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Individual and Combined Effects of Chronic T-2 Toxin and Aflatoxin B1 Mycotoxins on Rat Liver and Kidney

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ABSTRACT. Twenty-eight male albino rats were divided into four groups, one group was injected intraperitoneally with T-2 toxin, a second group was injected with Aflatoxin B1, a third group was injected with a mixture of the two mycotoxins and one group was used as control. The weight of each rat was recorded at weekly intervals. At the end of the study, all rats were killed, blood samples were drawn to determine white blood cell count and the packed cell volume. The liver, kidneys and other organs, which exhibited abnormalities, were weighed and processed for histopathological study.

Aflatoxin B1, and the mixture of AFB1 and T-2 toxin caused a significant ($p < 0.005$) drop in the average body weight of the rats after eight weeks of treatment. The highest PCV readings were recorded with rats injected with the mixture of AFB1 and T-2 toxin. Moreover, AFB1-treated rats and T-2 toxin-treated rats exhibited a significant ($p = 0.02$) decrease in the relative liver weight. The AFB1 and T-2 toxin mixture resulted in synergistic effects in most of the parameters of the study.

The histopathological study confirmed the hepatotoxicity of AFB1. One case of immunoblastic lymphoma was observed in this study; this observation is thought to be the first report that suggested a possible relationship between mycotoxicosis and lymphoma.

Many fungi that grow and contaminate stored foodstuffs are known to produce substances as secondary metabolites which exhibit harmfyl effects on various body tissues when consumed by animals or man. Aflatoxin B1 is the most abundant

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secondary fungal metabolite produced by certain strains of *Aspergillus flavus, A. parasiticus,* and some *Penicillium* species. It is the most potent hepatocarcinogen so far recognized, and is suspected of being a primary cause of human cancer in certain areas of the body (Wogan and Newbrene 1967) and is involved in the etiology of liver disease. The presence of aflatoxigenic fungi in the soil of Jordan is a contaminant that presents a potential hazard for man and animals through contamination of food and animal feed (Jarrar 1980 and Natour *et al.* 1993). The toxic effects of aflatoxins have been described in several studies, in a variety of species and strains of experimental animals (Butler and Barnes 1968). Infestation of wide variety of animals and human foodstuffs with *A. flavus* has resulted in speculations about the involvement of aflatoxins in the etiology of liver disease. Generally, the rat is considered to be the most sensitive mammal to aflatoxin carcinogenesis (F.D.A.). A probable carcinogenic effect of aflatoxin was observed in high incidence in renal epithelial neoplasm of male Wistar rats fed diets containing aflatoxin B1, for 147 days. Renal tumors developed in 8/14, 5/18 and 3/13 of male rats exposed to diets containing AFB1 levels of 1.0, 0.5, and 0.25 mg/kg respectively (Epstein *et al.* 1969).

T-2 toxin, produced by various species of *Fusarium* contaminants in foodstuffs, induced pathological and metabolic disorders in many animal species (Butler *et al.* 1969 and Deo *et al.* 1970). The adverse health effects associated with exposure to naturally occurring T-2 toxin is usually insidious, resulting from ingestion of comparatively small amounts of the toxin over a long period of time; most often death occurs after a relatively long period of illness (Poppenga *et al.* 1987). It was also reported that administration of T-2 toxin resulted in an enlargement, fatty degeneration and necrosis of the liver in rats (Sanega *et al.* 1984). The clinical signs observed in animals, when more than one mycotoxin is present in feed, are complex and diverse. Aflatoxin B1 and T-2 toxin were reported to be one of the most toxic combinations (Ward *et al.* 1975).

Materials and Methods

1. Animals:

Twenty-eight adult male white albino rats (*Rattus norvegicus*), 8-12 months old, were divided into four groups of seven rats each, housed in plastic cages and provided with commercial animal feed pellets and water *ad libitum.* The animals were allowed to acclimatize for two weeks prior to treatment and were weighed at weekly intervals for twenty weeks. The ear-punch code system was used to mark the rats.

2. Mycotoxins and treatments:

Two types of mycotoxins used in this study, T-2 Toxin, and AFB1. These toxins were dissolved in 25% ethanol. Rats of the four groups were intraperitoneally injected with these mycotoxins twice weekly for 20 weeks. The doses were administered in a volume of 1 ml./kg body weight as follows: Group 1 received 0.05 mg/kg T-2 toxin; Group 2 received 0.25 mg/kg AFB1; Group 3 received a mixture (1:1) of the above dosages of AFB1 and T-2 toxin and Group 4 received 1 ml/kg 25% ethanol (the control group).

3. Blood collection:

At the end of the experimental period, 1-2 ml of blood were drawn from the ansaesthetized rats by cardiac puncture, and kept in EDTA tubes to be used for studying the white blood cell counts and packed cell volume. After that, the animals were killed and immediately dissected.

4. Postmortem examination:

Postmortem examinations were carried out on rats immediately after death, the kidneys and liver of each rat were removed, weighed, and kept in screw-capped specimen jars containing formal-saline (10% formalin in 0.9% sodium chloride) as a fixative. Tissue slices (4 μm. thick) were taken from the liver, both kidneys and from other body organs, whenever abnormal gross pathology was noted in any of the animals.

Histological sections were prepared and microscopically examined. The histopathological changes were recorded and compared with sections taken from the control group. The total white blood cell counts, packed cell volumes, relative weight of the liver and kidneys were calculated for all the rats.

5. Statistical analysis:

The white blood cell count, packed cell volume, and the relative weight of the liver and kidney were calculated and the mean values were recorded for all groups and plotted for comparison. T-tests was employed for calculating the P value.

Results

1. Group 1; (T-2 toxin only):

The rats appeared weak, sleepy, and showed low food consumption after the twelfth week of treatment; after the eight week, rats developed recurrent diarrhoea. Upon necropsy, it was observed that the relative weights of the livers and kidneys

were higher than normal, during which a mild drop of body weight was observed, as compared with those of the control group. Other organs showed some abnormalities, such as lung abscesses and enlargement of the mesenteric lymph nodes. Microscopic examination of the liver revealed mild degenerative alterations, such as cytoplasmic vaculation, fatty changes, congestion and mild bile duct proliferation (Fig. 1). The average weights of the experimental animals are shown in Fig. (2).

2. Group 2; (AFB1 only):

The average weights of the rats showed a dramatic decline after the eighth week of treatment ($p = 0.003$) (Fig. 2). The rats developed diarrhoea after the tenth week. Two rats showed abdominal masses at the end of the study period. At necropsy, the liver and kidney, in most of the rats, showed enlargement, and three rats developed abscesses. Liver sections showed diffused centrilobular degeneration, with bile duct proliferation, hepatocytic regenerative activity, mild fatty changes, and severe liver sinusoidal congestion (Fig. 3). The major alterations in the kidneys were focal tubular cell necrosis, swelling of the glomeruli with hypercellularity and mesangial expansion (Fig. 4).

3. Group 3; (AFB1 + T-2 toxin):

Rats of this group developed a significant ($p < 0.005$) drop in average body weight after eight weeks of treatment (Fig. 2). Though all rats appeared to be sleepy, weak, and showed loss of appetite, only two of them developed abdominal abscesses and they also showed small yellow areas in the liver and lungs. Moreover, one rat developed ascites and enlarged mesenteric lymph nodes. Microscopic study of the liver sections of the rats of this group showed congestion with fatty changes (Fig. 5), and significant bile duct proliferation (Fig. 6). Hepatocytic changes which were characterized by moderate hyperplasia and dysplastic changes were also observed in this group. The major alterations in the kidneys were tubular epithelial degeneration, congestion, swelling of the glomeruli, as well as hypercellularity. The enlarged lymph nodes in one of the rats of this group was diagnosed as immunoblastic lymphoma.

4. Group 4:

The control group (25% ethanol only): This animal group did not show abnormal changes during the study period or at necropsy. They continued to be healthy and very active. The microscopic characteristics of the liver and kidney sections were considered normal (Figs. 7 and 8).

5. The effect of mycotoxins on the relative weight of the liver and kidneys of the rats:

5.1. Relative liver weight: The results indicated that relative liver weight of rats injected with AFB1 is significantly ($p = 0.02$) higher than that of the control group and that of rats treated with T-2 toxin (Fig. 9).

5.2. Relative kidney weight: Kidneys of rats treated with T-2 toxin showed a significant increase ($p = 0.014$) in kidney relative weight as compared with the control group (Fig. 10).

6. The effect of mycotoxins on rats white blood cells counts (WBC):

No significant effects were observed in white blood cell counts for any of the treated groups (Fig. 11).

7. The effect of mycotoxins on rats packed blood cell volume (PCV):

A significant increase in hematocrits was observed following the combined treatment with AFB1 and T-2 toxin, but not with either alone (Fig. 12).

An overall summary of changes observed in the experimental animals following treatments with mycotoxins is given below:

Parameter	T-2	AFB1	AFB1 and T-2
Body weight	+	+	+
Liver relative weight	+	+	–
Kidney relative weight	+	+	–
White cell count	–	–	–
Packed cell volume	–	–	+
Histopathology			
Kidney	–	+	+
Liver	+	+	+
Lymph nodes	+	+	+
Abscesses	+	+	+

(+) = There was an observable effect; (–) = No effect.

Discussion

The individual and combined effects of the T-2 toxin and AFB1 mycotoxins were studied on the liver and kidney of male albino rats. Several researchers have confirmed higher susceptibility of male rats to these toxins than females (Epstein *et al.* 1969 and Blunder *et al.* 1991). Due to the essential role of the liver in the metabolism and detoxification of a wide range of toxic materials, and that of the kidney in eliminating the waste products from the body, liver and kidneys were selected as target organs in this study.

The choice of these mycotoxins was due to their common occurrence in food and foodstuffs in Jordan and other countries (Natour *et al.* 1993), and due to the fact that these toxins induced pathological and metabolic disorders in many animal species (Lutsky *et al.* 1978).

Due to the fact that chronic ingestion of small amounts of toxins has more significance than acute exposure (Doerr *et al.* 1983), this research was conducted to study the chronic effects of the mycotoxins on experimental animals.

In this study, it was recognized that AFB1 was the more toxic. The toxicity of AFB1 is expressed by a decrease in growth rates and a significant ($p = 0.02$) increase in the relative weight of the liver. These individual effects of the AFB1 are in accord with previous study on chickens made by Huff *et al.* (1984).

The histopathological study of the liver showed that AFB1 caused significant changes in liver tissues, such as bile duct proliferation, hepatic degenerative activity and fatty changes with congestion. Such results confirmed that the liver is the target organ for AFB1 toxicity.

The T-2 toxicosis was characterized by a significant increases in the relative weight of the liver ($p = 0.020$) and the relative weight of the kidney ($p = 0.014$) as well as low food consumption and diarrhoea.

The liver of T-2 toxin-treated rats showed mild bile duct proliferation, mild fatty changes, hepatocytic degenerative activity, and congestion, while the kidneys shwoed mild degeneration of the tubular epithelium. The most serious toxicity was reflected upon the rats which were injected with a mixture of the two toxins (AFB1 and T-2). The effects of these two toxins for many parameters were significant and can be described as synergistic, and as a potent hepatotoxin and nephrotoxin.

Of all mycotoxin treatments, the PCV readings were significantly higher (p= 0.001) in rats treated with a mixture of AFB1 and T-2 toxin. It was also found that T-2 toxin, and AFB1 treated rats developed abscesses in the lung, abdomen and tests. The development of such abscesses may be due to the immunosuppressive properties of these mycotoxins. Similar conclusions have been reported by Sharma (1993).

Histopathological, the enlarged mesentric lymph nodes were found to be formed of massive plasma cell infiltration with lymphoid hyperplasia. An unexpected and probably, an important observation in this study was the development of lymphoma in one of the rats which were treated with a mixture of (AFB1 and T-2 toxin), a literature survey did not reveal such findings; hence, this observation is believed to be described for the first time and it calls for further investigation.

Acknowledgements

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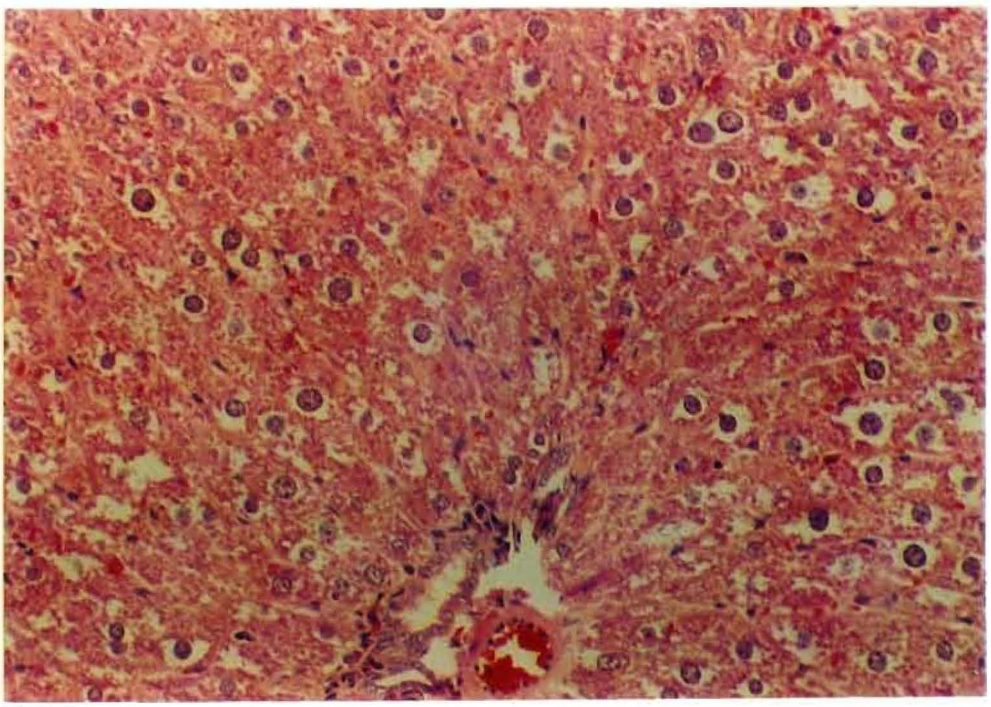

Fig. 1. Cytoplasmic vacuolization with hydropic degeneration in rats liver, after twenty weeks injection with T-2 toxin. (X 450).

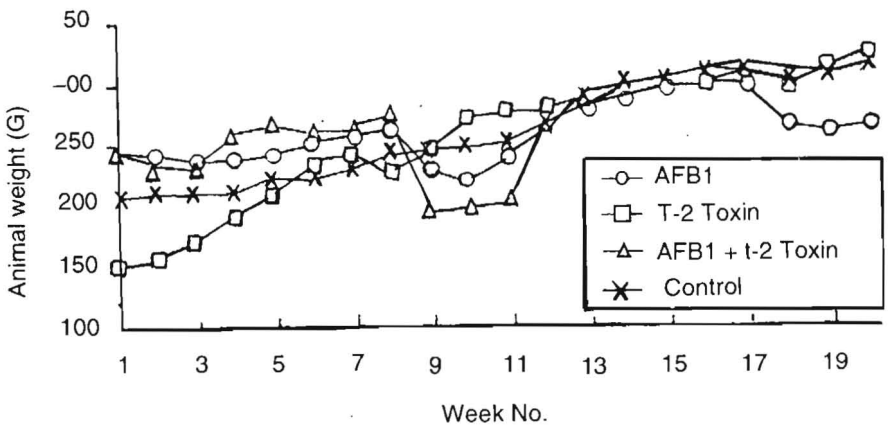

Fig. 2. Average body weight of the four groups of rats injected with mycotoxins at weekly intervals for twenty weeks. Data presented as the mean ± SD (n = 7).

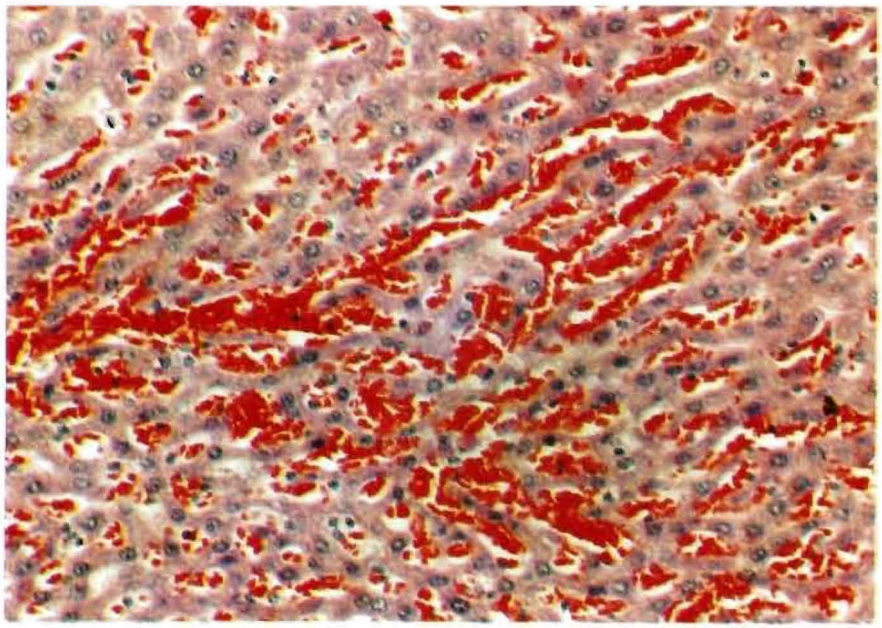

Fig. 3. Sinusoidal congestion of the rats liver, after injection with AFB1 for twenty weeks. (X 240).

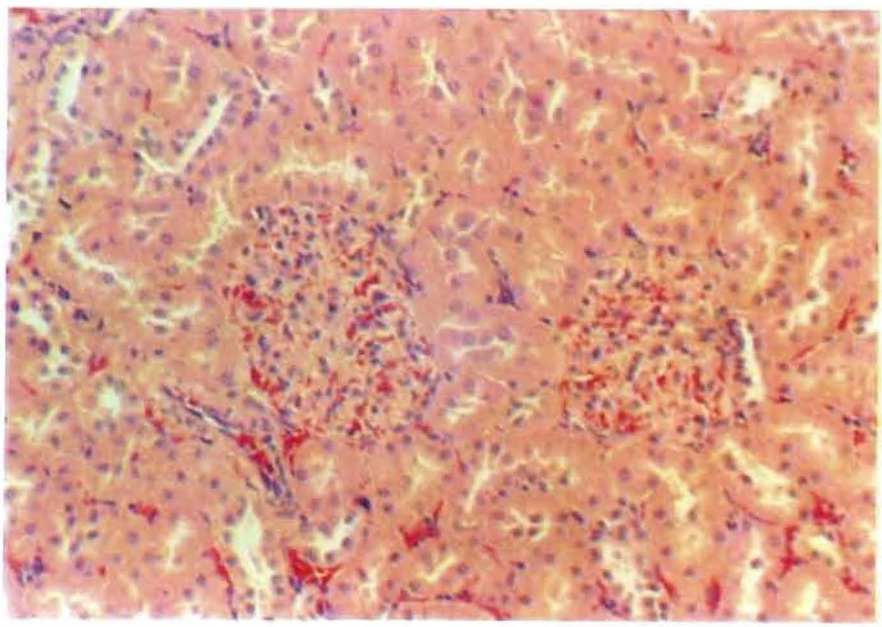

Fig. 4. Glomerular swelling, hypercellularity, mesangial expansion, and degeneration of the renal epithelial cells in kidneys of rats injected with AFB1 or with mixture of AFB1 + T-2 toxin for twenty weeks. (X 240).

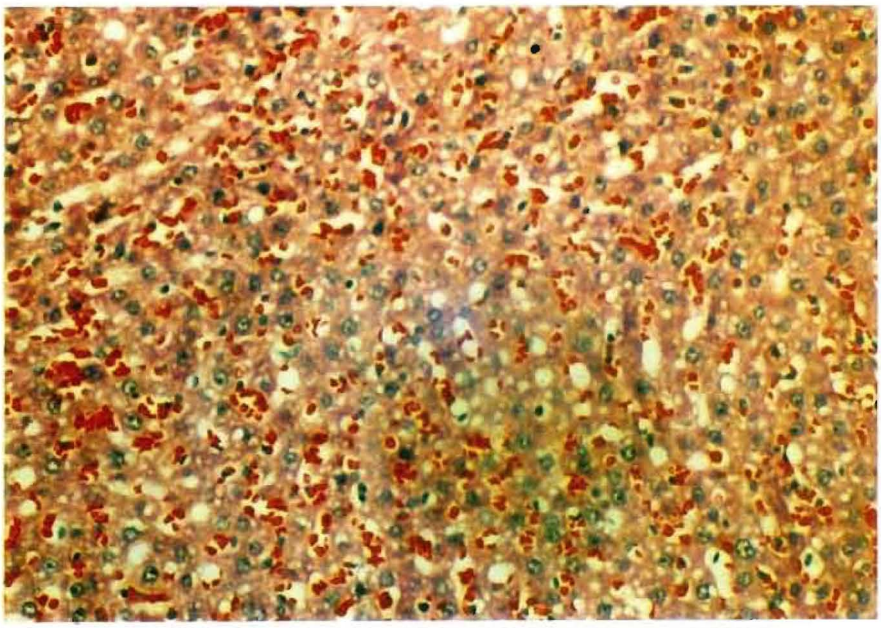

Fig. 5. Liver hepatocytes showing degeneration and fatty changes in rats injected with a mixture of AFB1 + T-2 toxin for twenty weeks. (X 240).

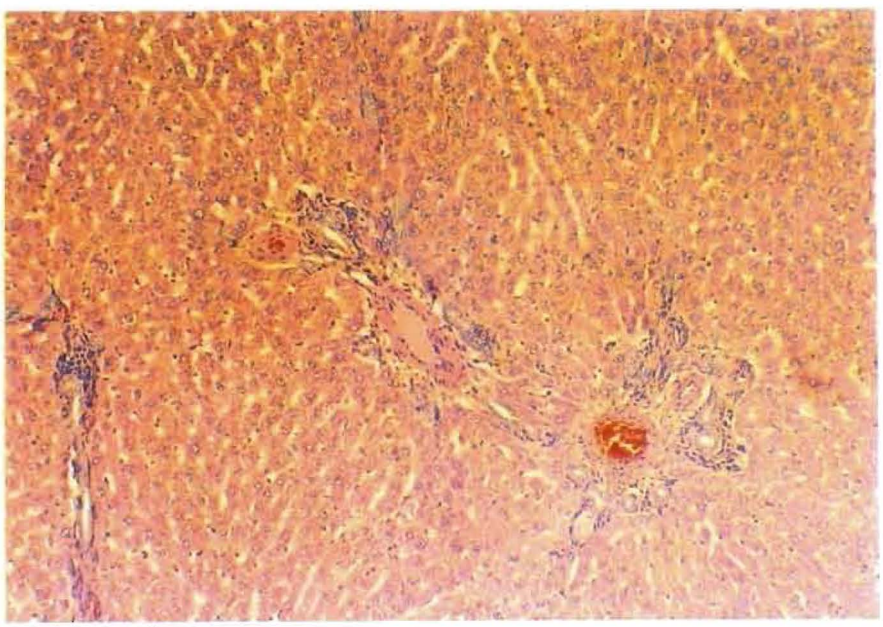

Fig. 6. Bile duct proliferation observed in liver of rats injected for twenty weeks with a mixture of AFB1 + T-2 toxin. (X 120).

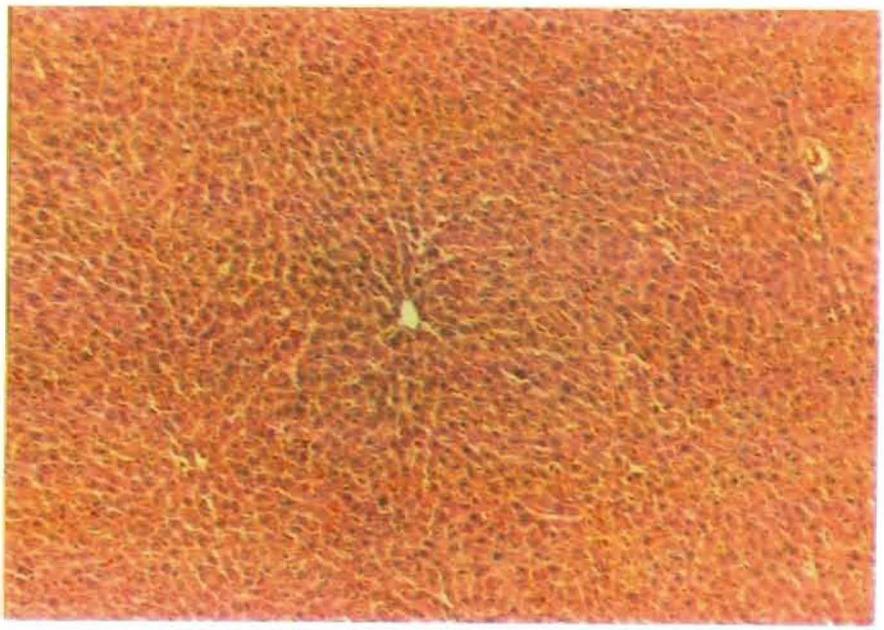

Fig. 7. Liver section from the rats of the control group. (X 120).

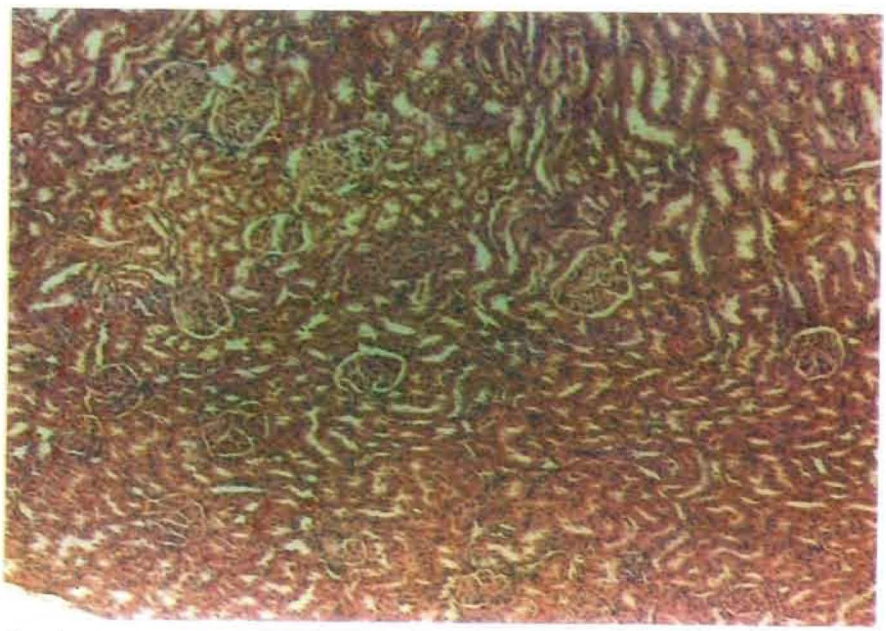

Fig. 8. Kidney section from the rats of the control group. (X 120).

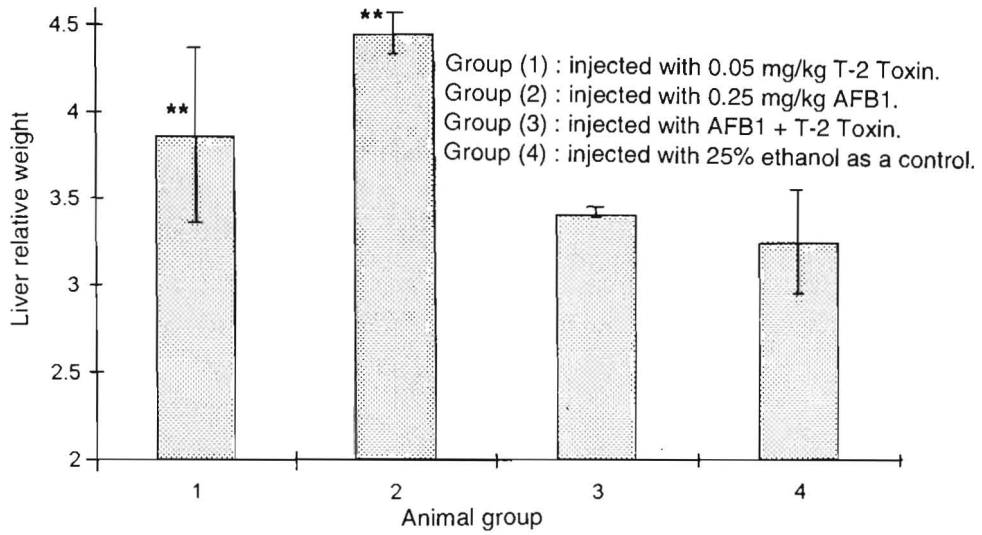

Fig. 9. Average liver relative weight of all rat groups of the study, twenty weeks after injection with the mycotoxins. Data are presented as mean ± SD (n = 7).
(**): Significantly different from the control ($p < 0.05$).

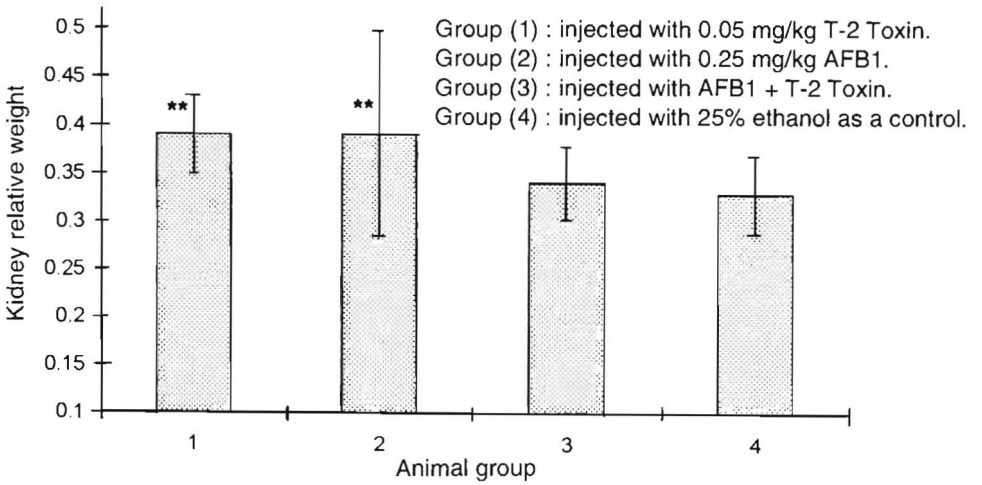

Fig. 10. Average kidney relative weight of all rat groups of the study, twenty weeks after injection with the mycotoxins. Data are presented as mean ± SD (n = 7).
(**): Significantly different from the control ($p < 0.05$).

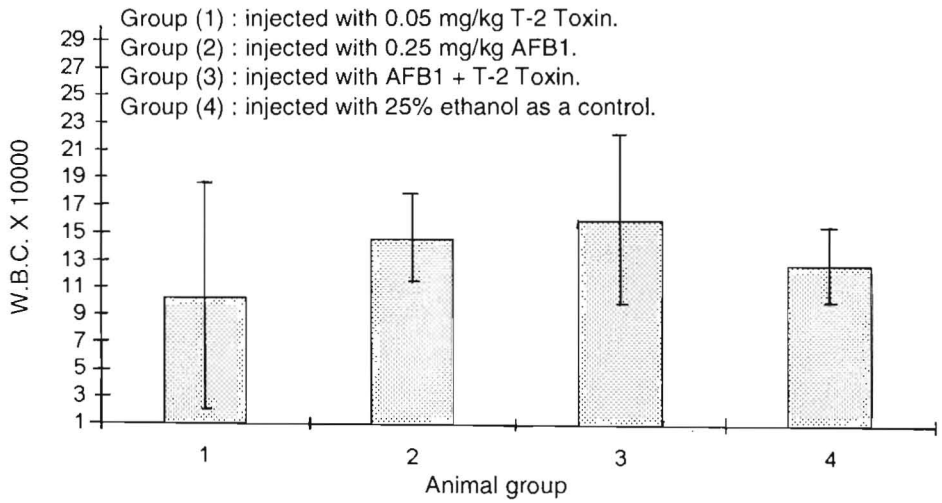

Fig. 11. Average white blood cell counts of all rat groups of the study, twenty weeks after injection with the mycotoxins. Data are presented as mean ± SD (n = 7).

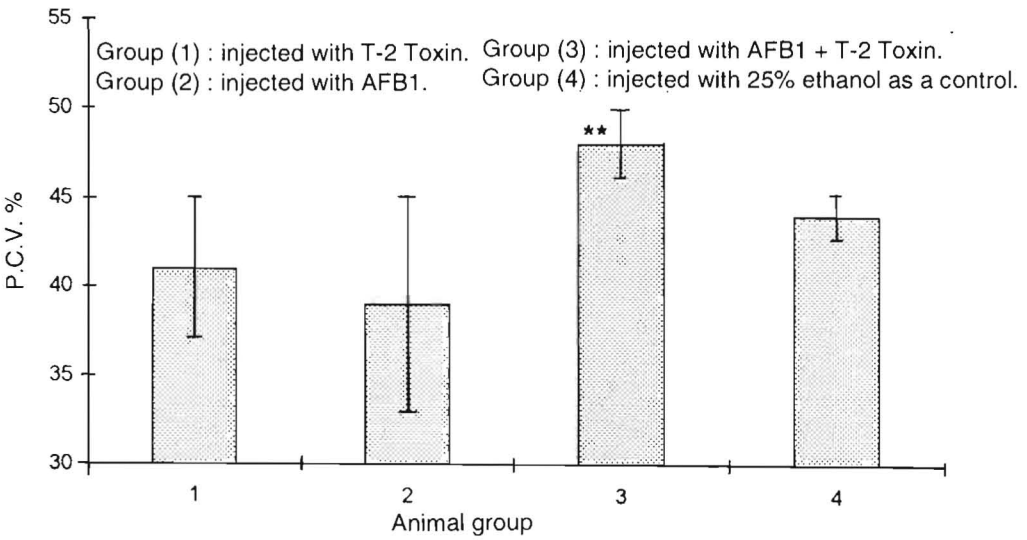

Fig. 12. Average packed cell volume (P.C.V) of all rat groups of the study, twenty weeks after injection with the mycotoxins. Data are presented as mean ± SD (n = 7).
(**): Significantly different from the control ($p < 0.05$).

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تأثير السموم الفطرية : أفلاتوكسين ب١ وسم ت-٢ ، على كبد وكلى الجرذ عند استعمالها منفردة أو مجتمعة

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تمت دراسة تأثيرات السموم الفطرية (أفلاتوكسين ب ١ ، وسم ت-٢) على كبد وكلى الجرذ بعد حقن هذان السمان منفردة أو مجتمعة .

لذلك تم اختيار ثمان وعشرون من ذكور الجرذان ، وقسّمت إلى أربع مجموعات ، حقنت المجموعة الأولى بالأفلاتوكسين ب ١ والمجموعة الثانية حقنت بسم ت-٢ والمجموعة الثالثة حقنت بمزيج من هذان السمان ، أما المجموعة الرابعة فقد استخدمت كشاهد للتجربة . وتم حقن جميع حيوانات الدراسة في تجويفها البطني مرتين كل أسبوع ولمدة عشرين اسبوعاً . وفي أثناء مدة التجربة ، تم تسجيل أوزان الجرذان مرة كل أسبوع . وفي نهاية مدة التجربة تم قتل جميع الحيوانات بعد أخذ عينات من الدم لدراسة مدى تأثير هذه السموم على عدد خلايا دمها البيضاء ، ونسبة حجم كريات الدم المتراصّة . وبعد ذلك تم تشريح الحيوانات وفصل الكبد والكلى وأي عضو آخر ظهرت عليه علامات مرضية . ثم تم تسجيل أوزان الكبد والكلى لمعرفة مدى تأثرها بالسموم الفطرية المستخدمة . وبعد ذلك تم تحضير أنسجة هذه الأعضاء لدراستها مجهرياً .

ولوحظ أن أوزان الجرذان التي حقنت بكل من الأفلاتوكسين ب ١ ، وبمزيج من الأفلاتوكسين ب ١ + سم ت-٢ كانت أكثر المجموعات تأثراً ، إذ

لوحظ هبوط معنوي في معدل أوزانها في منتصف مدة الدراسة ، إلا أنها قد استعادت أوزانها الطبيعية في النصف الثاني من زمن التجربة . فقد لوحظ أن مجموعات الجرذان التي حقنت بمزيج من الأفلاتوكسين وسم ت-٢ احتوت على أعلى قيمة معنوية لحجم كريات الدم المتراصّة .

كما لوحظ أن وزن الكبد نسبة إلى وزن الحيوان الكلي لدى مجموعة الجرذان التي حقنت بالأفلاتوكسين ب ١ كان لها أعلى قيمة معنوية تلاها مجموعة الجرذان التي حقنت بسم ت-٢ . لقد تبين من الدراسة ان مزيج السم الفطري ت-٢ مع أفلاتوكسين ب ١ كان له تأثير متكافل في جميع الحالات المدروسة ، ومن ضمنها الحالة المرضية النسيجية التي أصابت الكبد والكلى ؛ اذ ان أنسجة الكبد في مجموعة الجرذان التي حقنت بمزيج من الأفلاتوكسين ب ١ + السم الفطري ت-٢ ، كانت أكثر تأثراً من المجموعات التي حقنت بكل من هذين السمين منفردين .