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TOXIC EFFECTS OF FUMONISIN B2 PRODUCED BY *ASPERGILLUS NIGER* IN SOME ORGAN OF ALBINO MICE

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Abstract

High-Performance Liquid Chromatography (HPLC) technology was used for detecting toxins in fungal strains of *A. niger* isolates showed high levels of toxin and the highest percentage of Fumonisin B2 was 996.2 ppb.The effect of the *Pleurotus ostreatus* filtrate was studied as a biological treatment agent and calcium citrate as a chemical treatment factor to reduce the effect of the toxicity of the contaminated fungus. The results of the effect toxic pathogens *A. niger In-vivo* of albino mice showed the toxin caused changes in the weights of animals after ten days and after the end of the 21-day dose process and changes In levels of liver enzymes (GOT, GPT, ALP) as its level reached (159.3 ± 2.51, 85 ± 4.58, 227 ± 2) U/L respectively, compared to the levels of the control group that reached (95.6 ± 3.05, 47.3 ± 0.57, 153.3 ± 1.15) U/L respectively. And the efficacy of the Mycotoxin in the group gavaging with toxin caused pathological changes in the liver tissue, including an expansion in the hepatic sinusoid, presence of apoptotic bodies with necrosis. The laboratory results shown when examining the functions of the kidneys, there were significant differences in the levels of both urea and creatinine in the serum of laboratory animals treated with pathogenic fungus filtrate, as it reached the level of Urea mg/dl 45 ± 1.2 and creatinine ratio 1.41 ± 0.03 mg/dl compared to the control group in which the urea level reached 30 ± 1.73 mg/dl and creatinine 0.33 ± 0.02 mg/dl, thus damage to the kidneys of laboratory animals, which is atrophie in renal glomerulus, renal tubular expansion, degeneration and dissociation clear in the epithelial cells lining the urine tubule.

Key words: *Aspergillus niger*, HPLC, Fumonisin B2.

Introduction

Fumonisin is a Mycotoxin produced by some species of the *Fusarium* genus that are contaminated with grains such as wheat, barley, corn and rice. This Toxin was discovered by researchers Gelderblom and Colleagues in 1988 from the cultures of *F. verticillioides* MRC 826 (Gelderblom *et al*., 1988). Many species of *Fusarium* are capable of producing Fumonisins, the most important ones are *F. verticillioides, F. proliferatum, F. oxysporum, F. beomiforme, F. napiforme, F. dlamini, F. globosum, F. nygamai*, (WHO, 2000; Kumar *et al*., 2008; Yazar and Omurtag, 2008). Additionally, *Aspergillus niger* has the ability to produce Fumonisins such as FB2 and FB4 and the new series FB6 (Huffman *et al*., 2010). Fumonisin B2 carcinogenic toxins were first detected in the industrially important *Aspergillus niger*, the production of Fumonisin by *A. niger* is widespread as an industrial object It is extremely important and will have very negative effects on biotechnology, especially food safety. *A. niger* is used to produce citric acid and a product for extracellular enzymes, Certain strains of *A. niger* produce both Ochratoxin A and Fumonisins. so some foods and feeds may contain two types of Mycotoxins that cause cancer of this type (Jens *et al*., 2007) and through a study conducted by (Noonim *et al*., 2009) on Thai coffee beans (Coffeaarabica), it was found contaminated with the Fumonisin produced by the fungus *Aspergillus niger*, and non-species of *Fusarium* known to be produced by Fumonisin was shown, as the liquid chromatography technique showed HPLC detection that 67% of *Aspergillus niger* isolates from coffee beans are able to produce FB2 when grown on Czapek Yeast Agar with 5% NaCl.

Materials and Methods

Isolation of fungi from fodder grains

Several species of fungi associated with fodder grains collected from local stores and markets in AL-Diwaniya province were exposed to animal and human consumption and many were diagnosed. Some fungi isolates were

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tested in their ability to produce FB1, FB2 using HPLC technology which is: three strains from *Aspergillus niger* and strains of *Fusarium solani, Fusarium proliferatum* and *Fusarium* sp.

Filtrates preparation

The filtrate of fungi (*P. ostreatus and A. niger*), was prepared by using the PDB in flasks with an amount of 100 ml from the medium and sterilized by autoclave and after cooling, Chloramphenicol was added to the medium and then taking two piecesdiameter of 5 (mm) From fungus grown on PDA at the age of 7 days in each flask. Flasks incubated in the incubator at a temperature of 25°C. for a period of three weeks with continuous shaking every two days. After period ended, the fungalcultures were filtered for the fungus using Whatman No. filter paper. 1 Under sterile conditions and then sterilize the filtrate using Millipore Filters with a diameter of 0.22 microns, Filtrateswere prepared for detecting Mycotoxins with HPLC technology and for gavaging a laboratory animals. Calcium Citrate salt wasprepared according to instructions of the producing company.

Detecting Mycotoxins in fungal strains using HPLC technology:

Mycotoxins produced by fungi have also been detected, and among these toxins Fumonisins (B1, B2), dissolve these toxins in polar solvents and other solutions such as methanol and acetonitrile but they are insoluble in non-polar solvents.

HPLC condition:

Mobile phase = acetonitrile : D.W (60 : 40) , Column = C18 - ODS (25cm * 4.6mm)

Flow rate = 0.7 ml/min, Detector = florescent Ex = 365nm, Em = 445 nm.

Sample preparation:

The samples (5g) were sonicated in 50mL 70:30 v/v methanol : water for 40 min and centrifuged for 5 min, then 5 mL of the supernatant was drawn, diluted with 20 mL water and passed through the immune affinity column at no more than 3mL/min (the column was pre-viously conditioned with 20mL distilled water). The column was rinsed with 10 mL distilled water to remove the matrix components and the dried by passing air through to remove any remaining water. The final quantitative elution was accomplished by adding methanol (1.4mL) onto the column and fiushing with air. The eluate was diluted to 2mL with water and passed through a 0.45mm filter and the filtrate was injected into the HPLC. Using the following equation described before (1999) Akiyma and Chen, the concentration of Mycotoxins in the fungus filter samples was calculated.

Study effects of Fumonisin B2 *In-vivo*:

Laboratory animals:

White albino mice was used to find out the effect of Mycotoxins on it, which are biological methods that depend on the effect of changes in the tissues of laboratory animals after oral gavaging. 30 laboratory mice were used for male and female age ranges between (9-8) weeks and reached an average weight of 23 - 25 gm.

Body weight measurement:

The weight was measured before the start of the gavaging, during gavaging and before sacrifice, according to Al-Easwey (2003). Its purpose is to know or observe the effect of Mycotoxins on the weights of white mice during the experiment period.

Experimental design:

Doses were prepared and 0.5% dilution was chosen for the Mycotoxin concentration of *A. niger* Filtrate, taking into account the lethal dose LD50, according to (Mohammed *et al*., 2017) when studying the determination of the toxicity of Fumonisin on the male white mice. As for calcium citrate and *p. ostreatus*, the concentration was chosen 10%. gavaging started in 10/ 2/2020 every two days for (21) days (Mutouq, 2005) and (Rajani *et al*., 2012; Khafaji, 2017).

Distribution of laboratory white mice and doses:

Groups	Treatments
G1	Only gave water and feed and represented Control.
G2	Orally gavaging with *A. niger* filtrate every 48 hours 0.5 ml.
G3	Orally gavaging with *P.ostreatus* filtrate every 48 hours 0.5 ml.
G4	Orally gavaging with *A. niger* filtrate and 24 hours after the dose were administered with *P. ostreaus* filtrate 0.5 ml.
G5	Orally gavagingwith *A.niger* filtrate, after 24 hours, administered with calcium citrate 0.5 ml.
G6	Orally gavaging with *A. niger* filtrate, after 24 hours of dose, administered with *P. ostreaus* filtrate and calcium citrate 0.5 ml.

Two days after the last gavage, laboratory animals were sacrificed after being anesthetized with chloroform and anatomize by an opening in the abdominal cavity, then blood was drawn from the heart directly by stabbing the heart's heart puncture for the animal using a centrifuge syringe Putting blood drawn into a serum tube called JelTube after centrifuged at 3000 cycles per minute for

10 minutes for biochemical examination of measuring the level of ALP, ALT, AST and Renal function of the kidneys and measuring Urea and Creatinine.

Biochemical tests:

Measurement of levels of liver enzymes ALP, ALT, AST and Blood Urea, Creatinine in Serum by the AU480 Chemistry Analyzer, which is a fully automatic device that specializes in Clinical Biochemistry .After the completion of the blood collection , the organs (liver, Kidney) were removed then washed with Normal saline and later fixed in formalin 10% Preparation and completion were made and the tissue sections were prepared.

Statistical analysis: Use the statistical program known as the Statistical package for social science and the results of the study were analyzed using each of one way ANOVA and two way ANOVA with a value of less L.S.D (Least Significant), significant differences were determined at the 5% probability level (Schiefer, 1980).

Results and Discussion

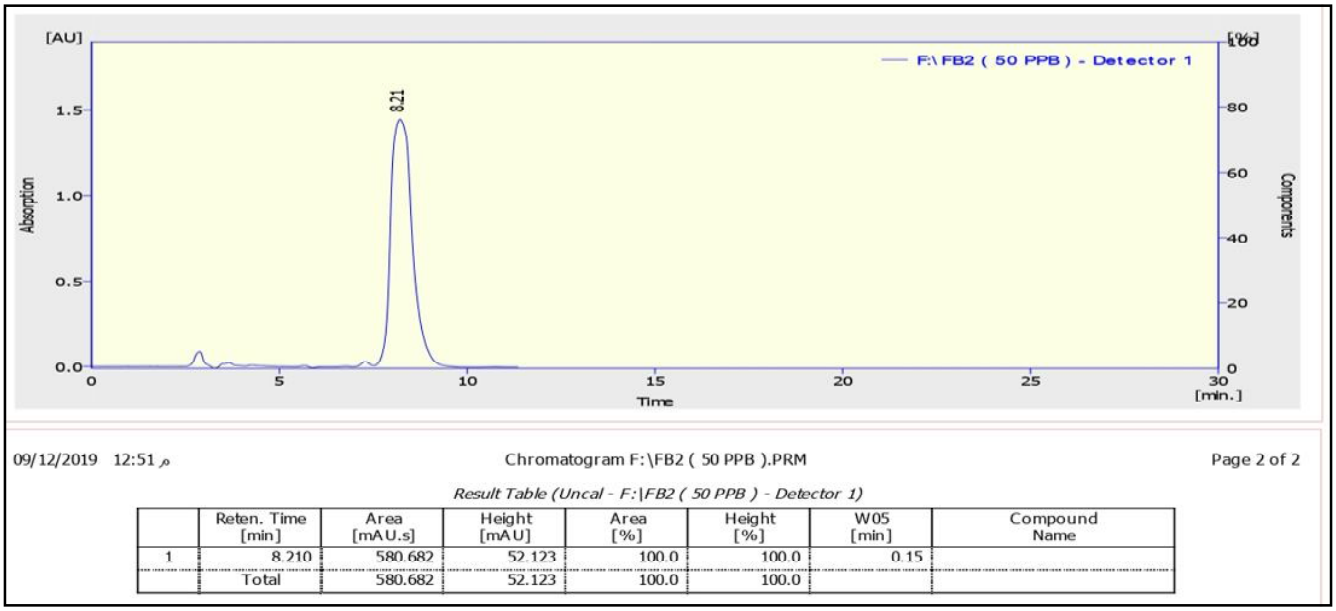
Use of HPLC technology to detect the Mycotoxins Fumonisin B1, B2 produced by isolated fungi:

Before starting this technique, some of the fungi strains that were previously diagnosed were selected to reveal the ability of these fungi to produce the fungal Mycotoxin Fumonisin, which are three isolates of the fungus *Aspergillus niger, F. solani, F. proliferatum, Fusarium* sp. And all of these isolates had the ability to produce Mycotoxin first type (FB1) and second type (FB2) and according to table 1 where the results showed the value of retention time (8, 210) minutes as it represents the standard area of the toxin as shown in the Fig. 1. Also, a simple test was done to ensure that this value belongs to the standard material, since the solvent was injected only once again, and no value was shown at this time, and the Stander solution was injected again, so it showed a value in the time of retention (8, 210) minute and this indicates that this value is due to the standard substance, Fumonisin Mycotoxin, after filtrates of fungal strains indicated in table 1 showed values of retention time (8.383) minute. These values indicate the presence of Fumonisin toxin in fungal isolates examined depending on the matching time of the standard material with The time at which these values appeared for the same conditions in which the standard material analysis was performed. To make sure that these values in the samples are belong to toxin and not to a compound that might resemble the time of retention, take 50 µl of the standard Mycotoxin and add to the previously injected sample, for which the analysis was performed again an increase in the sample area of the area for the same previously injected sample is observed and this is called Internal stander.

Table 1: Determination of Fumonisins in Fungal Strains.

No.	Strains	The calculated concentration of Mycotoxin ppb = (ng /ml)	
		FB1	FB2
1	*Aspergillus niger*	—	996.2
2	*A. niger*	—	554.2
3	*Fusarium solani*	533.7	—
4	*F.proliferatum*	388.2	—
5	*A. niger*	—	367.1
6	*Fusarium* sp.	70.8	—

The results of the HPLC examination shown in table



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8.210	580.682	52.123	100.0	100.0	0.15	
	Total	580.682	52.123	100.0	100.0		

Fig. 1: Standard Results for HPLC Technology for Fumonisin B2 Detection.

1 and the charts (1, 2) in the show that the fungi contaminated with fodder grains had the ability to produce Fumonisins, as the highest concentration of the toxin produced in the *Aspergillus niger* filtrate was for a poultry feed sample with a concentration of FB2 966.2 ppb, and the lowest concentration record in a local millet sample where the concentration of FB1 reached 70.8 ppb and these Mycotoxins are dangerous , carcinogenic and a real problem because they affect the health of humans and animals. These results were resemble to (Noonim *et al*., 2009) when he conducted a study on FB2 in *Aspergillus niger* strains using HPLC technique.

Effects of Fumonisin B2 *In-vivo*:

Exposure to *A. niger* filtrate led to a decrease in laboratory animal weights in both periods during the gavaging. The results shown in table 2 revealed differences between weights, In the second group theweight average before the gavaging was 24.2 ± 0.45g and a decrease in the average weights during the gavaging period ,after ten days it reached 23.8 ± 1.07g, while before sacrifice it amounted to 23.3 ± 0.91g and compared to the control group, weight average of before the gavaging was 24.4 ± 0.65g and after ten days it reached the average weight is 25.85 ± 0.63 g and before sacrifice 27.2 ± 0.64g These results are consistent with the above mentioned (Javed *et al*., 1992a) where gavaging Fumonis in to types of chicks caused low weight and mortality in addition to inflation in many organs including the liver, kidneys, heart and lung. While we note that there were significant differences in the third group, which were gavaging only by the *p. ostreatus* Filtrate before gavaging, the weights average reached 24.8 ± 0.75g, but after ten days it reached 26.03 ± 0.30g, while before sacrifice weights average 29.3 ± 1.52g and this is consistent with (Ibrahim., *et al*., 2006) indicates that *p. ostreatus* possess an enzyme system that is involved in metabolism and some nutrients are converted into fats by metabolism that occurs in the body of the organism. As for the other groups, a very small decrease was observed as a result of gavaging with the pathogenic fungus filtrate, consistent with another study that showed that feeding male rats with a food free of FB1 but containing FB2, FB3 resulted in renal and hepatic toxins Hepato- and nephrotoxicity and an effect on animal weights (Voss *et al*., 1996).

Table 2: Effect of Fumonisin B2 on albino mice weights.

Groups	Mice weights average			
	Before gavaging	After 10 days	before sacrifice	Treatment average± standard deviation
G1	24.4±0.65	25.85±0.63	27.2±0.64	25.81±1.4
G2	24.2±0.45	23.8±1.07	23.3±0.91	23.76±0.45
G3	24.8±0.75	26.03±0.30	29.3±1.52	26.71±2.32
G4	23.5±0.30	23.53±0.23	23.36±0.15	23.46±0.09
G5	25.4±0.91	25.23±0.66	25.2±1.31	25.27±0.10
G6	25.86±0.41	26±1.05	26.26±0.64	26.04±0.20
Periods average ± Standard Deviation				
	24.69±0.85	25.07±1.13	25.77±2.32	25.17±1.54
L.S.D	Periods 0.535	Treatment 0.757	Interfere 1.311	

*The values represent the mean ± standard deviation of laboratory animals.

Biochemical blood tests:

Measuring the levels of liver enzymes AST (GOT), ALT (GPT), ALP:

The effect of Fumonisin on the level of liver enzymes in the blood of treated white mice, it has reached (159.3 ± 2.51, 85 ± 4.58, 227 ± 2 U/L) respectively in the G2 compared to the control group G1 it reached (95.6 ± 3.05, 47.3 ± 0.57, 153.3 ± 1.15 U/L) and thus a significant increase in the second group compared to the control group. The results also showed a significant decrease ($P < 0.05$) in the levels of enzymes for others groups treatment compared to the second group as in table 3. These results are consistent with a study (Al-Badri, 2013) that showed the effect of Mycotoxins including the Fumonisin on enzymes the liver, which caused the rise of GPT, GOT enzymes. On the one hand, the effect of the *p.ostreatus* filtrate on laboratory animals by reaching levels of enzymes (102 ± 1.73, 38.6 ± 1.15, 105 ± 3.6 U/L) that the enzymes were not significantly affected by the filtrate and remained on their activity and representation in the liver and the results were close to the levels of the control group and according to a study conducted by the researcher (Timoz, 2015). The level of enzymes in other groups for the pathogenic *A. niger* Filtrate with calcium citrate or *P.ostreatus* filtrate has recorded a significant significant decrease ($P<0.05$) compared to G2 that gavage with Toxin only. Level of enzymes was significantly

Table 3: The effect of Fumonisin B2 on the level of liver enzymes.

Groups	ASTU/L	ALTU/L	ALPU/L
G1	95.6±3.05	47.3±0.57	153.3±1.15
G2	159.3±2.51	85±4.58	227±2
G3	102±1.73	38.6±1.15	105±3.6
G4	170.6±3.51	27.3±2.3	35.3±2.51
G5	115.6±0.57	57±2.08	72±1
G6	128±4.04	43.3±4.93	144±1.73
$LSD_{0.05}$	5.012	5.465	3.887

*The values represent the mean ± standard deviation of laboratory animals.

decreased compared of the control group, often reason to the control of the *P. ostreatus* filtrate in the survival of the liver cells.

Determination of the level of Urea and Creatinine:

Fumonisin toxin caused a significant increase (P <0.05) with the level of urea and creatinine concentration in serum of white mice gavaging with this toxin, as urea level reached 45 ± 1.2mg/dl and creatinine level was 1.41 ± 0.03 mg/dl compared with control group which reached urea level 30 ± 1.73 mg/dl and creatinine 0.33 ± 0.02 mg/dl, appearance creatinine in the blood is an indication of the performance of glomerulus because it is responsible for the filtration and its level changes in Blood in some pathogenic conditions, the most important of which is kidney failure (Bamanikar *et al*., 2016). And as shown in table 4, which shows significant differences in the levels of urea and creatinine between the groups studied, and the reason is due to the effect of the FB2 on the tissues of the kidney and caused by atrophy of the glomerulus and expansion of the renal tubules. These results are resemble to studyfor (Mohammed, 2015), as it was found that the fungal toxin Fumonisin was caused by an increase in the level of urea and creatinine concentration in the blood of treated animals compared to the control group, and in the gavaging of *P. ostreatus* filtrate on laboratory animals, the levels of urea and creatinine reached 35 .6 ± 3.05 mg/dl and 1.29 ± 0.07 mg/dl respectively, within the limits of normal proportions and indicates the control of this fungus to maintain the effectiveness of cells in the kidney, either in the other groups and gavaging with *P.ostreatus* filtrate or calicum citrate, observed a significant decrease in levels of urea and creatinine compared to the group gavaging with toxic fungus filtrate G2.

Table 4: Effect of Fumonisin B2 on the urea and creatinine.

Groups	Creatinine mg/dl	Blood Uera mg/dl
G1	0.33±0.02	30±1.73
G2	1.41±0.03	45±1.2
G3	1.29±0.07	35.6±3.05
G4	1.33±0.08	41.3±2.3
G5	1.30±0.08	41±3.4
G6	1.50±0.05	39±3.1
$LSD_{0.05}$	0.097	4.572

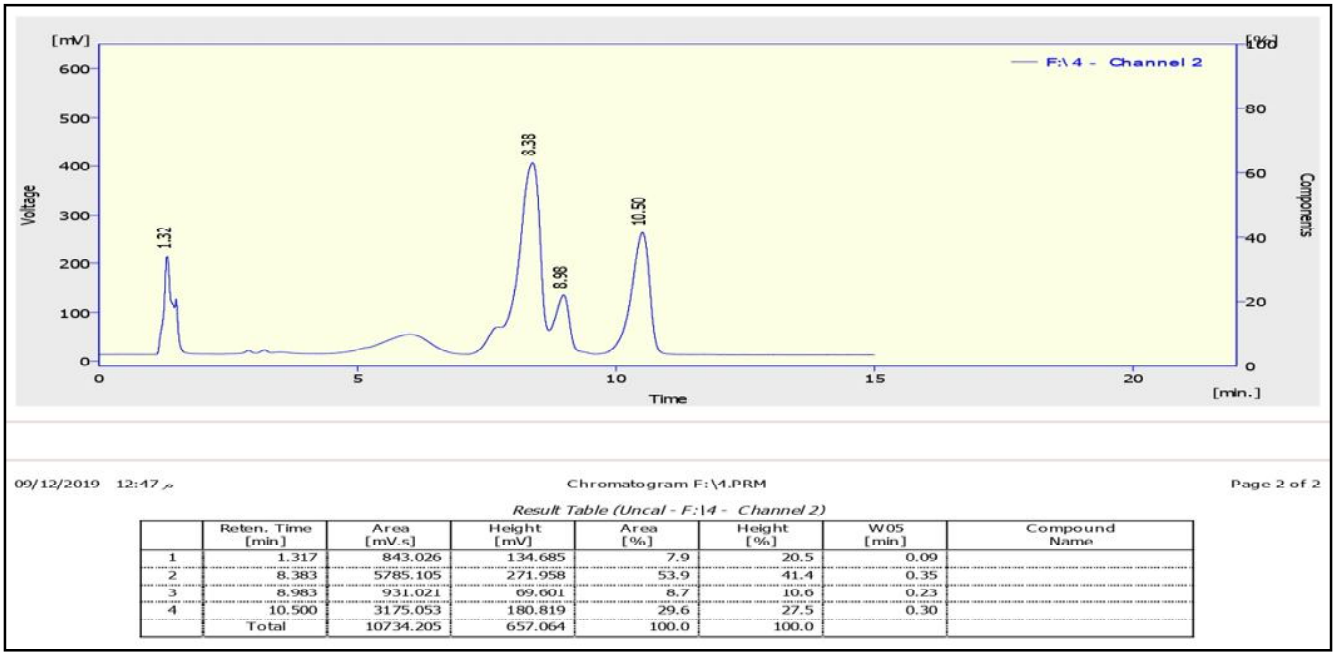
*The values represent the mean ± standard deviation of laboratory animals.

Histological study:

Effect of *A.Niger* filtrate on the liver and kidney of laboratory animals:

1- Liver

The results of the microscopic examination to diagnose the tissue sections taken from the livers of laboratory mice gavaging with *A.niger* Filtrate shown in Fig. 2 indicated the presence of clear histopathological changes, including loss of the radial arrangement of liver cells around the central vein, expansion of hepaticsinusoidis and infiltration of cells Inflammatory Cells, the presence of programmed death bodies called apoptotic Bodies, Necrosis is evident in the liver cells as Some Karyolysis cells appear and some have a Pyknotic nucleus with Hemorrhage in some tissue regions ,



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.317	843.026	134.685	7.9	20.5	0.09	
2	8.383	5785.105	271.958	53.9	41.4	0.35	
3	8.983	931.021	69.001	8.7	10.6	0.23	
4	10.500	3175.053	180.819	29.6	27.5	0.30	
	Total	10734.205	657.064	100.0	100.0		

Fig. 2: Determination of Fumonisin B2 produced by *Aspergillus niger* using HPLC.

Macrophage inflammatory cells accumulate and the reason is due to the effect of FB2 . These effects close to the results (Al-lobawi, 2016) when Study on the effect of Fumonisin FB1 on liver and kidney tissues on male mice orally gavaging with FB1, as results revealed that changes in liver tissue Kidneys in mice, FB1 at concentrations of 800 and 1200 ppb, showed increased degenerative changes and programmed death of cells compared to the control group. The result of control group showed the liver cells appear in their normal composition. As for the G3 of mice, which were gavaging by the filtrate *P. ostreaus* Fig. 3, no pathological changes were observed, but rather the regularity of the architecture of the liver tissue cells with the appearance of some binucleated cells.

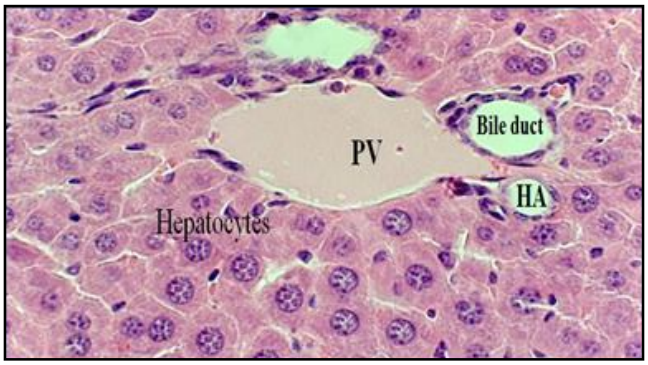


Fig. 3: Cross section of a mice liver from the Control G1 : The normal structure of the liver tissue is observed , one of the branches of the hepatic portal system that consists of a branch of the portal vein (PV), a bile duct bile duct, and a branch of the hepatic artery (HA). (X40 H&E).

These results are consistent with its findings (Timmos, 2015). When a study was conducted on the effect of the filtrate of this fungus, the sections taken from the livers of the mice did not show any toxicity and harm when gavaging these animals with *P. ostreaus* filtrate. The fourth group treated with both the *A. niger* and *P. ostreaus* Filtrates , structure was observed Regularly and radially to the hepatocytes around the central vein with congestion in one of the branches of the portal hepatic veins in addition to the multiplication of Kupffer Cells and this shows how the *P. ostreaus* Filtrate can control or reduce the toxicity of the fungus filtrate. The result in fifth group in Fig. 5 For the of toxic fungusfiltrate with calcium citrateobserved the geometric regularity of the liver tissue, the appearance of slight congestion in multiple areas, the presence of hydropic degeneration of the hepatic cells and the proliferation of Kupffe Cells. As last group G6a hyperplasia is observed,nuclear division of the hepatic cells where most of the binucleated Hepatocytes (BI) cells appear , indicating the emergence of regeneration and repair in some affected cells ,central vein congestion and proliferation of Kupffer cells Fig. 6, which indicates a less severe damage rate when compared to single gavaging of Mycotoxin in G2.

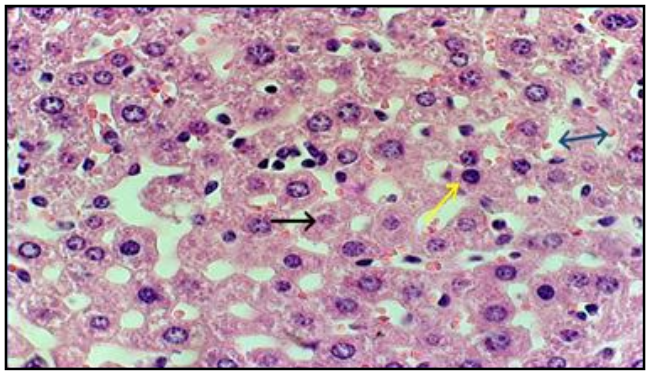


Fig. 4: Cross section of a mice liver from the G2 : Noticeable necrosis is observed in the liver cells (black arrow) and some of them have Pyknotic nuclei (yellow arrow) with hemorrhage in multiple areas (double-headed arrow). (X40 H&E).

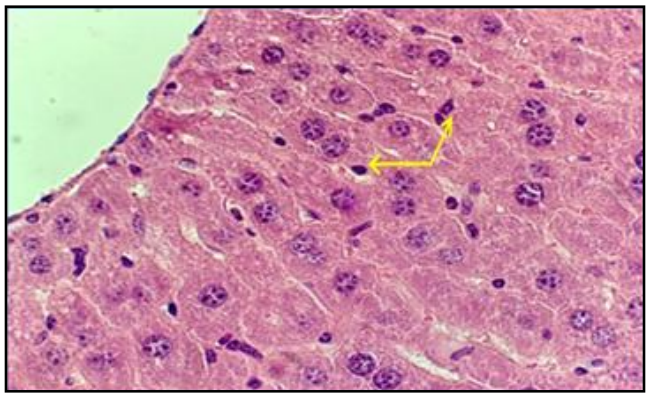


Fig. 5: cross section of a mice liver from the G3 : notes the regularity of the architecture of the liver tissue around the central Vein and some cells appear in a binucleated shape with the multiplication of Kupffer cells (yellow arrow). (X40 H&E).

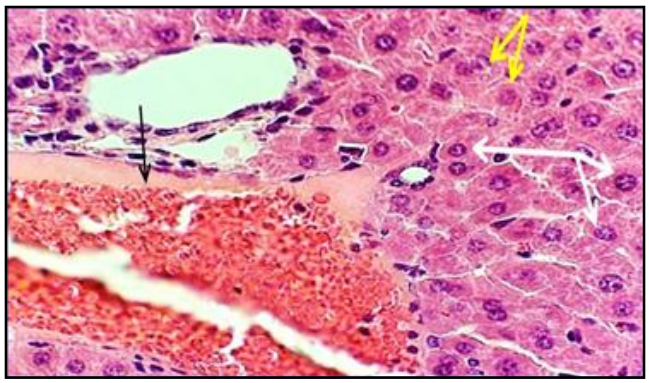


Fig. 6: Cross section of a mice liver from the G4 : Hepatocytes are normal and hexagonal (Triple arrow), simple necrosis in some hepatocytes (yellow arrows) and congestion in one of the branches of the portal vein (black arrow). (X40 H&E).

2- Kidney

In general, these Mycotoxins were found to be carcinogenic in rodents or tumor-causing in mice liver (Howard *et al*., 2001), as the liver and kidneys are the main organs targeted by these toxins and are most affected (Wang and Zhang, 2000; Carlson *et al*., 2001). Results of tissue sections taken from the kidney showed that there are histopathological changes in G1 with *A.niger* fungus filtrate Fig. 8 represented by simple atrophy of the renal glomerulus, expansion of the renal tubules, clear degeneration and dissociation of cells Epithelial lining of the urinary tubule as a result of the effect of FB2, compared to the control group that was

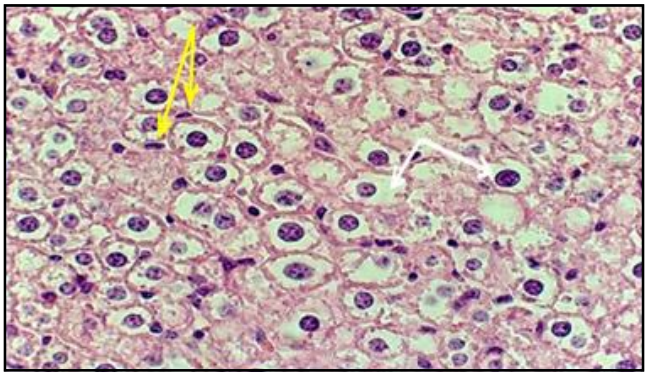


Fig. 7: Cross section of a mice liver from the G5 :Vacuolation appears (white arrows) and Kupffer Cells proliferates are observed (yellow arrows). (X40 H&E).

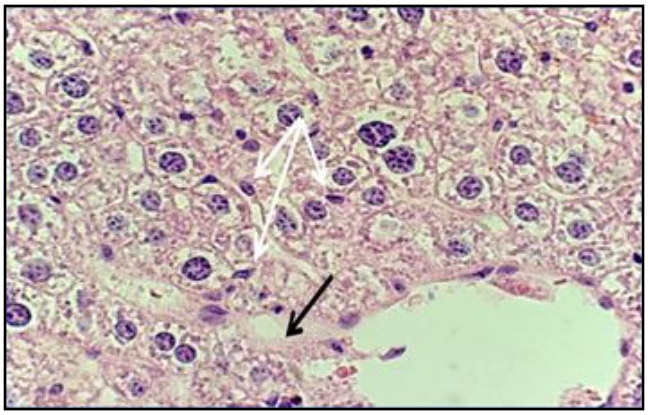


Fig. 8: Cross section of a mice liver from the G6 : Normal and hexagonal cells appearance, Kupffer Cells (white arrows), simple expansion of the sinusoid Hepatic (black arrow). (X40 H&E).

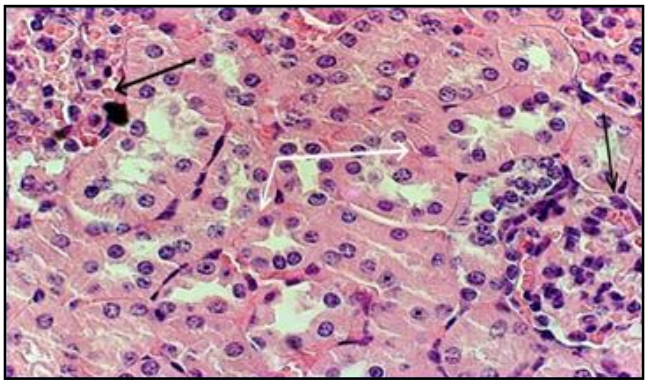


Fig. 9: Cross section of a mice kidney from the G1 : The renal glomeruli appear in their normal composition (black arrows) and normal renal tubules (white arrows). (X40 H&E).

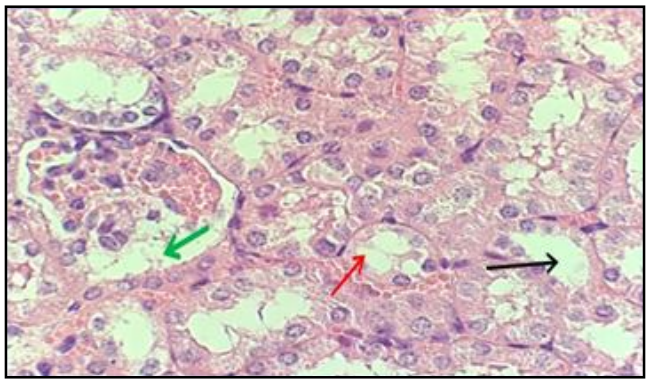


Fig. 10: Cross section of a mice kidney from the G2 : An atrophy of the renal glomerulus (green arrow) is observed , expansion of the Renal Convoluted Tubules (black arrow) with clear degeneration and dissociation of the epithelial cells lining the urinary tubule (Red arrow). (X40 H&E).

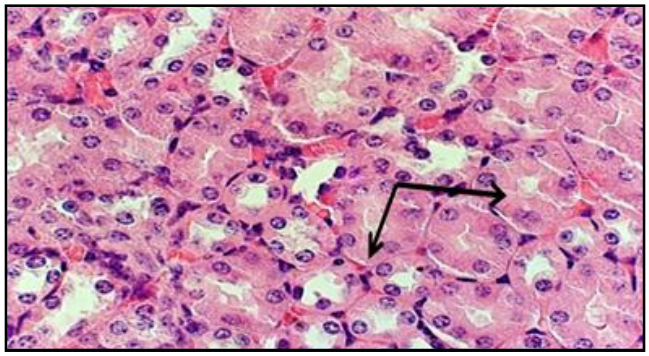


Fig. 11: Cross section of a mice kidney from the G3 : The renal tubules are natural and compact (black arrows). (X40 H&E).

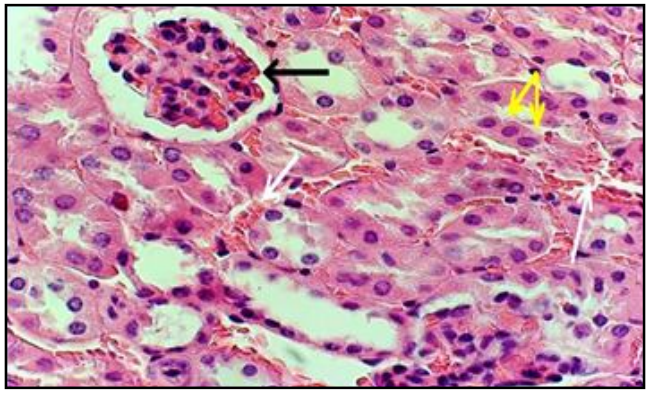


Fig. 12: Cross section of a mice kidney from the G4 : It appears as normal glomerulus (black arrow), slight hemorrhage in some areas (white arrows) and twisted renal tubules appear lined with cells natural cubed (yellow arrows). (X40 H&E).

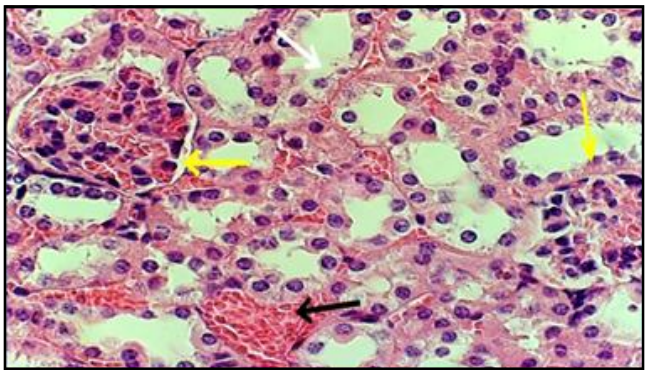


Fig. 13: Cross section of a mice kidney from the G5 : Hemorrhagic observed (black arrow), slight degeneration in the cells lining the renal tubules (white arrow) and the glomeruli appear round and normal (yellow arrow)). (X40 H&E).

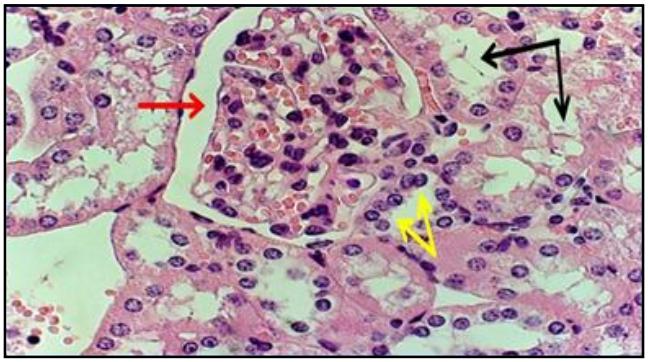


Fig. 14: Cross section of a mice kidney from the G6 : A slight shrinkage of the glomerulus is observed (red arrow) , hyperplasia of the cells lining the tubules (yellow arrows) and simple expansion of some renal tubules (black arrows). (X40 H&E).

free of pathological changes in the kidney tissue as shown in Fig. 7 where the natural structure of glomerulars and renal tubules appear naturally compact and lined with epithelial cells with circular nuclei. As for the cross section of the kidney Fig. 9, G3 gavaging with the *P. ostreatus* Filtrate, no changes were observed in the tissue of the kidneys the cells are normal and have not been degraded, The renal tubules appear natural and compact. The results of the examination of the mice in the group gavaging with *A. niger* fungu sand *P. ostreatus* filtrates indicated the appearance of natural and round glomeruli and high cellularity, simple hemorrhage in some areas and renal tubules and appear lined with normal cubic cells as shown in the Fig. 10. The group gavaging with *A. niger* filtrate and calcium citrate hemorrhage is observed in some areas ,simple degeneration in the cells lining the renal tubules and the glomeruli appear round and natural as shown in Fig. 11 and in the last group treated with two fungus filtrate with calcium citrate Fig. 12 showed a simple glomerular shrinkage, hyperplasia of the cells lining the tubules and slight expansion of some renal tubules indicating the ability of both treatments to reduce or limit the effect of the toxic fungus filtrate in the laboratory animal.

Conclusion

The ability of *A.niger* tested strains to production Mycotoxins, especially Fumonisin B2 , the pathogenic *A.niger* filtrate caused high histological and biochemical toxicity effects in male and female white mice, *p. ostreatus* filtrate and calcium citrate reduced effects of Mycotoxins FB2that produced by *A. niger*.

References

Akiyma, H. and D.Y. Chen (1999). Simle HPLC determination of aflatoxin B1. B2. G1. G2. in nut and corn, *J. of food Hygienic society of Japan,* **37(4):** 195-201.

Al-Badri, Dalia Abdual-Kareem (2013). Teratogenic and Toxic Effects of Fumonisin B1 in Mice and Its Detoxification by Cabbage Seed Extract . PhD thesis. College of Veterinary Medicine University of Baghdad.

Al-Esawey, N.S. (2003). Effect of experimentally induced on hypothyroidism adult male mice fertility. Higher Diploma thesis. High Inst Embry Res. O. Infertility treatment. Univ. Baghdad. Iraq.

Al-Khafajy, Nada A. Fayrouz (2017). Molecular characteristics of *A.niger* strains that Mycotoxins producing and contaminated feed grain and the possibility of controlling it laboratory. Master Thesis - College of Science - Al-Qadisiyah University.

Al-Loubawi, Abeer Abdul Zahra Jabbar (2016). Phenotypic and molecular characterization of *Fusarium verticillioides* fungus from maize (Fum1 and Fum6) which associated with Fumonisin toxin and test the effectiveness of some chemical compounds in the growth of fungus isolates. PhD thesis. College of Agriculture - University of Kufa.

Bamanikar, S.A., A.A. Bamanikar and A. Arora (2016). Study of Serum urea and Creatinine in Diabetic and non-diabetic patients in in a tertiary teaching hospital. *Journal of Medical Research,* **2(1):** 12-15.

Carlson, D.B., D.E. Williams, J.M. Spitsbergen, P.F. Ross, C.W. Bacon, F.I. Meredith and R.T. Riley (2001). Fumonisin B1 promotes aflatoxin B1 and *N*-methyl-N'-nitrosoguanidine initiated liver tumors in rainbow trout, *Toxicol. Appl. Pharmacol.,* **172:** 29-36.

Gelderblom, W.C.A., K. Jaskiewicz, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vleggar and N.P. Kriek (1988). Fumonisins-novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*, *Appl. Environ. Microbiol*., **54:** 1806-11.

Howard, P.C., R.M. Eppley, M.E. Stack, A. Warbritton, K.A. Voss, R.J. Lorentzen, R.M. Kovach and T.J. Bucci (2001). Fumonisin B1 carcinogenicity in a two-year feeding study

using F344 rats and B63C3F1 mice, Environ. *Health Perspect.,* **109:** 277-82.

Huffman, J., R. Gerber and L. Du (2010). Recent advancement in the biosynthetic mechanism for polyketide-derived mycotoxins. *Biopolymers,* **93:** 764-776.

Ibrahim, Diaa Khalil and Hammoudi, Sonbol Jassim and Al-Mashhadani Mohammed A. Shwail (2006). Addition effect The *P. nostreatus* to the feed of male broilers in some physiological characteristics and productivity Department of Animal Resources - College of Agriculture - University of Baghdad. *Iraqi Science Journal,* **3(19):** P. (29-20).

Javed, T., G.A. Bennett, J.L. Richard, M.A. Dombrink-Kurtzman, L.M. Côté and W.A. Buck (1992a). Mortality in broiler chicks on feed amended with a *Fusarium proliferatum* culture or with purified fumonisin B1 and moniliformin, Abst. 106th AOAC *Int. Ann. Mtg.,* Aug 31–Sept. **3:** 1992, Cincinnati, OH, p. 232.

Jens, C. Frisvad, Jørn Smedsgaard, Robert A. Samson, Thomas O. Larsen and Ulf Thrane (2007). *Journal of Agricultural and Food Chemistry,* **55(23):** 9727-9732.

Kumar, V., M.S. Basu and T.P. Rajendran (2008). Mycotoxins research and mycroflora in some commercially important agricultural commodities. *Crop Protection,* **27:** 891-905.

Mohammed, Sinai Waleed (2015). Detection of *Fusarium* Species Producing Fumonisin B1 on Maize Using Molecular Methods and Study Its Histopathological Effects on Albino Mice. PhD thesis. College of Science for Women - Baghdad University.

Mohammed, S.W., Khalid A. Habib, Salim R. Al-Obaidie, Hanan J. Nattef and Noorulhuda Khalaf (2017). Determination of the Toxicity of Fumonisin B1 on Male Albino Mice. Tropical Biological Research Unit, College of Science - University of Baghdad.

Mutouq, Zahraa Yusef Khudair (2005). The effect of some fungi on physiological parameters and changes Pathological histopathology of the white rat and the role of the fluoride biocide in protecting rice quotient from infection to it. Master Thesis. College of Science - University of Kufa. 41 pages.

Noonim, P., W. Mahakarnchanakul, K.F. Nielsen, J.C. Frisvad and R.A. Samson (2009). Fumonisin B2 production by *Aspergillus niger* in Thai co_ee beans. *Food Addit. Contam.,* **26:** 94-100.

Rajani, K.G., A.L. Zhenyuxu, S.L. Bertrand and M.A. Steinmetz (2012). Extensive Degradation of RNA Precursors by the Exosome in *Wild-Type Cells,* **48:** Pages 409-421 Issue.

Schiefer, W.C. (1980). Statistics for the biological sciences. 2nd ed. Addison. Wesley publ Comp. California, London.

Timmos, Sulav Hamid (2015). Molecular diagnosis of *Pleurotus ostreatus* and efficacy Some industrial waste in the production and storage capacity. PhD thesis. College of Education - Al-Qadisiyah University.

Voss, K.A., C.W. Bacon, F.I. Meredith and W.P. Norred (1996). Comparative subchronic toxicity studies of nixtamilized and water-extracted *Fusarium moniliforme* culture material, *Food Chem. Toxicol.,* **34:** 623-32.

Wang, H. and X. Zhang (2000). Recent progress in the study of common *Fusarium* mycotoxon apoptosis of cells. *Wei Sheng Yan Jiu.,* **29(3):** 181-193.

WHO, World Health Organization (2000). Fumonisin B1. (Environmental Health Criteria 219), International Programmed on chemical safety, World Health Organization, Geneva, 1-87.

Yazar, S. and G.Z. Omurtag (2008). Fumonisins, Trichothecenes and Zearalenone in cereals. *Internatio. J. Molecul. Scien.,* **9:** 2062-2090.