Pathological Effects of Crud Trichothecenes on Swiss Mice

التأثيرات الأمراضية لسم الترايكوثيسين الخام على الفئران المختبرية

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Abstract

Trichothecenes are natural secondary metabolites causing economic losses and health hazard to human and farm animals which are produced by several species of *Fusarium* and some other genera on different agricultural commodities. Study on trichothecenes mycotoxicosis revealed morphological, biochemical, and histopathological changes. After intraperitoneal injection of the toxin in male mice with different concentration for 35 days shows marked increase in body weight, dyspanea, shivering, bristling up of hair, hair falling, anomalies of eyes, and irritation around neck, also abdominal hemorrhage and clot accumulation in abdomen. In addition to inclusion (retention) cyst forms on liver. The biochemical studies on liver function by measuring GPT and GOT enzymes level have been done. An increase level of these enzymes in treated animal in comparison with control animal which indicating abnormal function of liver observed. The histopathological study on sections from the liver of treated animal with trichothecenes revealed many alterations in liver which includes congestion, kupffer cells hyperplasia, dilated sinusoids and mononuclear cells infiltration around the portal area.

المستخلص

تعد الترايكوثيسينات من النواتج الأيضية الطبيعية الثانوية التي تسبب خسائر اقتصادية و خطر صحي على الانسان و الحيوان و التي تنتج من عدة انواع من فطر الـ *Fusarium* وبعض الاجناس الاخرى حيث تسبب كذلك تلفا في المحاصيل الزراعية , أظهرت دراسة التسمم بالترايكوثيسينات تغيرات مظهريه , كيميائية حيوية و نسيجية . تم حقن الفئران المختبرية داخل الغشاء البريتوني بتراكيز مختلفة من سم الترايكوثيسين الخام لمدة 35 يوم , أظهرت زيادة ملحوظة في وزن الجسم و صعوبة في التنفس و ارتعاش و انتصاب الشعر و سقوطه و جحوض العين و تهيج حول الرقبه و نزف في المنطقة البطنية و تجمع الجلطات فيها بالاضافة الى تكون أكياس فوق كبد الفئران المعاملة بالسم . اظهرت الدراسة الكيميائية الحيوية للكبد من قياس مستوى انزيمات GPT وGOT زيادة في مستوى الانزيمات بالمقارنة مع حيوانات السيطرة و اما الدراسة النسيجية لمقاطع من كبد الفئران المختبرية المعاملة بسم الترايكوثيسين , اظهرت العديد من التغيرات و التي تتضمن الاحتقان و زيادة غير طبيعية لخلايا kupffer و توسع المنحنيات الجيبية للكبد وزيادة عدد الخلايا الاحادية النواة حول المنطقة البوابية .

Introduction

Mycotoxins are important fungal secondary metabolites with low molecular weight; they are representing a potential threat to human and animal. Mycotoxins present a challenge for scientists working on a wide range of disciplines such as microbiology, biochemistry,

structural chemistry, toxicology, pharmacology and genetics. Trichothecenes are about 148 type of closely related mycotoxins which are widely distributed in nature such as T-2 toxin, Diacetoxyscirpenol (DAS), Deoxynivalenol(DON) and HT-2 toxin. Trichothecenes are group of metabolites produced by species of the genus *Fusarium*, in addition to other genera including *Mythecium, Trichothecium, Verticimonosporium, Cefalosporium, Trichoderma, Gibberella, and Stachybotrys* [1].

The fate of Trichothecenes differs according to type of toxin and the animal receiving it. Generally muscle, liver and kidney much likely to be the target organs, for example, in pigs administered with HT-2 toxin, the percentage of administered radioactivity showed that muscle, liver, bile and kidney were the most target organs [2].

Trichothecenes comes after aflatoxin in toxicity and it affects both man and animal.This mycotoxin affects cereal grains, farm animal which consume contaminated feed, also affects man causing diseases, such as Alimentary Toxic Aleukia and Skeletal affliction (Kashin-Back disease)[3]. T-2 toxin is considered as one of the most toxic and common toxin in trichothecenes which is used in biochemical warfare and implicated in "Yellow Rain" used in Southeast Asia [4].

Many diseases reported after ingestion of moldy feed, these diseases characterized by feed refusal, depression, diarrhea, vomiting, and hemorrhage in intestine and muscles which lead to death of animals [5].

Materials and Methodes

1-Isolation and Identification of *Fusarium oxysporum*

The fungi isolated according to [6] about 30 randomly selected corn seeds were surface sterilized then seeds were cultured on Potato Dextrose Agar (PDA), 2 seeds for each plate then incubated at 25ºC for 7 days, then the grown *Fusarium* (Which was morphologically identified) purified by new subculturing (each one in single plate of Potato Sucrose Agar (PSA)) this purified isolates were used for identification.

Pure isolates were identified by direct examination with light microscope on glass slide and according to taxonomic system of Snyder and Hansen [7, 8].

2-Production, Extraction and Identification of Trichothecenes

To produce trichothecenes, spore suspension prepared according to [9], PDA inoculated with spore suspension(8.1×10^{6} spore\ml) and incubated at 25ºC for 1 week and at 27 ºC for 1 week and then at 8 ºC for 1 week. And according to [10], toxin extracted and kept in small vials in dry and cold place. By using TLC method trichothecenes have been detected and identified in comparison with standard T-2 toxin using benzene: acetone in 3:2 ratios as developing system for separation of trichothecenes and examined under U.V. light at 366(nm) Wave length (W.L.) with determination of Rf value [11], the amount of toxin was estimated in comparison with standard toxin using scanning densitometer [12].

3-Confirmatory test

According to [13] confirmatory test of trichothecene was done by using aluminum chloride solution cooled and examined under U.V. light at 366-wave length, the Rf value was estimated in comparison with standard toxin.

4-Laboratory animals

Mature Swiss mice, males and weighted (20.9-22.4) gr. were used in this study, those mice were divided into 4 groups (6 mice for each group) which injected intra peritoneal (IP) as following:-

Group I: Mice were treated daily (IP) with 0.1 ml of 10% chloroform for 35 days (5 weeks) and considered as control for low dose group.

Group II: Mice were treated daily (IP) with 0.1 ml of toxin (0.75mg/ml) for 35 days (5 weeks) and considered as low dose group.

Group III: Mice were treated daily (IP) with 0.3 ml of 10% chloroform for 35 days (5 weeks) and considered as a control for high dose group.

Group IV: Mice were treated daily (IP) with 0.3 ml of toxin (0.75mg/ml) for 35 days (5 weeks) and considered as high dose group.

After treatment blood sample were taken by heart puncture also kidneys were taken and stored in 10% formalin.

5-Biochemical study

The glutamic transaminase enzymes including serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT), measured by using the GOT and GPT- Kits.

6-Histopathological study

Samples were fixed in 10% formalin for 24 hr to be prepared for histopathological sectioning [14].

Resultes and Discussion

1-Isolation and Identification of *F. Oxysporum*

Maize has been selected as a natural source for isolation of *F. oxysporum*, when maize put on surface of PDA media gave a good result for isolation of *F. oxysporum* [7].

The average growth rate of cultures was about 5 ± 0.5 cm, the fungus is represented by white mycelium on the surface of the dish, but with purple to brownish tinge. Microconidia born on simple phialides arising laterally on hyphae or from short branched conidiophores. Generally microconidia abundant, cylindrical, straight and 1-2 septate.

Macroconidia are born on more branched conidiophores, they were thin walled, 3-5 septate and pointed at both ends, this results obtained according to [8].

2- Production and extraction of trichothecenes

The isolated fungi proved to be efficient producer for trichothecenes (750 ppb) in comparison with other isolates used for production of trichothecenes [15].

According to our experimental work, the method used was a suitable method for recovery of the toxin from the culture, which is characterized by clear red to pink fluorescence of trichothecene derivatives under U.V. light (366 nm) with Rf value 0.2 on silica gel plate after developing the chromatogram. The solvent system is efficient in separation the components of fungal culture extract.

3- Toxicity of crude trichothecenes

Marked increase in body weight was observed in treated mice when compared with the body weight of control animal, this increase sustended during the duration of treatment, these observations became slowly appeared in the last week, but in general they were dose dependent [16].

Body weight has significantly increased for high dose treated mice from 21.8 ± 0.36g to 28.2 ± 0.85g, and for low dose treated animals from 22.4 ± 0.37g to 26.9 ± 0.31g and that increase is significant according to ANOVA (<o.o5) as shown in Table (1).

Table (1): **Body weight of mice groups**:

Animal Groups	1 day	7 days	14 days	21 days	28 days	35 days
Group I	A 20.83 ± 0.41	A 21.46 ± 0.38	A 21.98 ± 0.35	A 22.63 ± 0.33	A 23.26 ± 0.3	A 23.95 ± 0.3
Group II	A 22.3 ± 0.3	B 23.18 ± 0.37	B 24.1 ± 0.4	B 25.15 ± 0.4	B 26.31 ± 0.37	B 27.21 ± 0.35
Group III	AB 21.25 ± 0.17	A 21.76 ± 0.19	A 22.43 ± 0.17	A 22.9 ± 0.13	A 23.56 ± 0.13	A 24.28 ± 0.12
Group IV	AB 21.85 ± 0.36	AB 22.62 ± 0.44	B 23.9 ± 0.5	B 25.9 ± 0.72	B 27.2 ± 0.73	B 28.21 ± 0.8

The increase in body weight of treated mice groups notably larger than in the control groups, this might be resulted from increase in water consumption and feed consumption, or might be attributed to enlargement of organs such as liver, kidney, spleen and pancreas due to mycotoxicosis [17].

Other signs observed for mycotoxicosis were loss of hair, shivering, dyspnea, redness around the neck and bristling of hair. Although of the aggressive behavior of mice, loss of activity observed at the last two weeks of treatment. All signs mentioned have referred to the action of toxin resulted from mycotoxicosis, similar morphological changes were observed in rat fed diet contaminated with aflatoxin B1 [18] which is attributed to the mycotoxicosis. Hemorrhage and clotting accumulation in the internal viscera are other signs of toxicosis; these signs resulted in giving abnormal dark red color for the internal viscera in comparison with control.

Crude toxin cause exudation case for blood vesicles, oozing of blood lead to hemorrhage and oxidation of hemoglobin in the internal viscera (stomach and intestine), also formation of clots and their accumulation gave the abdomen abnormal dark red color. This happened for most of the treated mice, but not all of them, this may be due to the difference in the animal behavior, built of body, and microanatomy between mice [19].

Inflammatory (retention) cyst was observed on liver of treated mice and those cysts occurred due to infection of liver or due to necrosis caused by toxicity [20].

4-Biochemical study

Generally trichothecenes affect the liver function, this effect can be seen in the evaluation of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzymes level in blood of treated mice, the effect is dose dependent Table (2). Treatment with the toxin induced hepatotoxicity by developing hepatic lesion which leads to this increase in enzymes level [21].

Similar results demonstrated by using aflatoxin B1 on chicken induced significant increase in GOT and GPT level causing them liver disfunctioning due to mycotoxicosis [22].

Table (2): Results of hematological testes:

	GPT IU/L	GOT IU/L
Group I	A	A
	110 ± 2.22	23 ± 1.76
Group II	B	B
	132 ± 6.14	35 ± 1.25
Group III	C	C
	195 ± 9.03	47 ± 1.58

Group I: control animals
Group II: low dose treated animals
Group III: high dose treated animals

5-Histopathological study (Light microscope examination)

Trichothecenes affects cell division, damage and death of cells or enhancement of the immune system and mechanical movement to control toxic materials and stop their separation or finding other means or ways to do so.

The hepatic cells of animals appeared with congestion and kupffer cells hyperplasia for low dose treated mice Figure (1) after 35 days of treatment with trichothecenes.

Mild mononuclear cells infiltration in the portal area, around the bile duct was noticed in addition to congestion, sinusoidal dilation and kupffer cells hyperplasia Figure (2, 3, 4) upon treatment with high dose.

Death of liver cells may explain the dilatation of sinusoid [23]. The mononuclear cells infiltration around the portal area can be explained in that those cells represent a defense mechanism against any inflammation and/or any infection with some toxic materials affecting the hepatic cells [24]. Similar effects were observed in liver of rats treated with aflatoxin B1 [18].

Kupffer cells hyperplasia means that increased numbers of macrophage cells appeared, this indicates that presence of an inflammation in hepatic cells, the hyperplasia act as a way to limit the inflammation that occurred because of the mycotoxin. Mycotoxicosis with other mycotoxins such as ochratoxin A cause also kupffer cells hyperplasia in rat liver [25].

Hepatotoxicity occurred when fuminsins administrated to rats, cause kupffer cell hyperplasia and inflammatory infiltrate [26]. So we can conclude from all the above results that trichothecenes like many other mycotoxins can affect significantly liver of

mice which considered as target organ and cause to several damages, toxicity on cell organelles, increase in GOT and GPT level, organ dysfunction, activation of the immune system and death of the animals, depending on the dose of the toxin and the time of exposure which is revealed by biochemical and microscopical examination.

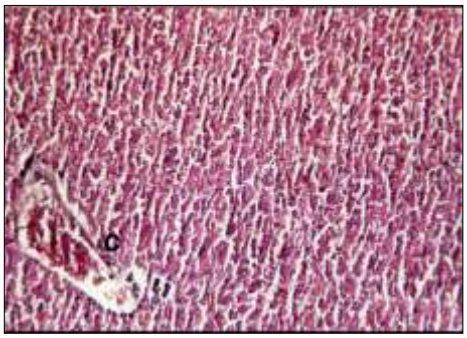

Fig (1): Liver section of animal treated with low dose of toxin showing congestion (c) and kupffur cells hyperplasia. Haematoxylin and

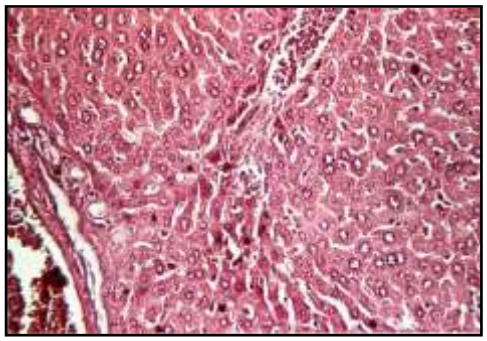

Fig (2): Liver section of animal treated with high dose of toxin showing congestion, sinusoidal dilation and kupffer cells hyperplasia. Haematoxylin and Eosin (40X).

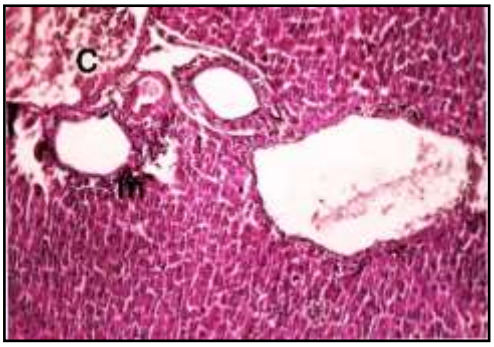

Fig (3) Liver section of mice treated with high dose of toxin showing mild infiltration of mononuclear cells in the portal area, around the bile duct (m). Haematoxylin and Eosin (10X).

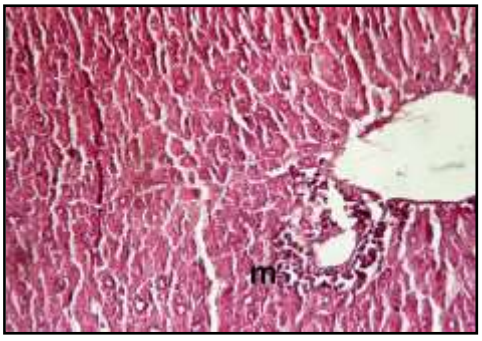

Fig (4) Liver section of animal treated with high dose revealed congestion (c) and inflammation cells infiltration in the portal area, around the bile duct (m). Haematoxylin and Eosin (10X).

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