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Determination of the Toxicity of Fumonisin B1 on Male Albino Mice

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Abstract

Fumonisin B1 is a toxic compound produced by *Fusarium verticillioides*. Liver and kidney are the major organs considered target to FB1 toxicity that is characterized by apoptotic, necrosis, and regeneration. Thirteen local isolates of *F. verticillioides* isolated form maize samples that collected from local markets and silos in Baghdad. Morphological identification are occurred and confirmed by PCR and their ability to produce FB1 was detected using ELISA techniques, Thirty six male albino mice were divided into six groups. Each group orally gavaged with different concentration of FB1. After 24hours, all treated mice were examined to determine the concentration which killed half of animals and was considered as LD50, the remaining groups were scarified after two weeks of oral administration. The LD50 of FB1 was 1800ppb which demonstrated to male mice after 24h. The significant elevations in liver enzymes (AST, ALT, and ALP) and kidney functions (Creatinine, Blood urea) have shown after orally gavaging of mice with FB1 at 800 and 1200 ppb concentrations in comparison with control group. The histopathological changes in the liver, kidney and spleen of treated mice with FB1 at 800 and 1200 ppb concentrations in comparison with control group, characterized by obvious increase in degenerative changes and apoptotic cells in comparison with control group.

Keywords: *Fusarium verticillioides*, FB1, histopathological changes, LD50

تحديد سمية الفيومنسين ب1 على ذكور الفئران البيضاء

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الخلاصة

فيومونسين ب1 مركب سام ينتج من قبل الفطر *Fusarium verticillioides*. يعد الكبد والكلى من الاعضاء المهمة التي تستهدف من قبل السم FB1 حيث يسبب التنخر والموت المبرمج والتجديد لهذه الاعضاء. ثلاثة عشر عزلة محلية من *F. verticillioides* عزلت من حبوب الذرة التي تم جمعها من الأسواق والمخازن المحلية في بغداد. و حددت الصفات المظهرية للعزلات وتم تأكيد تشخيصها بالطرق الجزيئية وقدرتها على انتاج FB1 باستخدام تقنية الاليزا، ست وثلاثين فأر ابيض ذكر قسمت الى ست مجاميع كل مجموعة جرعت فمويا بتراكيز مختلفة تراوحت بين 800 – 2000 جزء في البليون. بعد 24 ساعة تم

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تحديد نصف الجرعة القاتلة للسم (LD50) وكانت 1800 جزء في البليون، اما المجاميع الباقية ذات التراكيز الادنى من التركيز النصف القاتل قتلت بعد اسبوعين من التجريع وجمعت عينات الدم لغرض دراسة تاثيرات السم على انزيمات الكبد والكلى. اظهرت الدراسة وجود ارتفاع معنوي لانزيمات الكبد (AST، ALT و ALP) والكلى (يوريا الدم و الكراتينين) بعد تجريعها فمويا بالسم FB1 عند التركيزين 800 و1200جزء في البليون مقارنة مع مجاميع السيطرة. وظهرت تغيرات نسيجية في الكبد والكلى والطحال للمجاميع المجرعة بالسم عند التركيزين 800 و1200 مقارنة مع مجموعة السيطرة، تميزت بزيادة واضحة في التغيرات التنكسية والموت المبرمج للخلايا

Introduction

Fumonisins are toxic compounds formed by *Fusarium verticillioides* [1]. The most abundant and toxic fumonisin is Fumonisin B1 (FB1), representing about 70% of the total food and feed contamination [2]. Ingestion of FB1 causes species-specific target-organ toxicity such as: neurotoxic, nephrotoxic, hepatotoxic, teratogenic and pulmonary effects [3]. Studies have found that short term exposure to FB1 causes hepatotoxicity, whereas long term of FB1 leads to fibrous and chronic hepatitis that can ultimately result in liver cirrus and even sometimes in hepatic carcinoma. [4,5]. A recent study found that FB1 can cause histopathological changes in gastric gland cells like apoptosis and necrosis, leading to inflammation and infiltration of inflammatory cells such as lymphocytes which were observed in gastric gland parenchyma [6].

In Iraq, maize is an important grains and is produced annually in large quantities to be used in food industry, therefore this study aimed to determination LD50 and histopathological effect of FB1 on liver, kidney and spleen tissues and measure the enzymes in affected liver and kidney functions in experimental mice.

Methods

Isolation of *F. verticillioides*

Thirteen local isolates of *F. verticillioides* isolated form maize samples that collected from local markets and silos in Baghdad. Identification based on colony morphology and microscopic appearance on PDA, SNA and CLA according to the fungal keys of Leslie & summerell [7] and confirmed by species specific PCR Mule et al. [8].

Screening of *F. verticilioides* isolates for FB1 production:

FB1 production on patty maize medium was achieved according to Vismer *et al.* [9].

Partial Purification of FB1

The toxin purification was occurred according to [10], the concentration of toxin was measured by ELISA according to manufacture instruction of Biooscientific/ USA.

Determination the Median Lethal Dose of FB1

Thirty six male Swiss albino mice (4 weeks old, 24±2 gram weight) were divided into six groups. Each group orally gavaged with different concentration of FB1 as follows: 2000ppb, 1800ppb, 1600ppb, 1200ppb, 800ppb and control group (untreated group). After 24hours, all treated mice were examined to determine the concentration which killed half of animals and was considered as median lethal dose (LD50) [11]. The remaining groups were scarified after two weeks of oral administration.

Blood collection

The blood samples were collected at the end of experiment from remaining groups. About 0.5-1ml of blood was collected directly from heart through cardiac puncture. Blood was collected in a sterile test tube without anticoagulant left at room temperature, then centrifuged at 3000 rpm for fifteen minutes to obtain a suitable amount of serum, then kept in deep freeze -20ºC for biochemical examination included: AST, ALT, ALP, Creatinine and Blood urea.

Histopathological study

Fresh samples of liver, kidney and spleen of sacrificed mice were fixed in 10% formalin and histological preparations were preformed according to Luna [12].

Statistical analysis

All analysis was performed using the statistical package (SPSS) version thirteen. The data were expressed as mean, standard deviation, percentage. ANOVA was used to analyze repeated measurement.

Results

Determination of LD50 for male mice treated with FB1

LD50 of FB1 was detected by determinating the dose that caused 50% of death in laboratory animals. When oral gavaging of FB1toxin to mice, death occurred at 1800 ppb, and no death was observed in male mice in concentrations 800ppb and 1200ppb as shown in Table -1, therefore, 800 ppb and 1200 ppb used for studying liver enzymes (ALT, AST, ALP), kidney functions and histopathological effects.

Effect of FB1 on Liver Enzymes and Renal Functions of Male Mice

Table -2 showed the effect of FB1 on liver enzymes (ALT, AST and ALP) in mice. The results revealed a significant difference ($p \leq 0.05$) in liver enzymes elevation of both 800 ppb and 1200ppb concentrations when compared with control group (untreated), also significant difference between ($p \leq 0.05$) 800 ppb and 1200ppb concentrations for AST and ALP enzymes. However, no significant difference was seen between both concentrations for ALT enzyme.

Table -3 showed the effect of FB1 on kidney functions (Creatinine and blood urea). The results revealed that mice treated with FB1 at 800ppb and 1200 ppb concentrations caused creatinine elevation. There is a significant difference ($p \leq 0.05$) between both concentrations and control group. The results also revealed a significant difference in blood urea elevation of 800 ppb and 1200ppb concentration when compared with control group (untreated), but there was no significant difference ($p \leq 0.05$) between both concentrations.

The Histopathological Effect of FB1 on Male Mice

1: Effect on Spleen

Spleen section of control group showed normal structure appearance, contains normal white and red pulb as shown in Figure-1, in comparison, mice treated with FB1 at 800 ppb revealed widening of white pulp with degenerate of parenchymial tissue as shown in Figure-2.While at 1200 ppb concentration, the spleen showing follicular lymphoid hyperplasia and widening of white pulp with reduction of red pulp as shown in Figure-3.

2: Effect on Liver

In liver section of control group showing normal structure appearance of central vein and hepatocyte cells Figure-4, in comparison, mice treated with FB1 at 800 ppb revealed comment focal necrosis as shown in Figure-5 , while at 1200 ppb concentration of FB1 revealed mild mononuclear accumulation and degenerated cells as shown in Figure -6 .

3: Effect on Kidney

In kidney section of control group showed normal structure appearance which consists of glomeruli and renal tubules Figure-7 , in comparison, mice treated with 800 ppb concentration of FB1 revealed degenerative changes of renal epithelial tubular cells as shown in Figure -8 . While at 1200 ppb concentration, showing sever degenerative changes of renal epithelial tubular cells Figure-9.

Discussion

The effect of single dose of FB1 on the remaining groups of mice that treated with doses 800 and 1200 ppb by gavage route, then animals were sacrificed after two weeks of the treatment. This study observed the effect of this toxin on tested liver enzymes and kidney functions. The results showed elevation of ALT, AST, ALP, creatinine and blood urea in comparison with control group, and this elevation was good indicator for occurring histopathological changes in liver and kidney such as a significant increase in degenerative changes and apoptotic cells. These findings are similar to results observed in other studies on mice . This indicates that FB1 is hepatoxic and nephrotoxic in mice. Similarly, several studies reported that increase in liver and kidney enzymes indicated severe liver and kidney damage. This effect may be related to FB1 biotransformation which gives rise to various metabolites, which may covalently bind to protein and to DNA, leading to enzymatic processes alteration, such as glyconeogenesis, kreb's cycle, or fatty acid synthesis [15 - 17].

Other studies reported that FB1 induced organs lesions in rats, rabbits and broiler chick which were characterized by cell loss (apoptosis and necrosis) and proliferation (mitosis), and due to an imbalance between cell loss and replacement develops, making favorable conditions for carcinogenesis [18-21].

The toxicity of FB1 is due to inhibition of ceramide synthase and increase expression of tumor necrosis factor alpha (TNF-α) that lead to increase apoptosis, necrosis and carcinogenesis in liver and kidney of experimental animals receiving oral or intraperitoneal doses of toxin, or animals exposed to FB1 contaminated feed [22- 24]. Due to these histopathological changes, FB1 can be responsible for

several diseases of human and animal including hepatotoxicity, nephrotoxicity, neurotoxicity, immune suppression or stimulation, developmental abnormalities, liver and kidney tumors [4,18, 24, 25]. Finally, mycotoxins have different effects (acute and chronic) on humans and animals depending on strains and susceptibility of an animal within species [26].

Table 1- Percentage of died mice after orally gavaging with FB1.

Groups	FB1 concentration ppb	No. of mice	No. of death after 24hr.	Percentage of death %
1	2000	6	5	83.3
2	1800	6	3	50
3	1600	6	1	16.7
4	1200	6	0	0
5	800	6	0	0

Table 2- Effect of FB1 on liver enzymes in male mice.

Parameters Groups	ALT ng/ml (Mean±SD)	AST ng/ml (Mean±SD)	ALP ng/ml (Mean±SD)
Control	1.611±0.115[a]	6.875±0.871 [a]	46.43±4.70 [a]
800 ppb	2.381±0.156[b]	13.125±1.139 [b]	72.14±5.44 [b]
1200 ppb	2.738 ±0.111[b]	15.595±0.784[c]	102.86± 4.91[c]
**Different letter within the same column are significantly different (P≤0.05).			

Table 3- Effect of FB1 on renal functions in male mice.

Parameters Groups	Creatinine nmol/ml (Mean±SD)	Blood Urea mg/dl (Mean±SD)
Control	3.4756± 0.327 [a]	22.746±6.742 [a]
800 ppb	6.5854± 0.580[b]	55.493±8.529 [b]
1200 ppb	8.9228±1.361[c]	58.310±7.714[b]
**Different letter within the same column are significantly different (P≤0.05)		

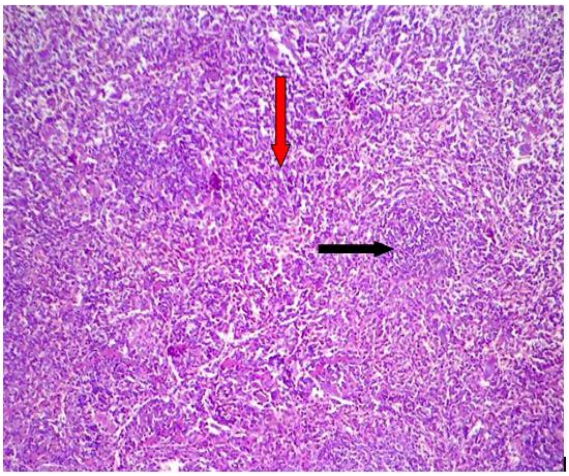

Figure 1- C.S in mouse spleen (untreated group) showing normal structure appearance which consist of white and red pulp. (X200; H&E). **Red arrow:** red pulp, **Black arrow:** white pulp.

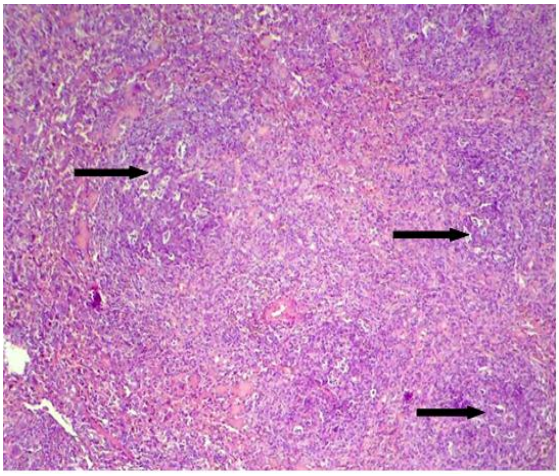

Figure 2- C.S in mouse spleen treated with 800 ppb of FB1 showing widening of white pulp with degenerate of parenchymial tissue (X200; H&E). **Black arrow:** follicular lymphoid hyperplasia.

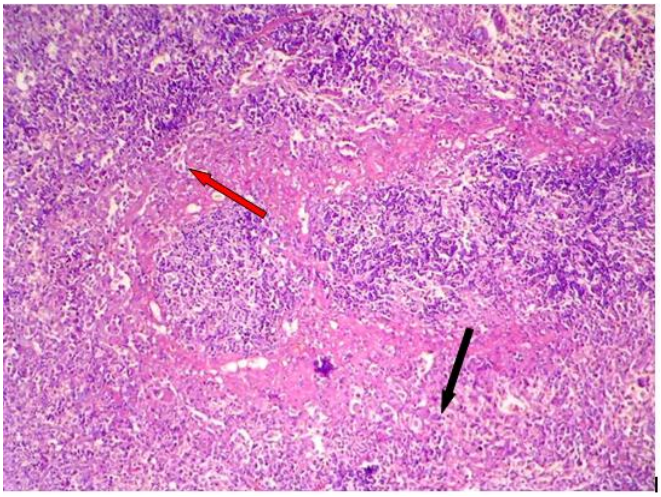

Figure 3- C.S in mouse spleen treated with 1200 ppb of FB1 showing follicular lymphoid hyperplasia with degeneration of parenchymal tissue (X200; H&E).

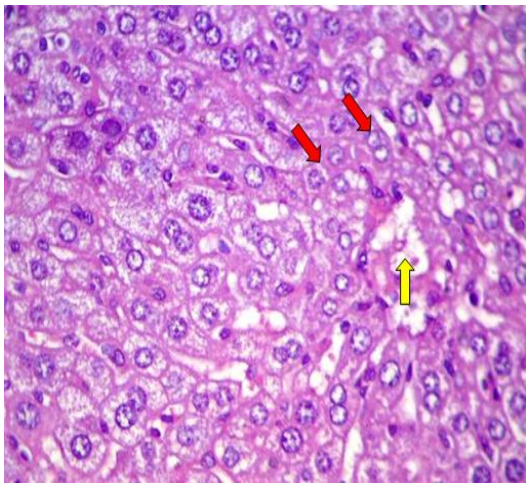

Figure 4-C.S in mouse liver (untreated group) showing normal structure appearance of central vein with threads of hepatocyte cells (X400; H&E). **Red arrow:** hepatocytes; **Yellow arrow:** central vein.

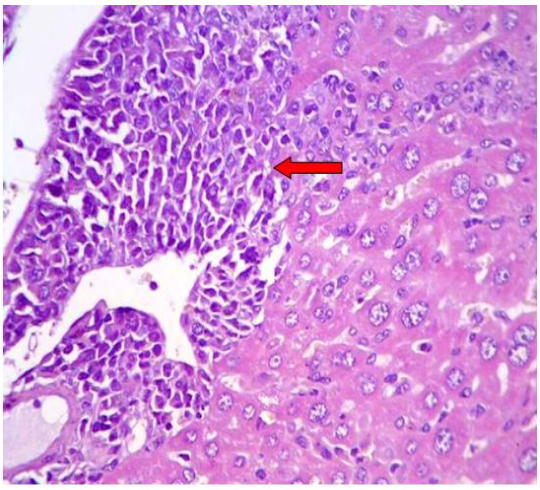

Figure 5- C.S in mouse liver treated with 800 ppb of FB1 showing comment focal necrosis (X400; H&E).

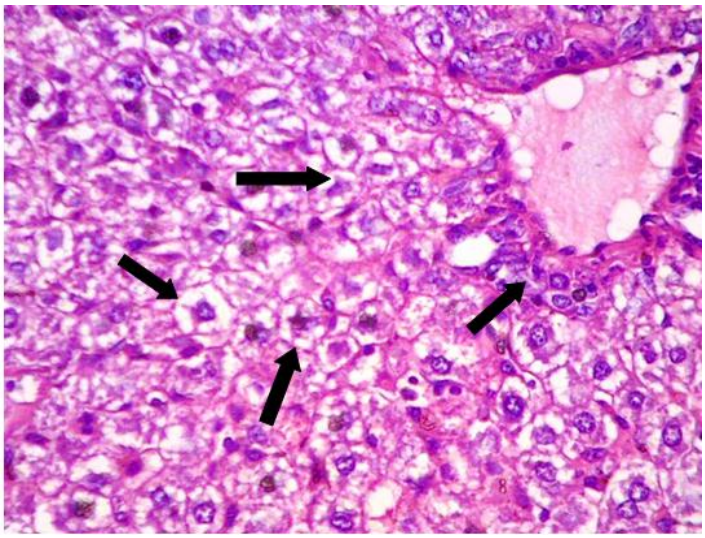

Figure 6- C.S in mouse liver treated with 1200 ppb of FB1 showing mild periportal mononuclear accumulation and degenerated cells (X400; H&E).

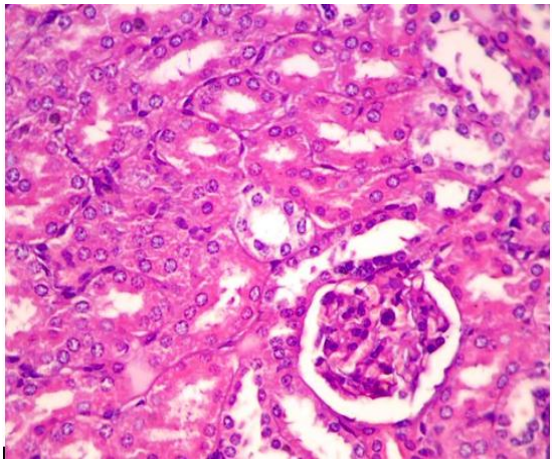

Figure 7- C.S in mouse kidney (untreated group) showing normal structure appearance (X400; H&E).

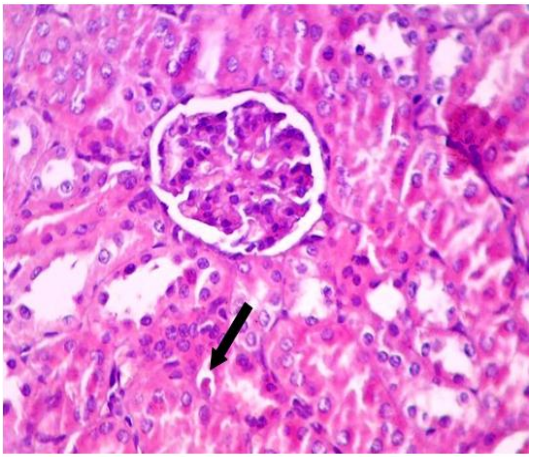

Figure 8- C.S in mouse kidney treated with 800 ppb of FB1 showing degenerative changes of renal epithelial tubular cells (X400; H&E).**Black arrow:** degenerative changes,

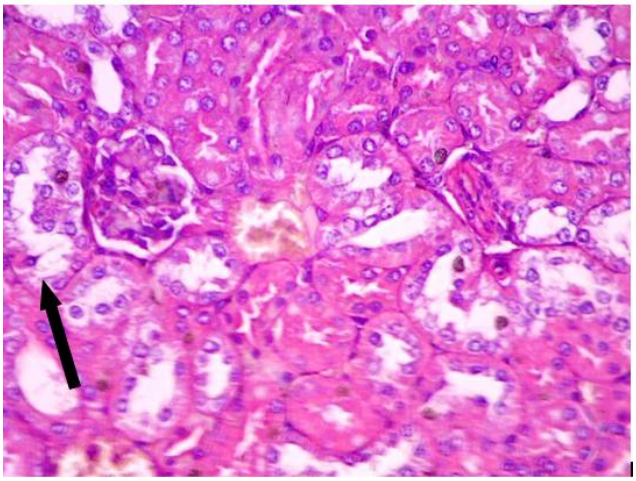

Figure 9- C.S in mouse kidney treated with 1200 ppb of FB1 showing sever degenerative changes of renal epithelial tubular cells (X400; H&E).

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