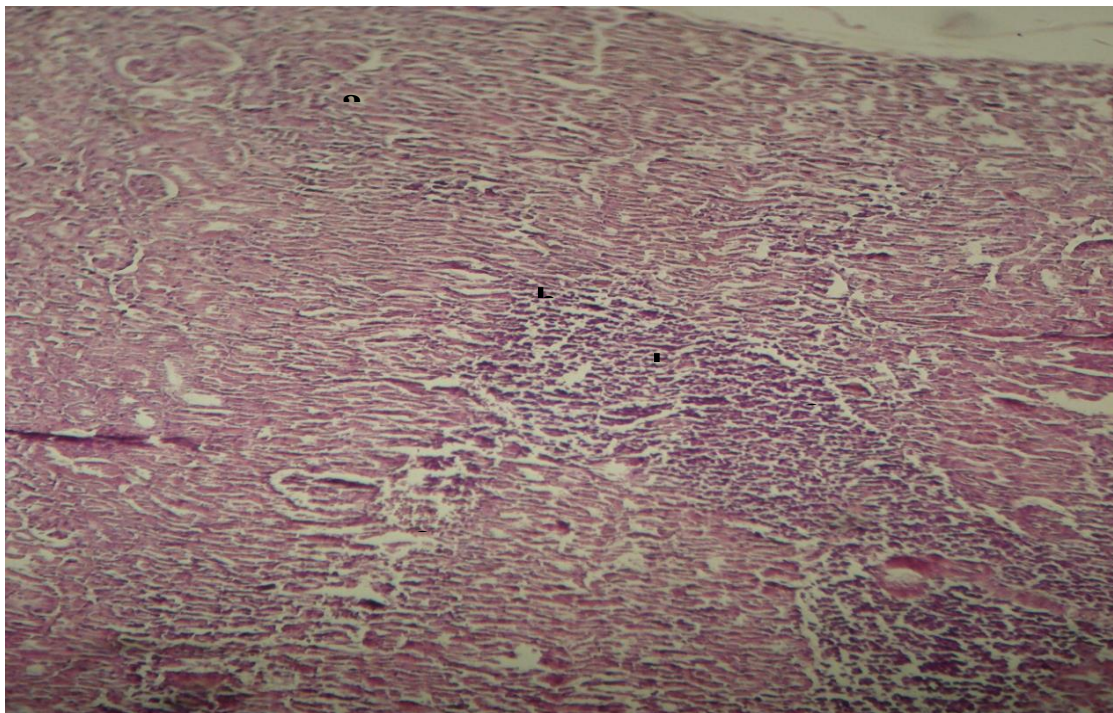


Fig. (12): Histological section of mice intestine , fed diet contaminated with 1ppm AF and injected with toxoplasma tissue cysts, showed mucus secreting cell proliferation and glandular proliferation (a), with sever lymphocytic infiltration (b) and tissue cyst (c). H&E, 115 X

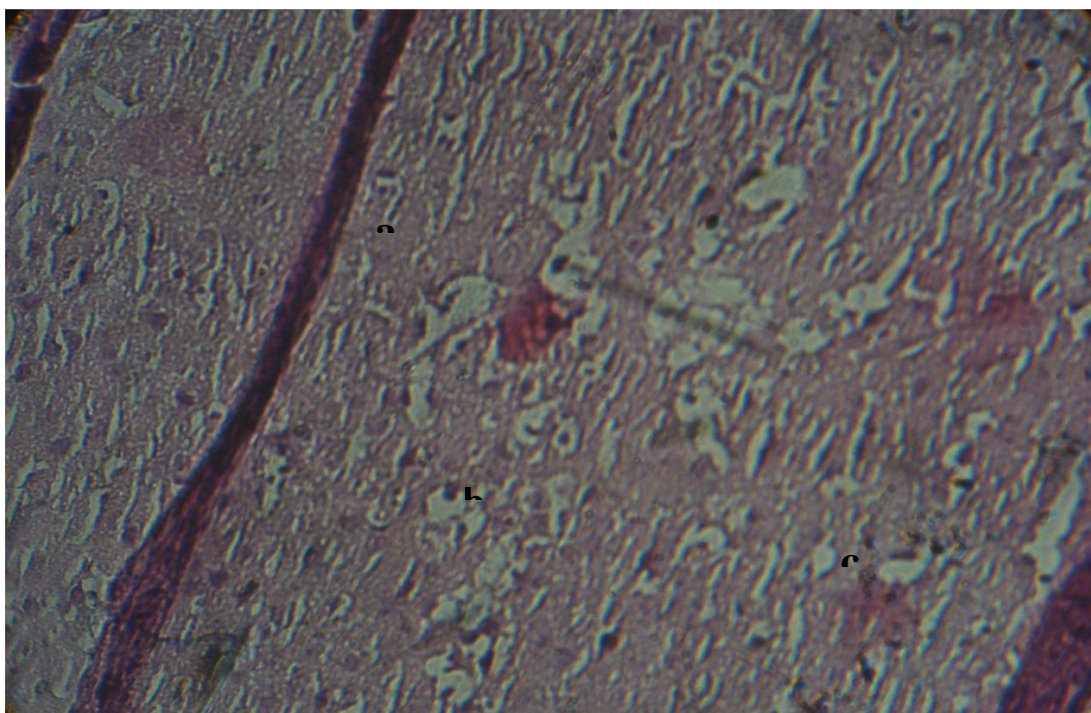


Fig. (13): Histological section of mice brain , fed diet contaminated with 0.5ppm AF and injected with toxoplasma tissue cysts , showed focal necrosis (a), foci of gliosis (b), mild lymphoid inflammation (c). H&E, 115 X.

دراسة نسجية كيموحيوية لتأثير الاصابة التجريبية بالمقوسات القندية والتسمم بذيوانات الافلاتوكسين في الفئران

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الخلاصة

أجريت هذه الدراسة لتحديد تأثير الأفلاتوكسين والتوكسوبلازما الكوندية من خلال التغيرات النسجية في بعض الأعضاء مثل الكبد والقلب والكلى والمخ في الفئران، وتم تقسيم الفئران إلى ست مجاميع بشكل عشوائي، المجموعة 1 (السيطرة غير المعالجة)، المجموعة 2 تم حقن بالبريتون مع التوكسوبلازما الكوندية، والمجموعات 3 و 4 تعرضت للأفلاتوكسين بالجرع 0.5 و 1 جزء في المليون على التوالي، المجموعتين 5 و 6 غذيت بالأفلاتوكسين 0.5 و 1 جزء في المليون وحقنت مع التوكسوبلازما الكوندية. واستمرت التجربة لمدة 40 يوماً.

وأظهرت النتائج تغيرات نسجية واضحة خصوصاً في المجموعة الخامسة والسادسة نتيجة تأثير الطفيلي والأفلاتوكسين ومنها ارتشاح الخلايا اللمفية وحدوث التخرات مع ظهور المراحل النسجية للطفيلي، وهناك زيادة في مستوى المالونديهايد وانخفاض في مستوى الكلوتاثيون وخصوصاً في المجموعتين 2 و 4 عند مقارنتها مع مجموعة السيطرة.
الكلمات الدالة:

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