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Individual and Combined Effects of Chronic Ochratoxin A and Zearalenone 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 ochratoxin A (OTA); a second group was injected with Zearalenone (ZEAR); a third group was injected with a mixture of the two mycotoxins and a fourth group was used as control. Injections were made twice a week, for twenty weeks. Animal weights were recorded on 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 studies.

Ochratoxin A, caused a significant ($P < 0.001$) drop in the average body weight of the rats after eight weeks of treatment and produced the lowest relative kidney weights. Rats treated with ZEAR had significantly ($P = 0.035$) low PCV readings. When ZEAR was combined with OTA it resulted in antagonistic effects in most of the parameters of the study.

The hispathological study confirmed the nephrotoxicity of OTA. One case of immunoblastic lymphoma was observed in this study. This observation suggests some relationship between mycotoxicosis and lymphoma.

Ochratoxin A is a fairly toxic substance, produced by several groups of *Aspergillus ochraceus;* the primary targets are the liver and the kidney (FAO 1982, and Albassam *et al.* 1987) and due to its accumulation in tissues it represents a possible source of danger to humans (Bohm 1992). This toxin also supresses the immune

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function (Kuiper and Scott 1989). Zearalenone is probably one of the most economically important mycotoxins produced by several *Fusarium* spp. and may contaminate grains in many countries (FAO 1982 and Juszkiewioz and Piskorska-Pliszcynska 1992). In Jordan, 11.3 % of the tested food samples were found to be contaminated with ZEAR (Dajani *et al.* 1990). Zearalenone leads to fertility disturbances by altering oestrogen production in experimental animals (Lopez *et al.* 1988). The clinical signs observed in animals when more than one mycotoxin is present in feed are complex and diverse (Huff and Dorr 1981).

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 and kept in the animal room in wire mesh cages, with plastic bottoms. The animals were weighed at weekly intervals for twenty weeks.

2. Mycotoxins and treatments: The two types of mycotoxins used in this study, OTA and ZEAR were dissolved in 25% ethanol. The four groups of rats were intraperitoneally injected with the mycotoxins, twice weekly for 20 weeks. The doses were administered in a volume of 1 ml/kg body weight as follows:

Group 1 received 0.20 mg/kg OTA; group 2 received 0.10 mg/kg ZEAR; group 3 received a mixture (1:1) of the OTA and ZEAR dosages used above; group 4 received 1 ml/kg 25% ethanol (the control group).

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

4. Postmortem examination: Postmortem examinations were carried out on rats immediately after killing. Kidneys and liver of all the rats were removed, weighed, and fixed in 10% formaldehyde. Tissue slices (4 µm. thick) were prepared from the liver and both kidneys as well as from other body organs, whenever abnormal gross pathology was noted in any of the animals.

Histological sections were prepared and the histopathological changes were recorded and compared with those 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 (OTA only): The average body weight of the rats of this group showed a significant ($P = 0.001$) decrease at the ninth weeks of treatment (Fig. 1); one rat of this group showed a large abdominal swelling, and ascites, with enlargement of its mesenteric lymph nodes (Fig. 2). At necropsy, the kidneys of most of the rats were atrophic and surrounded by a large amount of fatty tissue. The histopathological study of the livers of this group showed mild degenerative and regenerative activity and mild fatty changes. The pathological alterations of the kidney sections consisted of moderate dilatation of proximal convoluted tubules (Fig. 3). Four rats showed mesangial matrix expansion with hypercellularity along with swelling of the glomeruli (Fig. 4). In three rats, the kidneys showed proteinaceous casts in renal tubules. The enlarged lymph nodes contained large aggregations of monotonous large immunoblastic proliferation with prominent mitoses, this change was diagnosed as immunoblastic lymphoma (Fig. 5).

2. Group 2 (ZEAR only): Rats of this group did not show a significant change in body weight (Fig. 1). Two rats showed abdominal masses and three rats developed testicular abscesses, during the last month of the experiment. Microscopic study of the liver sections of this group revealed mild bile duct proliferation with mild centrilobular degeneration. Kidney sections revealed mild degeneration of the tubular epithelium and the glomeruli showed a mild swelling and dilatation of Bowman's capsule.

3. Group 3 (OTA and ZEAR): Rats of this group showed changes in body weight similar to these observed in controls (Fig. 1.). Renal injury in the form of tubular degeneration was observed in most of the kidneys. Swelling and dilatation of Bowman's capsule and degeneration of tubular epithelium were also observed (Fig. 6).

4. Group 4 (25% ethanol only): This group did not show any abnormal changes during the study period or at necropsy; they continued to be healthy and active. The microscopic features of the liver and kidney sections were considered normal.

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

5.1. Liver relative weight: The results indicated that relative liver weight of rats, injected with either OTA or ZEAR did not show any significant changes as compared to the controls, while injection with the combination of both mycotoxins caused mild decrease (Fig. 7).

5.2. Kidney relative weight: As given in Fig. (8) rats kidneys, injected with OTA were significantly (P = 0.003) lower than those of the control group.

6. The effect of mycotoxins on rats white blood cells counts (WBC): No significant differences in white blood cell count were observed among the four groups (Fig. 9).

7. The effect of mycotoxins on rats packed blood cell volume (PCV): Rats injected with a mixture of OTA and ZEAR exhibited a significant increase (P = 0.015) in the PCV readings, while rats injected with. ZEAR alone exhibited a significant decrease (P = 0.039) in it (Fig. 10).

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

Parameter	OTA	ZEAR	OTA & ZEAR
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 mycotoxins; OTA, and ZEAR, were studied on the liver and kidney of male albino rats. 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 elimination of the waste products in the body, liver and kidney were selected to be the target organs in this study.

The choice of these mycotoxins was due to their occurrence in feed and foodstuffs in Jordan and other countries (FAO 1982 and Dajani *et al.* 1990) and due to the fact that these toxins were known to induce pathological and metabolic disorders in many animal species.

Moreover, due to the fact that the ingestion of small amounts of toxins, over a long period, may be of more significance than acute intoxication (Kuiper *et al.* 1989 and Bohm 1992), this research was conducted to study the chronic effects of the mycotoxins on experimental animals. Accordingly, four groups of rats were intraperitoneally injected with these mycotoxins, to determine their individual and combined effects on rat livers and kidneys.

Treatment of the experimental animals with OTA alone caused a significant ($P = 0.0027$) drop in the animals' body weight. On the other hand, treatment with ZEAR alone did not cause such an effect (Lopez *et al.* 1988). Zearalenone did not inflect any serious effects on the rats' liver and kidney, but it may affect other tissues, such as those of the testes as reported by Lopez *et al.* (1988). However, when the animals were treated with a mixture of both toxins, their weight was not affected. Aside from this, treatment with OTA alone caused a significant drop in kidney weight, while treatment with ZEAR alone did not cause any noticeable effect. Yet, treatment with a mixture of both toxins did not cause any effect on kidney weight. Such results, suggest ZEAR, when mixed with OTA, antagonizes the toxic effect of OTA in the experimental animals.

The histopathological studies of the liver and kidney of the rats, treated with a mixture of OTA and ZEAR, revealed nearly the same effects caused by ZEAR alone. From these observations, it can be suggested that ZEAR may be used in low concentrations as an anti-mycotoxin.

In all mycotoxin treatments, the PCV readings were significantly higher ($P = 0.001$) in rats treated with a mixture of OTA + ZEAR ($P = 0.015$), while rats injected with ZEAR alone exhibited a significant ($P = 0.039$) decrease in it's (PCV) readings.

The histopathological changes noted in the sections takens from the enlarged mesenteric lymph nodes were diagnosed as plasma cell infiltration with lymphoid hyperplasia (Fig. 5). An unexpected and probably an important observation in this study, was the development of one case of lymphoma in rats treated with OTA; a similar case was observed in rats injected with a mixture of AFB1 and ZEAR and in another case with rats injected with a mixture of AFB1 and T-2 Toxin (Halabi *et al.* 1997); a literature survey did not reveal such finding and hence this observation may suggest some relationship between mycotoxicosis and lymphoma and calls for further investigations.

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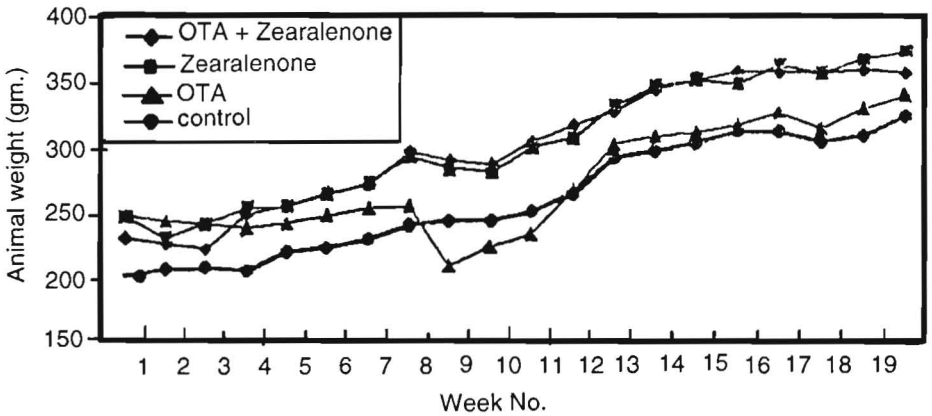

Fig. 1. Average body weight of the four groups of rats treated with mycotoxins at weekly intervals for twenty weeks. Data presented as the mean (N = 7).

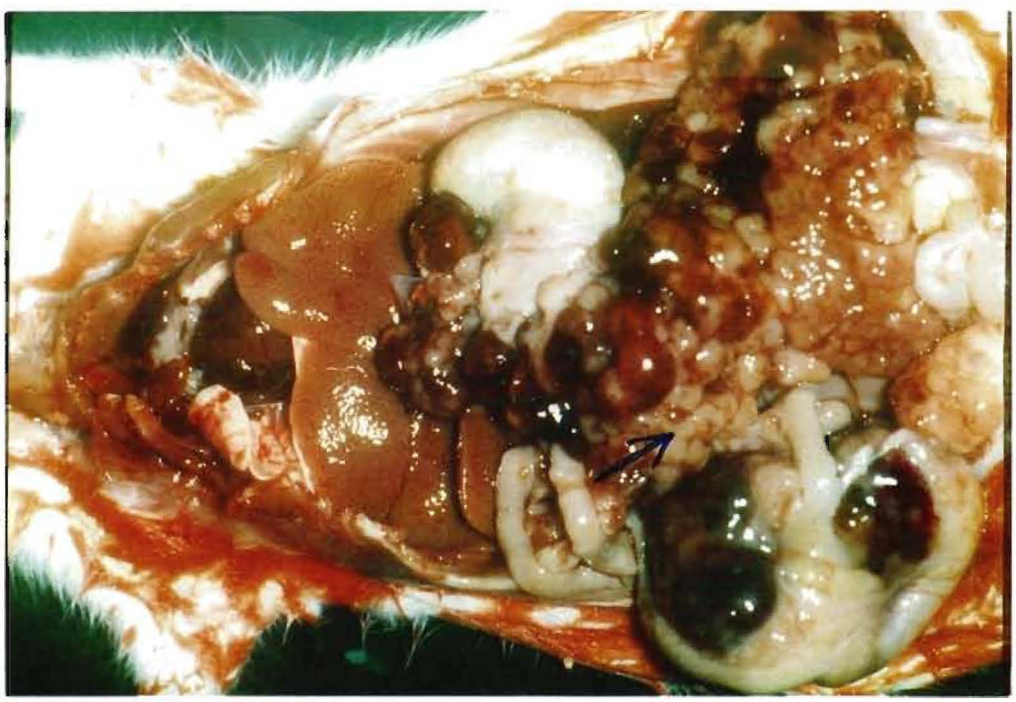

Fig. 2. Enlarged mesenteric lymph nodes (arrow) in one of the rats of group (1) which developed lymphoma after twenty weeks of injection with OTA.

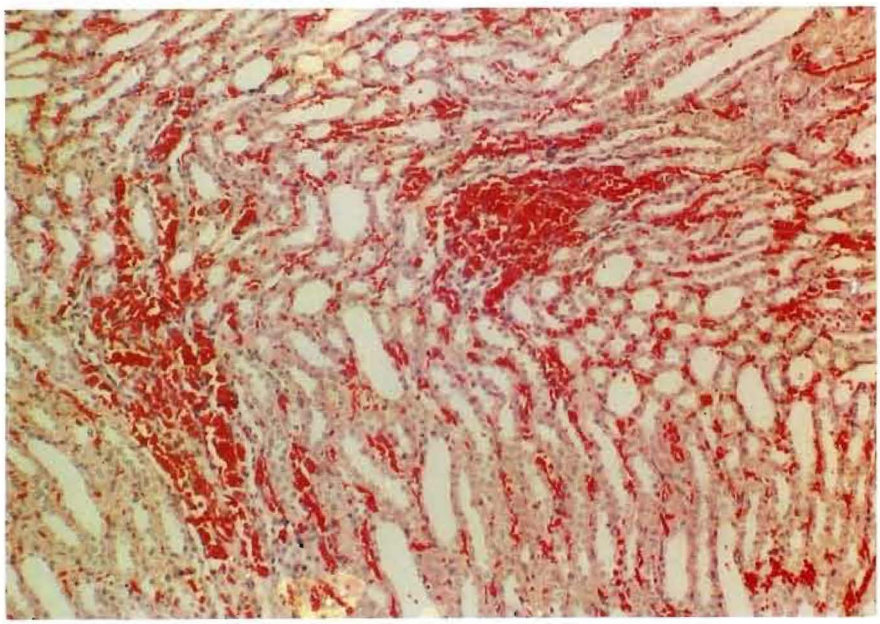

Fig. 3. Dilatation of renal tubules in the kidneys of rats injected (i.p) with OTA for twenty weeks. (X240).

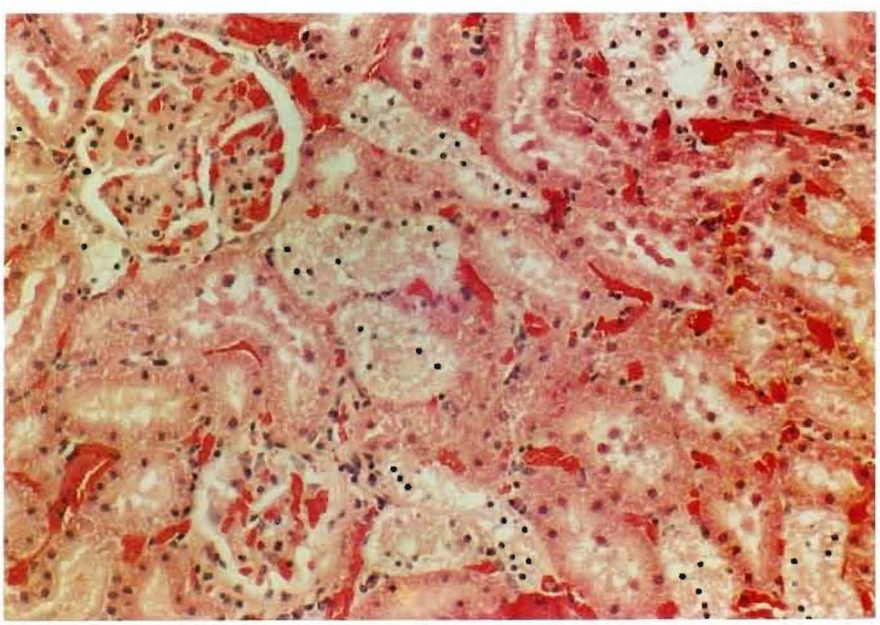

Fig. 4. Degeneration and tubular cell necrosis, and glomerular swelling with mesangial expansion, in kidneys of rats after twenty weeks of injection (i.p.) with OTA.(X480).

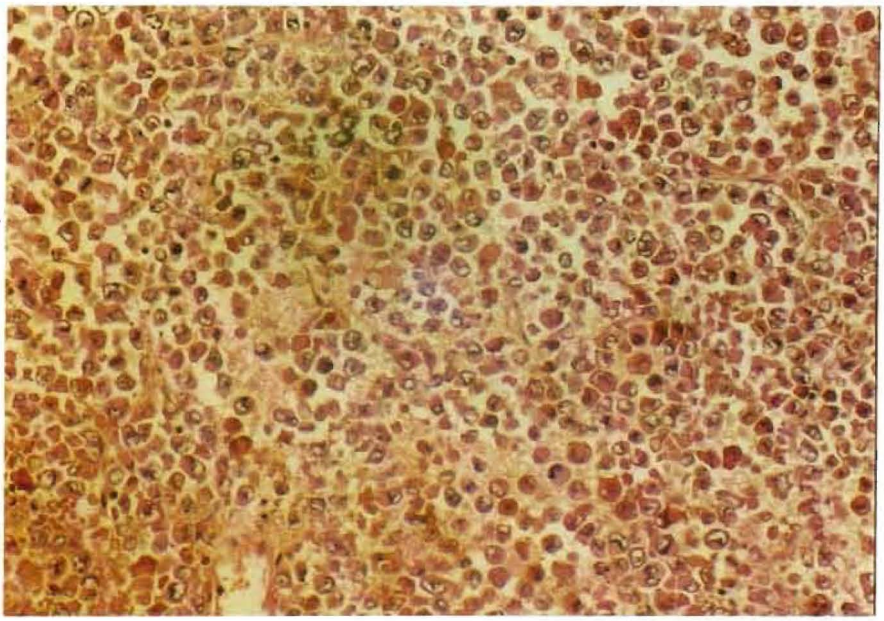

Fig. 5. Section from the lymph node of the rat which diagnosed as immunoblastic lymphoma, after twenty weeks of injection (i.p) with OTA. (X480).

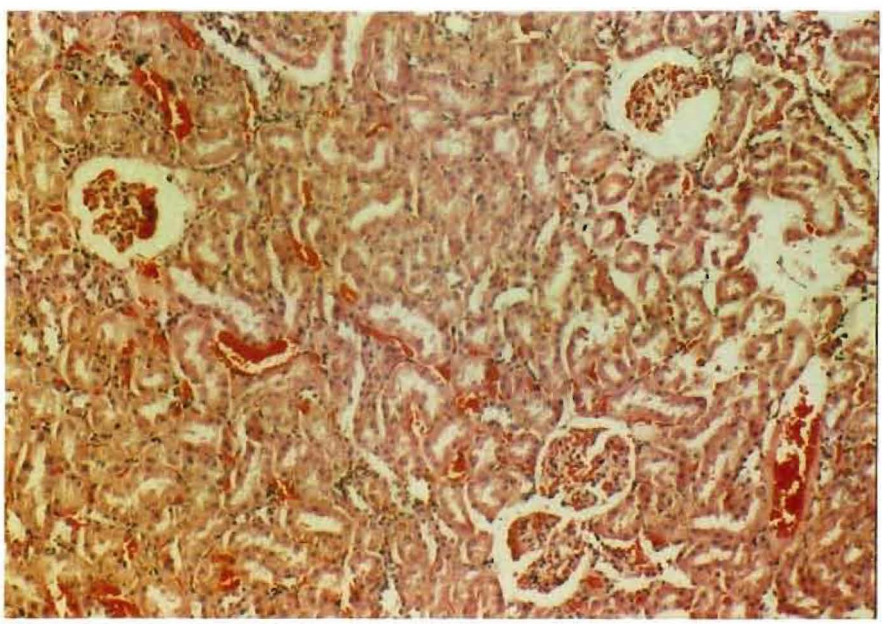

Fig. 6. Swelling and dilatation of Bowman's capsule and degeneration of tubular epithelium, in the kidneys of rats injected (i.p) for twenty weeks with a mixture (1:1) of OTA + ZEAR. (X240).

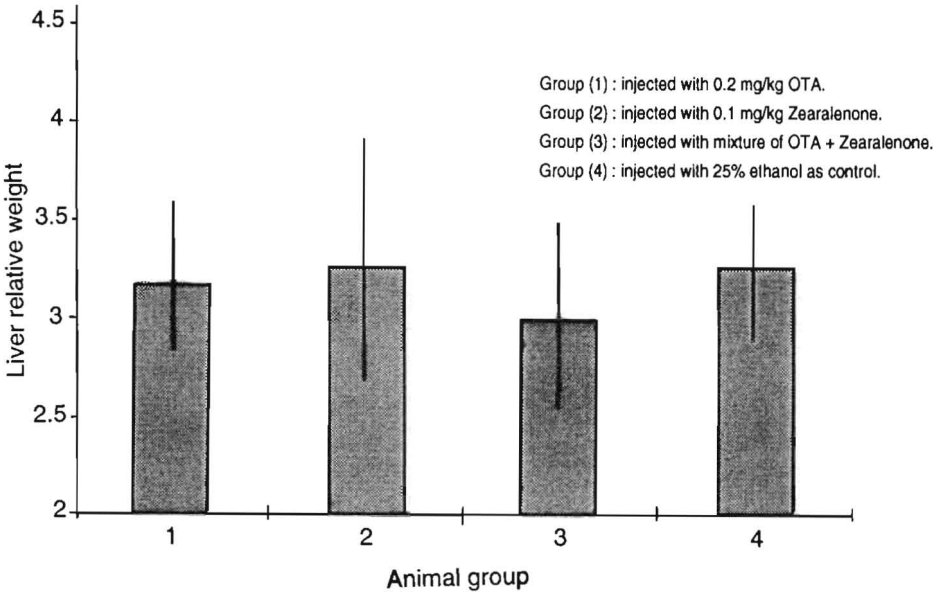

Fig. 7. Average liver relative weight of all rat groups of the study, twenty weeks after injection with the mycotoxins. Data are presented as mean ± SD (N = 7).

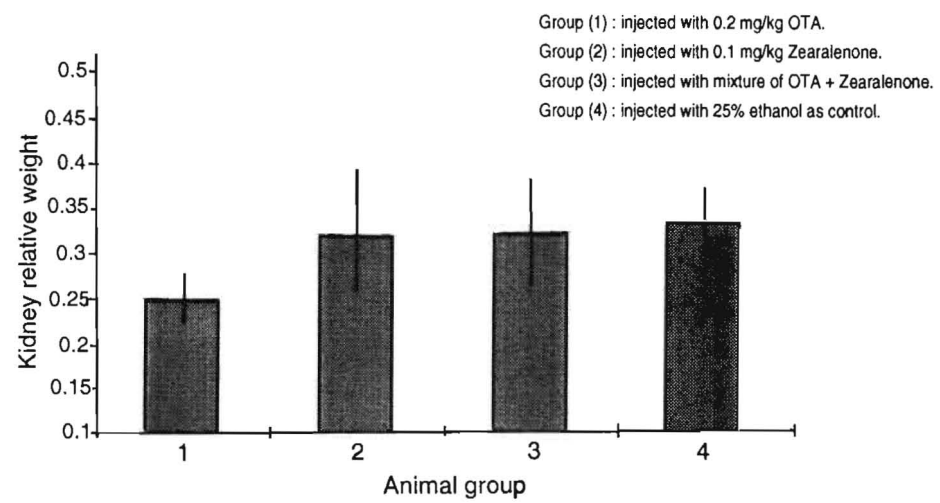

Fig. 8. Average kidney relative weight of all rat groups of the study, twenty weeks after injection with the mycotoxins. Data are presented as mean ± SD (N = 7).
(**) Significantly different from the control ($P < 0.05$).

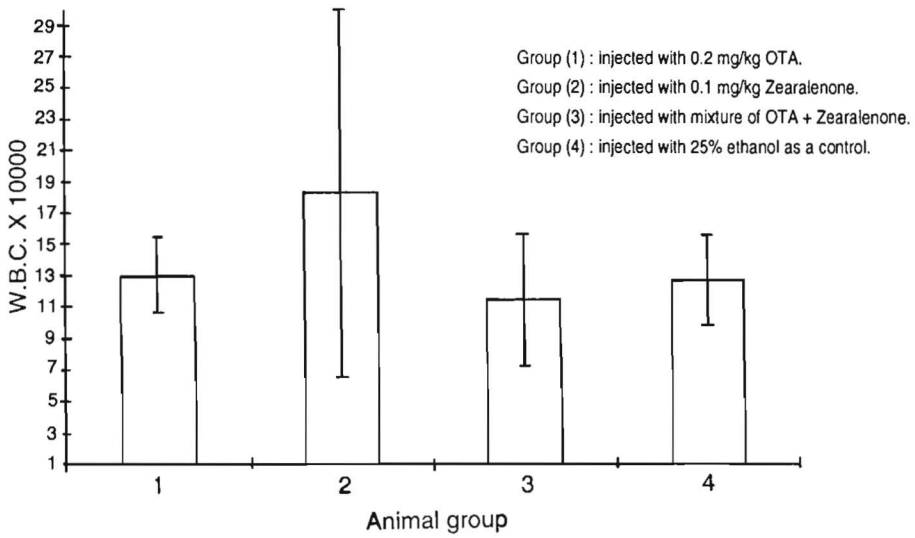

Fig. 9. Average white blood cell counts of all rat groups of the study, twenty weeks after injection with the mycotoxins. Data presented as mean ± SD (N = 7).

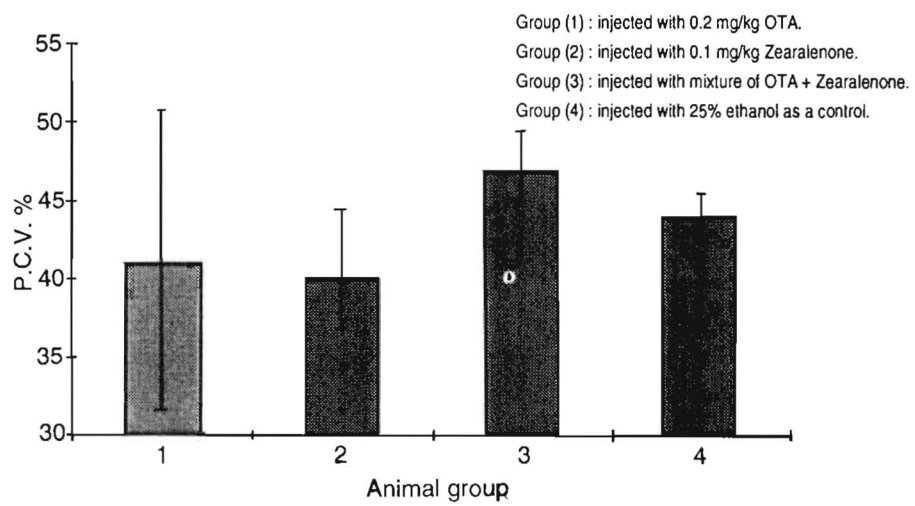

Fig. 10. Average packed cell volume (PCV) of all rat groups of the study, twenty weeks after injection with the mycotoxins. Data are presented as mean ± SD (N = 7).
(**) Significantly different from the control ($P < 0.05$).

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تأثير السموم الفطرية : أو كراتوكسين أو الزيرالينون على كبد وكلى الجرذ عند استعمالها منفردة أو مجتمعه

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أجري هذا البحث على تأثير السموم الفطرية (أوكراتوكسين (أ) والزيرالينون) على كبد و كلى الجرذ عند حقنها منفردة أو مجتمعة ، حيث تم اختيار ثمان وعشرون من ذكور الجرذان ، قسمت إلى أربعة مجموعات . حقنت المجموعة الأولى بالاكراتوكسين (أ) والمجموعة الثانية بالزيرالينون والمجموعة الثالثة بمزيج من هذين السمين ، أما المجموعة الرابعة فقد أستخدمت كشاهد للتجربة . وقد حقنت جميع الحيوانات في تجويفها البطني مرتين أسبوعياً ولمدة عشرون أسبوعاً . وفي أثناء مدة التجربة تم تسجيل أوزان الجرذان مرة كل أسبوع . في نهاية الدراسة تم قتل جميع الحيوانات بعد أخذ عينات من الدم لدراسة مدى تأثير هذين السمَّين على عدد خلايا دمها البيضاء ونسبة حجم كريات الدم المتراصة . بعد ذلك تم تشريح الحيوانات وفصل الكبد والكلى وأي عضو آخر ظهرت عليه علامات مرضية وتم تسجيل أوزان الكبد والكلى وبعد ذلك تم تحضير أنسجة هذه الأعضاء لدراستها مجهرياً .

لقد لوحظ أن أوزان الجرذان التي حقنت بالاكراتوكسين (أ) كانت أكثر تأثراً من المجموعات الأخرى ، اذ لوحظ هبوطاً واضحاً في معدل أوزانها في منتصف مدة الدراسة الا أنها استعادت أوزانها الطبيعية في النصف الثاني من مدة الدراسة ، كما لوحظ أيضاً أن الأوزان النسبية لكلى الجرذان المحقونة

بالاكـراتوكسين (أ) كـانت أقل ، كـمـا بينت هذه الدراسـة تفاعل تضـادي بين السمَّين الزيراليـنون والاكراتوكسين (أ) لدى حقنها معاً في حيوانات التـجربه . وأثبتت نتائج هذه الدراسة ان السمية اكراتوكسين (أ) له تأثير حاد على كلى الجرذ لدى حقنه منفرداً ولكن هذه السمه قد خفت حدتها لدى حقن الجرذان بكلا السمَّين .

اضـافة إلى ذلك فقـد ظهـر في هذه الدراسة حـالة ورم سـرطاني أصـابت الخلايا اللمفاوية لجرذ حقن بالاكراتوكسين (أ) ، مما يحتمل وجـود علاقة بين أورام الغدد اللمفاوية والتسمم الفطري .