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Immunohistochemical Expression of P16 Protein and TGF β1 in Mice Liver Exposed to Fumonisin B1

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Abstract:

Fumonisin B1 (FB1) is a mycotoxin produced in some grains (mainly corn) by *Fusarium* species. Due to a structural similarity between FB1 and sphinganine, sphingolipids metabolism is inhibited. Such inhibition plays a critical role in cell to cell singling and structure of lipoprotein; therefore FB1 has been suggested to have a relationship with human and animal cancer. This research is planned to study the effect of FB1 on male mice at two doses (20 and 30 µg/ ml) on the expression of TGF-β1 and p16 in liver cells. Three groups of Swiss albino male mice; each group was orally administrated with FB1 toxin as the following: normal saline (control group); 20 and 30 µg/ ml. All groups were sacrificed after two weeks of oral management. Liver samples were collected and prepared for immunohistochemistry technique (IHC) using anti-TGF-β1 and anti-p16 antibodies. The results showed that exposure to FB1 caused significant elevation of TGF-β1 in both doses (76.74 ± 2.387% and 80.62 ± 7.277%, respectively) in comparison with the control group (46.79 ± 2.404%). The level of p16 protein was decreased at 20 µg/ml (76.63 ± 2.349%) and then increased at 30 µg/ml (81.25 ± 6.263%) but the expression was lower than that of control (90.00 ± 0.805%). In conclusion, FB1 has a significant effect on TGF-β1 and p16 protein expression at both doses (20 and 30 µg/ml), and therefore, its role in cancer development is suggested.

Key words: Fumonisin B1, p16 protein, Transforming growth factor-beta (TGF-β).

Introduction:

Fungal toxins are toxic compounds with low molecular weight, produced by a few species of fungi in the field during the period of harvest (1). A broad spectrum of *Fusarium* species causes diseases in plants and produces important mycotoxins like trichothecenes, zearalenone, and fumonisins, which are the major threats to animals and humans (2). These toxins can cross the epithelium of intestine causing a diverse effect in the immune system by impairing the function of macrophages, neutrophils, decrease the activity of lymphocyte, as well as the production of antibody (3). The host becomes susceptible to infection with microorganisms during exposure to *Fusarium* toxins (4), depending on host factors such as; age, dose, and exposure time to the toxin (5).

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Fumonisin B1 (FB1) is one of these toxins, and its carcinogenic effects in human and laboratory animals have been suggested. It deactivates the enzyme ceramide synthase and increases the concentration of sphinganine (Sa) and sphingosine (So) in tissue (6). Therefore, FB1 toxicity affects mainly the liver organ, which is characterized by apoptotic, necrosis, and regeneration (7). A recent study has found that FB1 can cause changes in the tissues of liver, lung, and kidney such as apoptosis and necrosis, leading to infiltration of inflammatory cells which were observed in these organs (8). Due to these histopathological changes, FB1 can be responsible for several diseases in human and animal including hepatotoxicity, nephrotoxicity, and neurotoxicity (9). Transforming growth factor-beta (TGF-β) is a cytokine that plays a serious role in the regulation of different cellular processes, and also important for homeostasis of tissues and organs (10). In contrast, p16 protein acts as a negative regulator to cell cycle progression (11).

Recent studies recorded that TGF-β1 and p16 protein serves as biomarkers for malignant progression (12, 13), and since the contamination of

human diet with mycotoxin is still a serious problem, so this study was planned to determine the effect of single exposure to FB1 on male mice at two doses; 20 and 30 µg/ml, on immunohistochemical (IHC) expression of TGF-β1 and p16 in liver cells.

Material and Methods:

Preparation of FB1 solution

The stock solution was prepared by dissolving 5 mg of FB1 (Enzo life Science) in 5 ml of acetonitrile: water and from this stock solution the doses were prepared.

Estimation of TGF-β1 and p16 protein by IHC

Three groups of Swiss albino male mice (each of six animals) were treated with two single oral doses of FB1 (20 µg/ ml and 30 µg/ ml) as previously described (8). The first group was given normal saline, while groups 2 and 3 were administrated with the second and third doses of FB1, respectively and the animals were scarified after two weeks of the oral management. Liver samples of sacrificed animals were fixed in 10% formalin and histological preparations were performed. The paraffin blocks of mice liver were used for immunohistochemistry technique (IHC) using anti-TGF-β1 and anti-p16 antibodies. IHC was carried out according to manufacturer instructions of Cambridge Science Company. The TGF-β1 and p16 protein expression were measured by enumerating positive cells with brown cytoplasmic staining (14).

Statistical Analysis

The results were given as mean ± standard deviation (SD, N= 6). Significant differences between means were assessed by ANOVA (analysis of variance) followed by LSD (least significant difference). A probability (p) value ≤ 0.05 was considered significant. The statistical package SPSS version 16.0 was used to carry out these analyses.

Results and Discussion:

Due to little information about the effect of FB1 on some liver biomarker, this study was conducted by using IHC technique to study the effect of single dose of FB1on mice that were treated with 20 and 30 µg/ml *via* orally gavage route on the expression of TGF-β1 and p16 protein in liver tissue. The results showed that exposure to FB1 caused significant elevation of TGF-β1 in both doses (76.74 ± 2.387% and 80.62 ± 7.277%, respectively) in comparison with the control group (46.79 ± 2.404%) (Figs. 1 and 2). The level of p16 protein was decreased at 20 µg/ml (76.63 ± 2.349%) and then increased at 30 µg/ml (81.25 ± 6.263%) but the expression was lower than that of control (90.00 ± 0.805%) (Figs. 1 and 2). The results revealed a significant difference in the expression of liver biomarker between treatment and control groups ($p \leq 0.05$). A previous study demonstrated an increased expression of TGF-β1 by hepatocytes and caused apoptosis and fibrosis as seen in FB1-induced liver injury and engaged in the tumor-promoting effects of FB1 (15, 16).

Further research reported that FB1 induced organ lesions in some animal, which were characterized by apoptosis, necrosis, and proliferation, and due to this an imbalance between cell loss and replacement develops, causing good conditions for carcinogenesis (5,17).

Another study suggested that the level of the p16 marker was not a significant sign to predict for oral squamous cell carcinoma samples (12). Further study found that p16 protein is a key player in preventing tumorigenesis, while, suppression of p16 and p53 proteins was the causes of human tumors (18).

Conclusion:

FB1 has a significant effect on TGF-β1 and p16 protein expression at both doses (20 and 30 µg/ml), and therefore, its role in cancer development is suggested.

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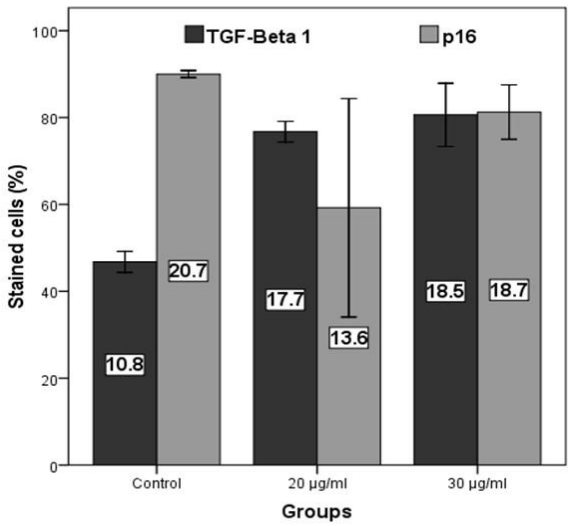

Figure 1. Level of TGF-β1 and p16 expression in mice liver at three doses; Control (normal saline), 20 & 30 µg/ml of FB1 toxin

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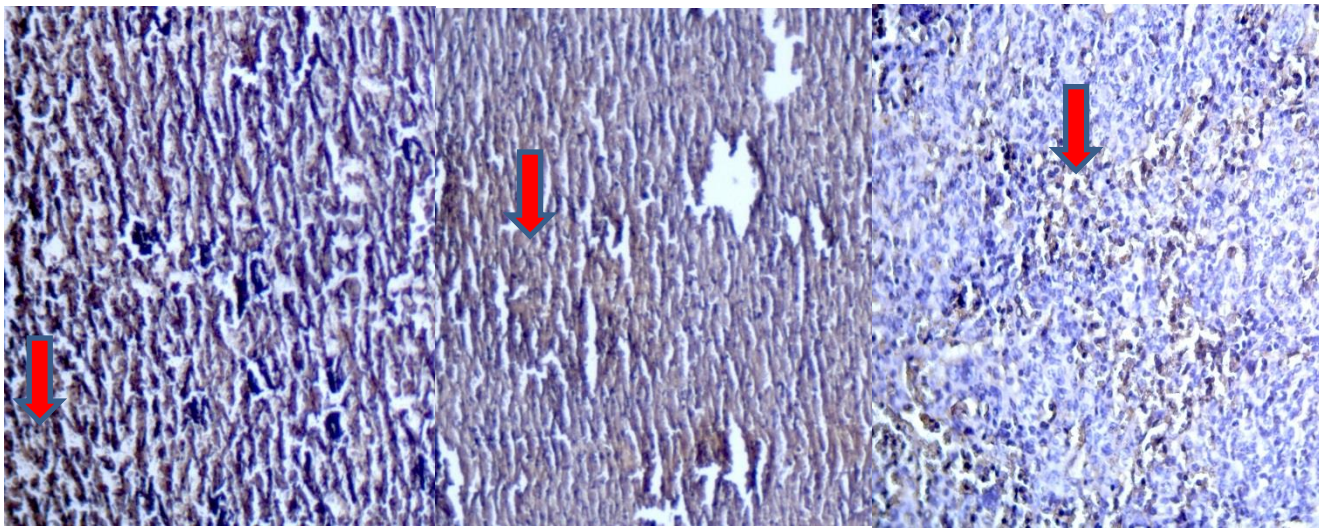

Figure 2. Distribution of TGF-β1 in the liver of mice treated with the FB1 toxin: (A) 20 µg/ml, (B) 30 µg/ml, (C) Control, (X200, Red arrow: Positive cells)

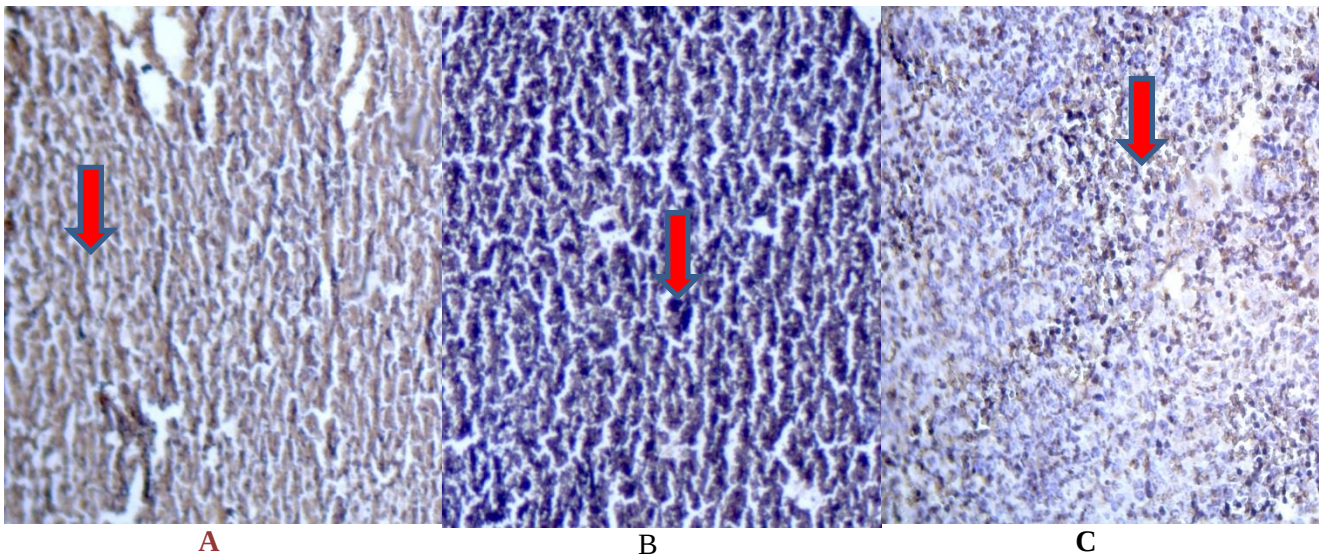

Figure 3. Distribution of p16 protein in the liver of mice treated with the FB1 toxin: (A) 20 µg/ml, (B) 30 µg/ml, (C) Control, (X200, Red arrow: Positive cells)

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

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التعبير المناعي النسيجي للبروتين ب16 و عامل النمو المحول بيتا1 في كبد الفئران المعرضة للسم فيومنسين ب1

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الخلاصة:

فيومنسين ب1يعد من السموم الفطرية التي تنتج في بعض الحبوب (الذرة) عن طريق الأنواع *Fusarium*. وبسبب التشابه التركيبي بين FB1 و Sphinganine ، يتم تثبيط عملية بناء Sphingolipids. ويلعب هذا التثبيط دوراً حاسماً في إفراز الخلايا وبنية البروتين الدهني ؛ لذلك يعتقد ان FB1 له علاقة بسرطان الإنسان والحيوان. صممت الدراسة الحالية للكشف عن تأثير FB1 على ذكور الفئران بجرعتين (20 ميكروغرام / مل و 30 ميكروغرام / مل) على تعبير عامل النمو المحول بيتا1(TGF-β1)و بروتين ب16 (p16)في خلايا الكبد. ثلاث مجاميع من الفئران البيض السويسرية ، كل مجموعة جرعت فمويا بالسم FB1 على النحو التالي: مجموعة السيطرة و 20 ميكروغرام / مل و 30 ميكروغرام / مل. تمت التضحية بجميع المجاميع بعد أسبوعين من التجريع. ثم جمعت عينات الكبد وجهزت لتقنية النسيجية المناعية (IHC) باستخدام TGF-β1 و p16 كأجسام مضادة. وأظهرت النتائج أن التعرض ل FB1 يسبب ارتفاعا واضحا في قيم تعبير TGF-β1 في كلا المجموعتين حيث كانت 76.74 ± 2.387 %و 80.62 ± 7.277% على التوالي بالمقارنة مع مجموعة السيطرة (46.79 ± 2.404%). في حين أن مستوى البروتين p16 ينخفض عند 20 ميكروغرام / مل (76.63 ± 2.349%) ثم يرتفع عند 30 ميكروغرام / مل (81.25 ± 6.263%) ولكنن تعبيره لا يزال منخفضا عن سيطرته (90.00 ± 0.805%). استنتجت الدراسة ان FB1 كان له تأثير كبير على تعبير البروتين TGF-β1 و p16 عند كل من الجرعات (20 و 30 ميكروغرام / مل). ويستنتج من هذا أن FB1 له القدره على تطور السرطان.

الكلمات المفتاحية: فيومنسين ب1، بروتين ب16، عامل النمو المحول بيتا1