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RESEARCH ARTICLE

Study the Expression of Tumor Growth Factor β (TGF β) in Mice Organs after Exposure to Aflatoxin B1 using Immunohistochemistry Technique

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Abstract

Background: Aflatoxin B1(AFB1) had high toxicity and carcinogenecity effects on human and animals tissues and capable of affecting both the cellular and humoral immune responses resulting in impairments in cellular immunity hence decreasing the host resistance to infections. Objectives: The current study aimed to evaluate the effects of purified aflatoxine B1 on the expression of TGF ß in liver, spleen and kidney of experimental mice and estimate it's concentration by using immunohistochemistry IHC technique. Methodology: Mature Swiss albino mice (male) were used in this study, animal were provided from the National Centre for Drug Control and Research /Baghdad. To study the expression of Transforming growth factor beta (TGF-ß) in mice, sixteen mice (divided into four groups) were orally admenestred with (9mg/k.g.b.w., 6mg/k.g.b.w. and 3mg/k.g.b.w.) of purified aflatoxin B1 (isolated and extracted from *Aspergillus flavus*) with control group (without any treatments) and the mice of each group were sacrificed after two weeks. The blood samples of the mice were collected and samples from liver, kidney and spleen were dissected out from each mouse. The expression of TGF ß in these organs was estimated using immunohistochemical technique. Results: Immunohistochemistry technique revealed that an increase in the expression of TGF ß in mice liver, kidney and spleen in comparison with control group. The concentration of TGF ß increased with the increase in the concentrations of purified AFB1 that had been given to the treated mice. The highest expression and concentrations of TGF ß were investigated in liver, spleen and kidney respectively after treated the mice with 9m.g. /b.w. (90%) concentration of purified AFB1 which represented the affected toxic dose of this toxin. AFB1 at concentrations 6m.g/b.w. (60%) and 3m.g/b.w. (30%) also increased the expression of TGF ß but lesser than (90%).Conclusion This study can be concluded that purified aflatoxin B1 (9mg/k.g, 6mg/k.g and 3mg/k.g) which represented (90%, 60% and 30%) concentrations respectively were elevated the expression of TGF-ß of mice tissues that treated with these concentrations after comparison with the control. The expression of TGF-ß in mice organs increased with the increased in the concentrations of purified aflatoxin B1 using immunohistochemistry technique. AFB1 90% concentrations represented the affected toxic dose and had the highest effectiveness onto the expression of TGF-ß in mice organs (liver, spleen and kidney) after treated with this aflatoxin and the highest expression of TGF-ß was noticed in liver, spleen and kidney respectively. Further investigations are required to evaluate the acute and /or chronic effect of aflatoxin B1 on other cytokines expression with determining the histopathological studies of acute and /or chronic effect of aflatoxin on other organs such as lung,muscles, heart, brain, spleen and intestine are required.

Keywords: *TGF-β3, Gliotoxin, immunohistochemical technique*

Introduction

Aflatoxins are the most important widespread classes of mycotoxins which represent potent carcinogens in association with hepatitis B virus and responsible for many thousands of deaths of human mostly in non-industrialised tropical countries. *Aspergillus flavus* and *Aspergillus parasiticus* metabolite these mycotoxins and exist in nature world widely. The common aflatoxins are B1, B2, G1 and G2. The aflatoxin B1 is the most toxicity among mycotoxins followed by G1; the toxicities of B2 and G2 are relatively week.

Because of the high toxicity and carcinogenicity to human and animals, many countries and regulatory agencies impose strict limits on aflatoxins [1, 2]. TGF-β was originally named because of its ability to stimulate fibroblast growth in soft agar; but it can also serve as a potent inhibitor of epithelial cell proliferation and macrophage [3]. This cytokine is virtually secreted by all type of cells and has overlapping receptor usage [4]. The elevation in production and activation of latent TGF-β have been linked to immune defects associated with malignancy and autoimmune disorders, to susceptibility to opportunistic infection, and to the fibrotic complications associated with chronic inflammatory conditions.

TGF-β1 is produced by every leukocyte lineage, including lymphocytes, macrophages, and dendritic cells, and its expression serves in both autocrine and paracrine modes to control the differentiation, proliferation, and state of activation of these immune cells .TGF-β3 was isolated from a cDNA library of human rhabdomyosarcoma cell line; It's mRNA is present in lung adenocarcinoma and kidney carcinoma cell lines; interestingly, umbilical cord expresses very high level of TGF-β3 [5, 6].

The essential function of TGF-β3 in normal palate and lung morphogenesis and implicated in epithelial-mesenchymal interaction. TGF-β3 signaling controls a diverse set of functions mainly those related to cell proliferation, development, differentiation, adult hemostasis and disease TGF-β3 also plays an essential role in controlling the development of lungs in mammals, by regulating cell adhesion and Extracellular matrix (ECM) formation; Lung tissue is one of the tissues that express TGF-β3 in significant levels [7, 8]. Therefore, this study aimed to evaluate the effects of purified aflatoxine B1 on the expression of TGF β in liver, spleen and kidney of experimental mice and estimate it's concentration by using immunohistochemistry IHC technique.

Material and Methods

Aflatoxin B1 Production and Extraction

Fungal Isolate and Preparation of Spore Suspension of *Aspergillus flavus* Isolates

Pure culture of a single isolate of *Aspergillus flavus* which isolated from patients with aspergillosis and maintained in SDA medium (slants and Petri dishes) sealed with parafilm.

It placed in refrigerator at a temperature from 4 to 8 °C as stock culture, and every two months the fungi was transferred to other plate [9]. After the isolate was cultured on SDA at 28 °C for 7 days, fungal colonies were removed by adding 7 ml of distilled water and a drop of tween 80 per slant. Then the suspensions were prepared by gently agitation of the surface with a loop. The slant was shaken to give a uniform suspension of spores. The spore suspension was filtered through sterile gauze, and then the filtrate was transferred to a sterile test tube. Inoculums quantification was made by counting the spores using haemocytometer by added one drop of the suspension to haemocytometer by Pasteur pipette, spores were calculated under high power 40X of light microscope using the following equation:

Concentration of spores / ml = X * 4 * 106 / N

Where:

X = total number of spore count

N = total number of small square of hemocytometer

4=number of large square of hemocytometer

10^6= dilution factor

The tubes of suspension were used to inoculate each 25g of substrate [10].

Aflatoxins Production (in Rice Medium)

The production of aflatoxin from A. flavus was done according to the method described by [11, 12].

Extraction of Aflatoxins

After incubation time, the moldy rice was soaked overnight with 75 ml of chloroform 99.45% in dark place. Then, the soaked medium was homogenized with electric homogenizer for 15 min. The extracted solution was filtered through gauze then, through a Whatman No.1 filter paper. The residue of moldy rice was washed with 50 ml of chloroform, and filtered. Fractions of chloroform were pooled and dryness by evaporation was made at 50°C. The extract (sticky paste) stored at 4°C until use [13].

Preparation of Aflatoxin Standards B1

It was prepared by diluting in absolute methanol, 10mg/ml for B1. Then 100 µl aliquot of aflatoxin solution was mixed well in a 2ml vial. The mixture was diluted in series to 100,000 folds in water: methanol (7:3v/v) then stored at -70 ºC in deep freeze until using [14].

High Performance Liquid Chromatography (HPLC) Analysis

This experiment was conducted as recommended by Horwitz [15]. It was done at the Ministry of Science and Technology / Environment and Water Research and Technology Directorate (EWRTD). The conditions for the HPLC analysis were as follow:

- Instrument: Sykam- Germany
- Analytical column: C18 (30 cm x4.6 mm)
- Injection volume : 20 µl
- Detection- EX :365 EM : 455
- The mobile phase: ACN: H2O (40:60)
- Flow rate: 1 ml/min.
- Temperature: 30ºC.

As mentioned in (2.1.d) the standard of aflatoxins B1 was dissolved in methanol and injected for the HPLC analysis to determine retention time RT and relative peak area. The concentrations of aflatoxins in crude extract were calculated according to the following equation: Cs.Ast= CstAs.

CSt. = concentration of standard aflatoxin (ppm)

ASt = peak area of standard aflatoxin (count)

CS. = concentration of aflatoxin sample (ppm)

AS. = peak area of aflatoxin sample (count)

Determination of the Toxic Effects of Aflatoxins

According to [16] and depending on the results of a preliminary experiment, the 9mg /kg b. w of aflatoxins B1 purified extract was determined as a toxic effect dose which represented 90% concentration of aflatoxin. From this concentration, 60% and 30% concentrations were also used which represented 6mg/kg. b.w and 3mg/kg.b.w respectively of purified aflatoxin B1.

Experimental Animals

Sixteen of Swiss albino mice (male) were purchased from the National Centre for Drug Control and Research /Baghdad were used, their ages were ranged (10-12) weeks. The mice were acclimatized for two weeks before treatment. They housed in plastic cages containing hard wood chips for bedding, in controlled animal house at 25± 2Cº, 4/10 hour's light / dark cycle. The animals were given water and fed with suitable quantity of complete diet. They were housed at the animal house in Biotechnology Research Center/ Al- Nahrain University.

Experimental Design

The sixteen mice were orally administrated, divided into four groups as shown below:

Group 1: Control in which animals without any treatments.

Group 2: Animals were gavaged with purified extract of aflatoxins B1 (9 mg / kg b.w.) which (represented 90% of concentration) two times in a week (for two weeks).

Group 3: Animals were gavaged with purified extract of aflatoxins B1 (6mg / kg b.w.) which (represented 60% of concentration) single dose daily (for ten days).

Group 4: Animals were gavaged with purified extract of aflatoxins B1 (3mg / kg b.w.) which (represented 30% of concentration) single dose daily (for ten days).

Preparation Tissues for Immunohistochemical Technique

Tissue Processing and Sectioning

Tissue processing, sectioning were carried out according to [17] At the end of each experiment, and after taking blood samples the mice were dissected. Samples from liver, kidney and spleen were dissected out, and washed in saline solution for removal blood.

Then the organs were fixed in plastic containers containing 10ml of formalin 10 % for 24 hours. After that the organs samples were dehydrated for one to two hours in each ascending concentration of alcohol (70%, 90% and 100% v/v). The dehydrated tissues were cleared in xylene for one to two hours.

Then the samples were submerged in melting paraffin wax for one to two hours, left to cool and embedded in paraffin wax and cut by a microtome into section of 4-5 µm thickness. The sections were floated out in a warm water bath to lay on slides and dried on oven 50-55 ° C.

Estimation of TGF-β using immunohistochemical Technique

Evaluation of TGF in the affected liver, kidney and spleen for immunohistochemical (IHC), the sections were deparaffinized in the hot air incubator at 80ºC for 70 min using adhesion microscope positively charged slides and then rehydrated in graded alcohols. At room temperature backed slides were immersed sequentially for the indicated times in the following solutions: Xylene for 30 minutes, Fresh xylene for 30 minutes, absolute ethanol for 5 minutes, 90% ethanol for 5 minutes, 70% ethanol for 5 minutes, 50% ethanol for 5 minutes and distilled water for 5 minutes.

By using polyclonal anti-TGF-β antibody these sections were subjected to IHC evaluations. Cooling of slides were made for 20 minutes at room temperature, then marked the edges surrounding the sections by a pap pen liquid blocker to avoid the distribution of the materials out of the sections during the run of the IHC staining. To avoid the drying of the samples the slides were transferred quickly to the strainer racks.

Hydrogen Peroxide were added (enough drops) and blocked then incubated for 10 minutes and washed two times in buffer then Protein block 20µL was applied and incubated for 10 minutes at room temperature in order to block the nonspecific background staining then washed one time in buffer. Primary antibody (anti-TGF-β) about 40µl was applied onto the tissue section and incubated for 30 minutes at 37˚C in a humid chamber.

The slides were drained and blotted gently and then transferred to refrigerator for 24 hrs. After 24 hrs, the slides were placed in washing buffer bath for 5 min, drained and blotted gently, and 20µl of the secondary antibody (the complement) was applied onto the sections, and the slides were placed in a humid chamber and incubated at 37ºC for 10 min, rinsed and placed in washing buffer bath as before, excess buffer drained and blotted gently. After that, 20µl of HRP conjugate was placed onto each section of tissue and incubated for 15min at 37˚C in a humid chamber; the slides were placed in washing buffer bath for 5min, drained and blotted gently.

DAB Chromogen was added to DAB Substrate (one drop to 50 drops) then mixed by swirling, and then applied to tissue and incubated for 1-10 min, then rinsed 4 times in buffer. The slides were immersed in a bath of Mayer's Hematoxylin for 1 min and washed three times in distilled water, 1 min each; then drained and blotted gently and dehydrated by placing the prepared slides in the following solutions: 50% ethanol for 5 min, 70% ethanol for 5 min, 90% ethanol for 5 min, absolute ethanol for 5 min, xylene for 5 min and fresh Xylene for 5 min. Finally, a drop of DPX was applied to the xylene wet sections and covered with cover slips gently to remove excess and air bubbles then left to dry overnight [18].

Evaluation of immunostaining For TGF-β Expression

The expression of TGF-β protein was measured by counting the number of positive cells with brown (DAB) cytoplasmic staining under light microscopy 40X. For the evaluation of TGF-β expression, immunostaining was assessed semi quantitatively using a scoring system for both intensity and extent of staining as shown in Table-1 [19].

Table -1: Quantitative scoring system for TGF-β Immunostaining

TGF-β	Score	Intensity	Stained cells (%)
Negative	0	No staining	<10
Positive	1	Weak	10-30
	2	Moderate	31-50
	3	Strong	>50

Results and Discussion

Identification of *Aspergillus flavus*

The cultural characters of *A. flavus* on SDA medium after incubation at 28°C for seven days showed that the fungus colonies were green or yellowish velvety smooth or powder,

radial or vertical disjointed vary in specifications of isolation to another.

The microscopic features of *A. flavus* after staining with lactophenol cotton blue showed typically radiate conidial head, later splitting to form loose columns, biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose, pale green and conspicuously echinulate as in Figure (3-3). These features were identical to those which mentioned in the key by [20, 21].

Detection of the Types of Aflatoxins in Rice Culture Extract by High Performance Liquid Chromatography (HPLC)

The HPLC analysis for aflatoxins in rice culture extract revealed that, the solvent system composed of ACN: H2O at ratio (40:60v/v) was very satisfactory, because it gives one peak at retention time 6.2 minutes for AF B2 whereas 8.5 minutes for AFB1 in comparison with standard aflatoxins, and the concentration of each was 0.06264 ppm and 2.50503 ppm respectively as shown in figure (1). HPLC technique considered the method of choice for the analysis of a wide variety of compounds. Separation of a mixture into its components depends on different degrees of retention of each component in the column.

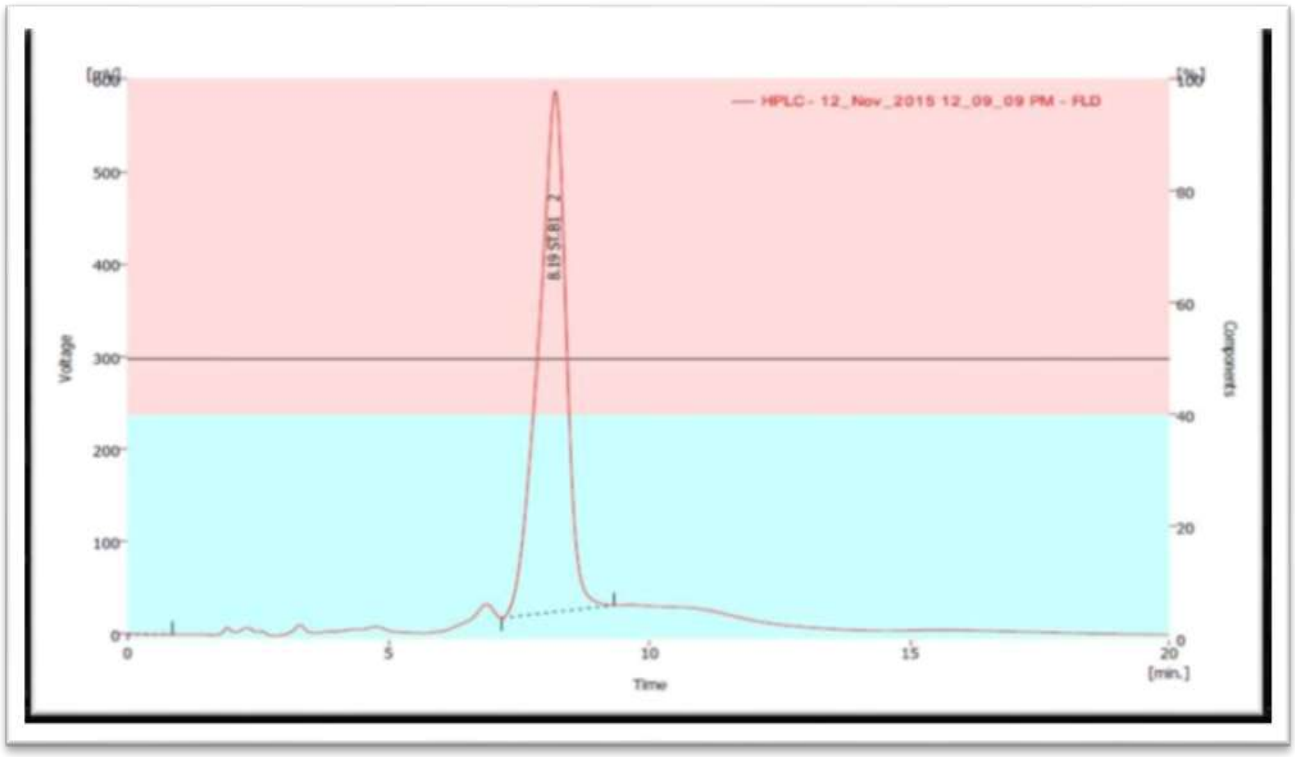
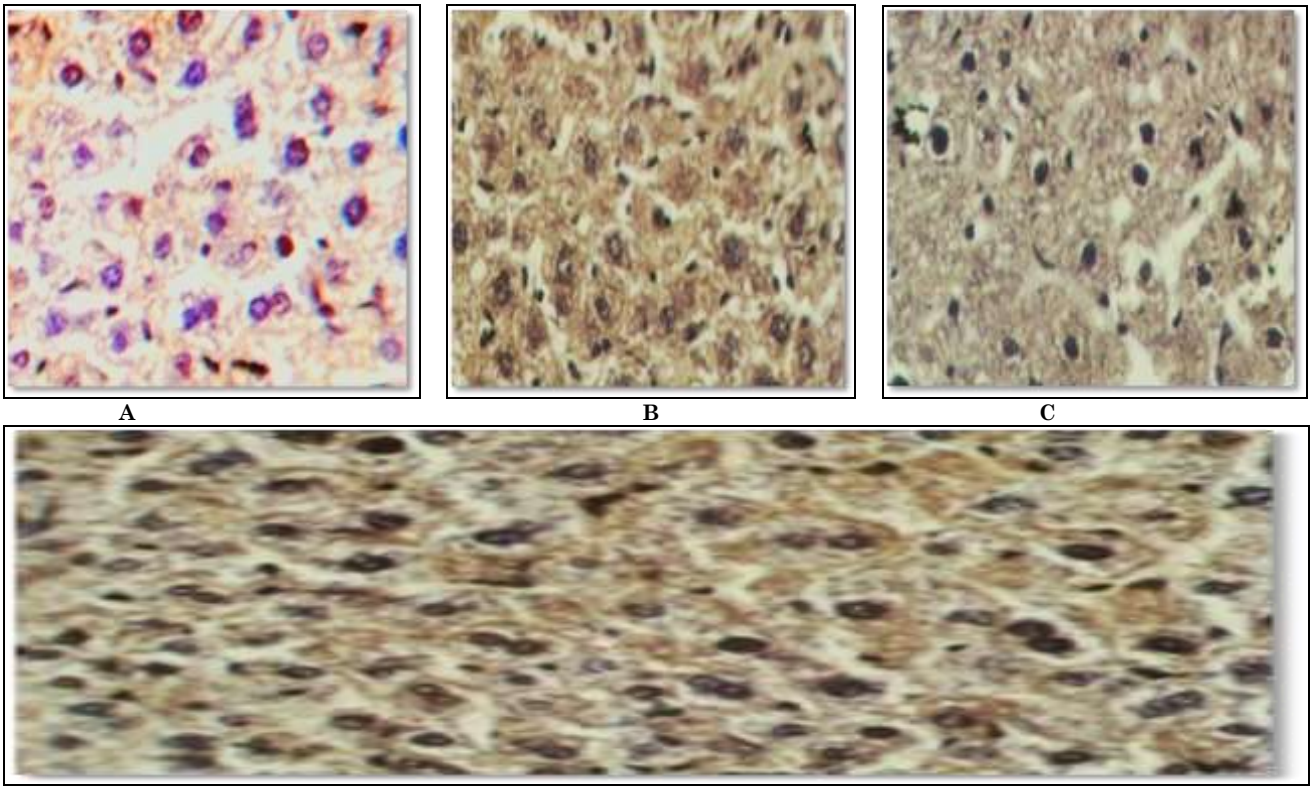


Figure 1: HPLC analysis detection of standard aflatoxin B1, (B1=1.625ppm)
Analytical column: C18 (30 cm x4.6 mm)
Injection volume: 20 µl
Detection- EX: 365 EM: 455
The mobile phase: ACN: H2O (40:60)
Flow rate: 1 ml/min.
Temperature: 30°C.

Estimation of TGF-β using Immunohistochemical Technique

Histological examination of the liver section from control mice showed the normal architecture of hepatocytes (Figure-2a) and the expression of TGF ß from the control mice was shown in table (2) and recorded to be (**75.00 ± 1.52***) while, after treatment with (30%, 60% and 90%) concentrations of aflatoxin B1 and examination of liver from mice treated with AFB1, results shown in figure (2 b,c and d) and table (2), The number of hepatocytes increases with the concentrations of aflatoxin B1 and the expression of TGF ß increases also with the increase in the concentrations of the aflatoxin B1. The highest expression of TGF ß (**151.33 ± 0.88***) was determined after treatment with the affected toxic dose of aflatoxin B1 (90% concentration) 9 mg/kg b.w. The lowest concentration of TGF ß (**89.67 ± 1.45***) was estimated with (30% concentration) of aflatoxin B1.



A **B** **C**

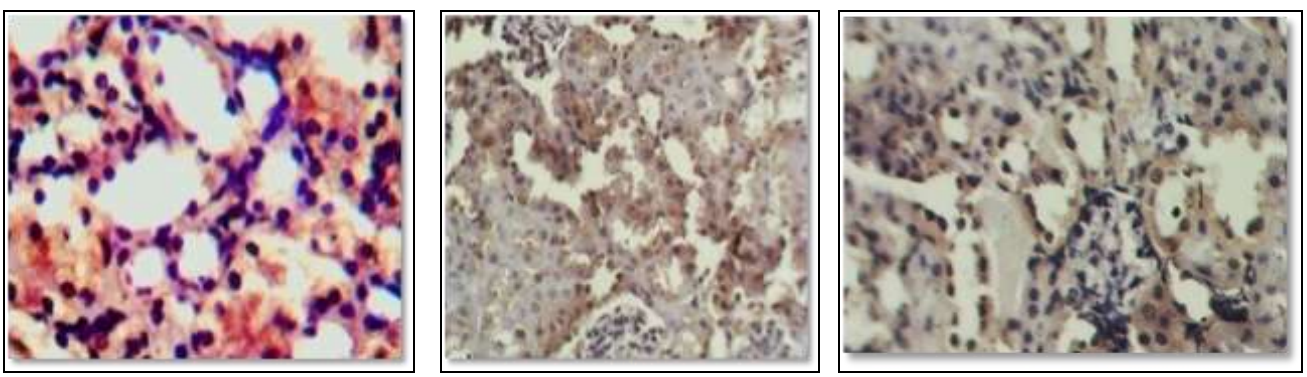
D

Figure (2): Liver sections of albino mice (a) without any treatment (control), (b) Mouse treated with 30% AFB1 single dose daily (for ten days) (H&E stain40X). (C) Mouse treated with 60% AFB1 single dose daily (for ten days). (d) Mouse treated with the effected toxic dose 90% AFB1 two times in a week (for two weeks)

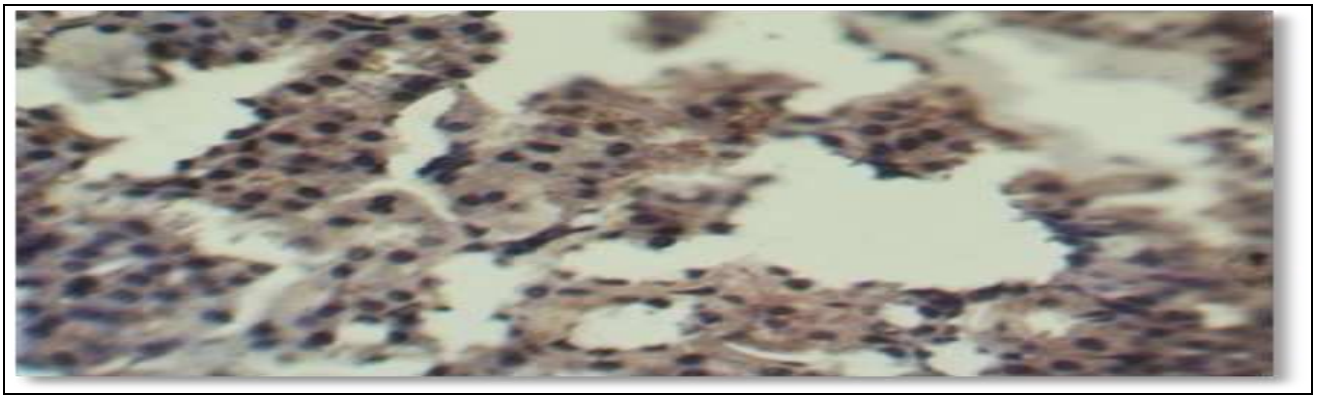
Histological examination of the kidney section from control mice showed the normal architecture of kidney cells (figure-3a) and the expression of TGF ß from the control mice was shown in table (2) and recorded to be (**28.00 ± 1.52***) while, after treatment with (30%, 60% and 90%) concentrations of aflatoxin B1 and examination of kidney from mouse treated with AFB1, results shown in Figure (3 b, c and d) and table (2), the number of cells increased with the concentration of aflatoxin B1 and the expression of TGF ß increases also with the increase in the concentration of the aflatoxin B1. The highest expression or concentration of TGF ß (**83.56 ± 0.38**) was determined after treatment with the affected toxic dose of aflatoxin B1 (90% concentration) 9 mg/kg b.w. The lowest concentration of TGF B (**29.03 ± 0.54**) was estimated with (30% concentration) of aflatoxin B1.

Table 2: Semi quantitative scoring system for TGF-β expression IHC in Liver, Kidney and spleen after mouse treated with 30%, 60% and 90% AFB1.

	Stained cells (%) (Mean± S.E.)			
organs	**Control**	**30%**	**60%**	**90%**
Liver	**75.00 ± 1.52***	**89.67 ± 1.45***	**98.23 ± 0.39***	**151.33 ± 0.88***
kidney	**28.00 ± 1.52***	**29.03 ± 0.54***	**74.33 ± 1.76***	**83.56 ± 0.38**
Spleen	**51.00 ± 1.52***	**54.00 ± 2.08***	**83.33 ± 2.40***	**122.46 ± 1.30***



A **B** **C**

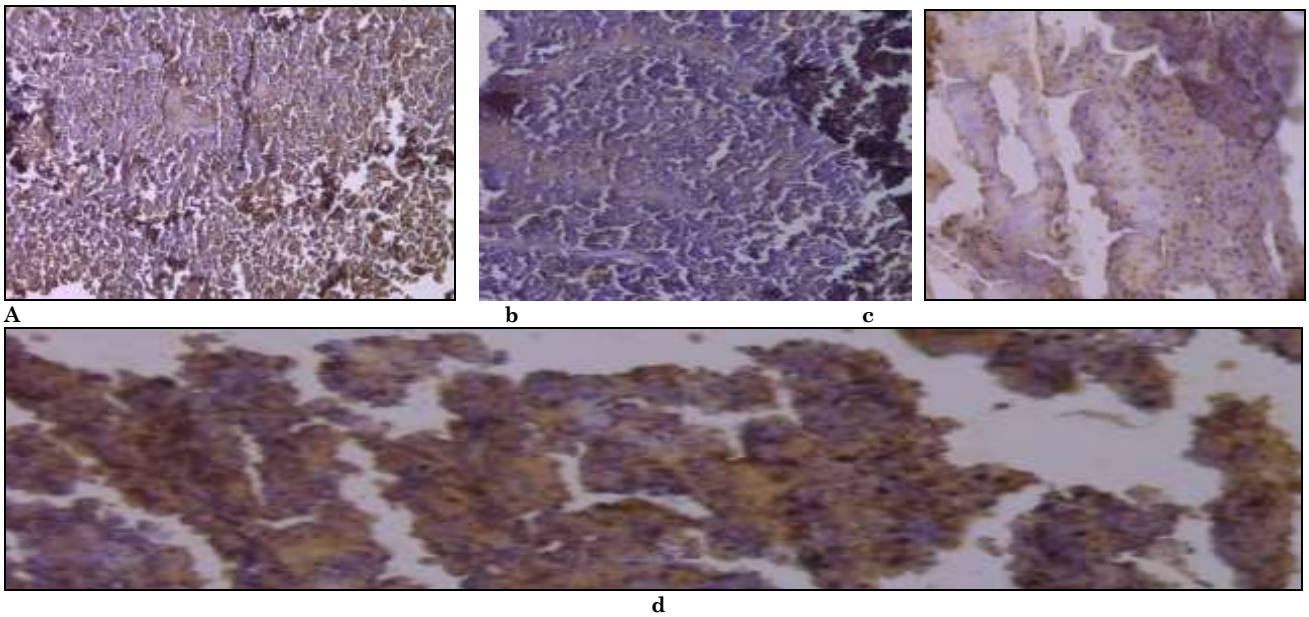


d

Figure 3: Kidney sections of albino mice (a) without any treatment (control), (b) Mouse treated with 30% AFB1 single dose daily (for ten days) (H&E stain40X). (C) Mouse treated with 60% AFB1 single dose daily (for ten days). (d) Mouse treated with the effected toxic dose 90% AFB1 two times in a week (for two weeks)

Histological examination of the spleen section from control mice showed the normal architecture of spleen cells (figure-4a) and the expression of TGF ß from the control mice was shown in Table (2) and recorded to be (51.00 ± 1.52*).

While, after treatment with (30%, 60% and 90%) concentrations of aflatoxin B1 and examination of spleen from mouse treated with AFB1, results shown in figure (4 b, c and d) and Table (2), The number of cells increased with the concentration of aflatoxin B1 and the expression of TGF ß increases also with the increase in the concentrations of the aflatoxin B1. The highest expression or concentration of TGF B (122.46 ± 1.30*) was determined after treatment with the effected toxic dose of aflatoxin B1 (90% concentration) 9 mg/kg b.w. The lowest concentration of TGF ß (54.00 ± 2.08*) was estimated with (30% concentration) of aflatoxin B1. By noticing the levels or expressions of TGF ß in the three organs of mouse as shown in table (2), the highest concentrations or levels of TGF ß (151.33 ± 0.88*, 122.46 ± 1.30* and 83.56 ± 0.38*) were determined in (liver, spleen and kidney) respectively, after treating the mouse with (90% concentration) of AFB1, and these results confirmed that 90% concentration of AFB1 represented the affected toxic dose of this toxin. Results also clarified that liver is the strongest affected organ with AFB1 followed by spleen and kidney.



A b c

d

Figure 4: Spleen sections of albino mice (a) without any treatment (control), (b) Mouse treated with 30% AFB1 single dose daily (for ten days) (H&E stain40X). (C) Mouse treated with 60% AFB1 single dose daily (for ten days). (d) Mouse treated with the effected toxic dose 90% AFB1 two times in a week (for two weeks)

The results of this study showed that there was a high expressions of TGFß in mouse liver, spleen and kidney treated with aflatoxin b1 only due to that aflatoxin B1 has a range of biological activities, including a cute toxicity, teratogenicity, mutagenicity and

carcinogenicity [22], and all these may interfere with normal process of protein synthesis as well as inhibition of several metabolic systems thus causing damages to various organs especially the liver, kidney and heart [23, 24]. The World Health Organization (WHO) classifies AFB1 as a class 1 carcinogen [25, 26]. The extent of toxicity depends on the organ affected especially the liver.

The lethal toxicity of aflatoxin B1 varies in different animals from extremely susceptible (Sheep, Rat, Dog) to resistant species (Monkey, Chicken, Mouse). However, there is no toxicity in humans though epidemiological data from studies in Africa, South Africa, South East Asia and India implicate aflatoxins in the incidence of liver cancer especially the hepatobiliary carcinoma and death of children due to malnutrition, kwashiorkor and marasmus [27; 28].

Aflatoxins have been associated with various diseases like aflatoxicosis and other health problems in humans, livestock and domestic animals globally. All species of animals are susceptible to aflatoxicosis and the susceptibility of individual animals to aflatoxicosis varies considerably depending on dose, duration of exposure, species, age, sex and nutrition. AFB1, AFB2 and AFM have been detected in liver, gall bladder, spleen, heart, muscle and kidney of growing swine when protein and protein-free portions of the diet were separately fed [29].

Aflatoxin's cancer causing potential is due to its ability to produce altered forms of DNA adducts. The primary disease associated with aflatoxin intake is hepatocellular carcinoma (HCC, or liver cancer). Liver cancer has an increasing incidence that parallels the rise in chronic hepatitis B (HBV) and hepatitis C (HCV) infection [30, 31].

Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) can progress to advanced liver disease, including cirrhosis. Aflatoxin B1 is a potent liver carcinogen in a variety of experimental animals. It causes liver tumours in mice, rats, fish, marmosets, tree shrews and monkeys following administration by various routes. Types of cancers described in research animals include hepatocellular carcinoma (rats) colon and kidney (rats), cholangiocellular cancer (hamsters), lung adenomas (mice), and osteogenic sarcoma, adenocarcinoma of the gall bladder and carcinoma of the pancreas (monkeys) [32,34].Chronic consumption of aflatoxin-contaminated foods has been reported to cause immunosuppression in both humans and animals worldwide [35; 36].

In human, aflatoxins affect both the cellular and humoral immune responses where they alter immunological parameters in participants with high AFB1 levels resulting in impairments in cellular immunity hence decreasing the host resistance to infections. The exposure to aflatoxin has been shown to cause suppression in the immune system, especially in cell-mediated responses [37,38].

Chronic exposures of individual to aflatoxins decrease the delayed hypersensitivity reactions and the phagocytic efficiency of the phagocytes in birds. Aflatoxins also reduce the cell populations of the thymus; decrease the bone marrow and the red and white blood cells count, numbers of macrophage and the phagocytic activity of the cells [39]. It also depresses the T-cell functions dependent of splenic lymphocytes in mice.

Aflatoxin decreases the concentrations of immunoglobulins IgM, IgG and IgA in birds as well as decrease complement activity in chickens [40 and 41]. The AFB1 in low dose is capable to slightly decrease both mRNA and protein levels of lymphocytic IL-2, IFNγ and it preferentially affects the function of macrophage as well as IL-1α, IL-6 and TNF production by these cells [42,43]. Aflatoxin made suppression to the immune system therefore subjects the individual to high risk of susceptible to infectious diseases like parasitic, bacterial and viral infections [44].The transforming growth factor ß (TGF-ß) family of proteins are a set of pleiotropic secreted signaling molecules with unique and potent immunoregulatory properties.

This cytokine can modulate expression of adhesion molecules, provide a chemotactic gradient for leukocytes and other cells participating in an inflammatory response, and inhibit them once they have become activated. Increased production and activation of latent TGF-ß have been linked to immune defects associated with malignancy and autoimmune disorders, to susceptibility to opportunistic infection, and to the fibrotic complications associated with chronic inflammatory conditions. TGF-ß1 is produced by all lineage of leukocyte, including

lymphocytes, dendritic cells and macrophages, and its expression serves in both autocrine and paracrine modes to control the proliferation, differentiation, and state of activation of these immune cells.

Collectively, TGF-ß inhibits the development of immunopathology to self or non-harmful antigens without compromising immune responses to pathogens [45, 46]. Transforming growth factor ß (TGF-ß) plays a vital role in the initiation and progression of tumor, functioning as both a suppressor and a promoter. TGF-ß exerts systemic immune suppression and inhibits host immunosurveillance.

Neutralizing TGF-ß enhances CD8+ T-cell- and NK-cell-mediated anti-tumor immune responses. It also increases neutrophil-attracting chemokine's resulting in recruitment and activation of neutrophils with an antitumor phenotype. In addition to its systemic effects, TGF-ß regulates infiltration of inflammatory/immune cells and cancer-associated fibroblasts in the tumor microenvironment causing direct changes in tumor cells. [Yang *et al.,* 2010].

Many immune and non-immune cells can produce TGF-ß, but it is always produced as an inactive complex that must be activated to exert functional effects. Thus, activation of latent TGF-ß provides a crucial layer of regulation that controls TGF-ß function. [47].

For these reasons the expression of TGF-ß in this study elevated after affected of mice organs with purified aflatoxin B1. [48], studied the effect of Immunization against Aflatoxin B1 on the Expression of COX-2 in Liver, Kidney and spleen of Rabbits treated with aflatoxin and concluded that the expression of COX-2 was high in liver, kidney and spleen of animals treated with aflatoxin (0.7 mg/kg) only, and the immunization of animals treated with aflatoxin B1 by aflatoxin B1 BSA conjugate (100 µg and 200 µg) make the expression of COX-2 reduce or remove, on the other hand using 200 µg of vaccine was more effective in reduction or removal the expression of COX-2 than 100 µg.[2].

Described the histopathological changes and expression of Transforming growth factor beta (TGF-ß3) in mice liver, lung and kidney exposed to Gliotoxin. They found that in both sample and standard

Gliotoxin groups, the high concentrations of the mycotoxin resulted in over expression of TGF-ß3 in liver. In lung, the expression of TGF-ß3 decreased at 250µgmL^{-1} then increased with the higher GT concentration. While in kidney highest expression of TGF-ß3 was at 250µgmL^{-1}. In addition, mice included in this study were suffering from histopathological symptoms caused by GT.

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