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Research Article

Aflatoxin B1 Quantities in Wheat Grains and Their Pathogenicity on Rats

Mary M. Fletcher[1], M.M. Shahin [2] and Hanaa A. Nasseem [3,*]

[1] Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, QLD 4108, Australia
[2]Queensland Health Forensic and Scientific Services (QHFSS), Australia Hospital & Health Care Laboratory, Queensland, Australia.
[3] Plant Protection Department, Faculty of Agriculture, Tanta University.Egypt.
*Corresponding Author: Hanaa A. Nasseem (hanaa-nassem @agr.tanta.edu.eg)

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Abstract:

Wheat grain samples were randomly collected from various shops and markets in Brisbane City, Queensland, Australia, and were found to be contaminated with *Aspergillus* sp. fungi. The quantification of the Aflatoxin B1 (AFB1) levels was determined by using an ELISA test. Results showed that all wheat grain samples contained high levels of AFB1, ranging between (30.0-40.5) µg/ kg. Biochemical analyses demonstrated that feeding rats with wheat grain samples contaminated by AFB1 for 3 weeks led to significant weight loss and gain enlargement of the liver and kidney of rats, comparing with the control. The quantities of HC and RBC were increased, while the number of WBC was dropped. In addition, the activities of liver enzyme of AST, ALT, and ALP were significantly increased, and the concentrations of kidney marks of urea and creatinine were significantly increased too comparing with the control. Histological analysis showed that liver of control rats exhibited normal morphology. In contrast, the livers of treated rats showed marked disseminated portal fibrosis and congestion, infiltration by inflammatory cells and necrosis. Whereas, kidney of control rats showed typical kidney morphology. While kidney of treated rats showed severe disseminated tubular necrosis, infiltration by inflammatory cells, and disseminated segmental glomerular necrosis. The results confirmed that AFB1 quantities in grains affected seriously on health and made risks through biochemical and histopathological changes in vital organs, liver and kidneys. Effective management strategies and strict regulations should be taken to control Aflatoxin B1 levels in agricultural grains for avoiding risks in food and feed.

1. Introduction

Wheat (*Triticum aestivum*) is a globally cultivated crop, it is the most widely grown as a first crop for its grains comparing with all other grains or food crops, because of its exceptional adaptability to a wide range of conditions around the world. It serves as the primary source of food for both humans and animals. Further, this is an essential source of minerals, nutritional fibers, vitamins, and carbohydrates in huge quantities. Furthermore, it is source of vegetable proteins by 13% (Alassi et al., 2020).

However, wheat grains can be contaminated with fungi upon biological, environmental, and physiological conditions. Some fungi infect cereals and produce toxic compounds which are considered poisonous to humans and animals, these toxins are known as mycotoxins, (Cong et al.,2024).The most important mycotoxins that have an effect on grains, food, and feed are Aflatoxins (AFBS).These toxins are primarily produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Arafat and Khan, 2017).

Aflatoxins are toxic compounds that contaminated agricultural products, particularly grains, and leading to severe health risks in animals and humans upon consumption. AFB1 is the most toxic and prevalent form among the Aflatoxins. The contamination with high percentage of it can lead to immediate loss of life in humans and animals. The adverse effects of AFB1 in humans range from acute hepatotoxicity to persistent diseases collectively with liver most cancers, and immune-suppression (Eraslan et al., 2017).

The aims of this study were to quantify the levels of AFB1 in contaminated grain samples collected from shops and markets in Brisbane city, Queensland, Australia, using ELISA kits. Additionally, the study aimed to evaluate the effects of AFB1 contamination on biochemical parameters and histological changes in rats organs (liver and kidney).

2. Materials and Methods

2.1. Wheat Grain Sample

A total of 10 Kg from Each wheat grain samples were randomly collected from shops and markets in two sits in Brisbane city at Queensland, Australia, known for high contamination rates. Four replicates of wheat grains were collected and stored under storage conditions recommended for human consumption, as outlined in Food and Agriculture Organization (FAO) report, (FAO, 2000).

2.2. AflatoxinB1 Detection in wheat grain samples

The study was carried out at the Toxin Research institution, Forensic and Scientific Services Laboratory, Archerfield City, Queensland, Australia to determine AflatoxinB1 quantities in stored wheat grains samples. Aflatoxins (AFS) quantities were quantitatively determined using an ELISA device (Elisa Plate Reader Statfax Chromate 4300, Sigma Company, England) by using a

test kit specifically designed for AFB1 detection. For each test, 10 grams of wheat grain samples were ground using an electric grinder. The grinder was cleaned and sterilized after each use to ensure sample integrity. From the ground sample, 1 gram was taken and placed in a 50 mL centrifuge tube. Five milliliters of a pre-prepared extraction solution, as specified in the test kit manual, was added to the tube. The mixture was centrifuged at 4000 rpm for 10 minutes at 18°C.After centrifugation, 100 µL of the supernatant was mixed with 700 µL of the re-dissolving solution provided in the test kit. This mixture was stirred continuously for 10 minutes. Finally, 100 µL of the prepared sample was used for analysis. The concentration of AFB1 was calculated according to the instructions provided in the manufacturer's manual for the ELISA test kit.

2.3. Animal Study

Wistar rats (Male weighted 100-150 g) were used in study and were fed with a standard pellet, water and labium (5L79, PMI Nutritaion International, St Louisa, and University of Queensland). The rats were kept in standard conditions (temperature 25-28°C and 12h light/12h dark). Animals were obtained from Charles River Laboratories (Queensland university- Brisbane, Australia). Rats were divided into a control group and an experimental group exposed to AFB1 (administered via diet). The experimental group was fed a diet containing AFB1 at sub-lethal concentrations for 3 weeks.

2.4. Biochemical parameters

The biochemical analyses were conducted at the Biological Research Facility, Forensic and Scientific Services Laboratory, Archerfield, Queensland, Australia. Blood samples were collected at various intervals (1, 2, 4, and 8 weeks post-exposure).

Blood serum measurements included concentrations of various parameters: blood-related measures such as hemoglobin levels, red blood cell (RBC) count, and white blood cell (WBC) count; liver enzyme levels, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP); as well as kidney function markers, specifically creatinine and urea concentrations.*2.4.1. Red blood cells count* (RBC)

RBC counts in blood samples were performed using a 1:200 dilution ratio with RBC diluting fluid (commonly the Hayem's Fluid) which preserves and fixs the red blood cells. A drop of the cell suspension was placed in a specific counting chamber for this motive after blending the blood well with the dilution solution. The red blood cell count (n) was determined within five medium squares. After placing the glass cover on the counting slide, the sample was allowed to rest for 1–2 minutes to enable the erythrocytes to stabilize before calculating the total count, as outlined by (Hassan, 2014).

2.4.2. White blood cells count (WBC)

WBC counting was performed following the method described by Jayaratne et al. (2020). A specialized pipette was used to collect the blood, and then diluted with Turk's solution. The blood and diluents were thoroughly mixed, and a drop of the mixture was placed on the counting slide, discarding the first few drops. The slide was left undisturbed for two minutes to allow the cells to settle. WBC counts were performed by examining the central square of the counting chamber under 40X magnification.

2.4.3. Hemoglobin concentration (HC)

HC was measured by adding 0.2 ml of blood to a test tube containing 5 ml of Drabkin's solution. The mixture was thoroughly blended using a rotary mixer and allowed to stand for 10 minutes. The transmittance was then measured using a spectrophotometer set to a wavelength of 540 nm, after calibrating the device with Drabkin's solution. The hemoglobin level was determined using a standard curve that correlates transmittance with hemoglobin concentration per 100 ml, as described by (John and Lewis, 1984).

2.4.4. Evaluation of Aspartate Amino Transferase (AST)

The AST enzyme activity in blood serum was measured using a kit assay provided by Randox Company (UK). The method utilizes the enzyme's ability to act on the substrate (aspartic acid and alpha-ketoglutaric acid), converting aspartic acid into alpha-ketone (pyruvic acid). This product is then reacted with the reagent 2,4-dinitrophenylhydrazine to form a reddish-brown derivative. The absorbance of the resulting compound was measured at a wavelength of 505 nm using a spectrophotometer, following the procedure outlined by (Kogbe and Adediran, 2003).

2.4.5. Evaluation of Alanine Amino Transferase (ALT)

ALT enzyme activity was evaluated in the blood serum by using a kit supplied by Randox Company (UK).The method involves the enzymatic reaction of alanine and alpha-ketoglutaric acid, converting alanine into pyruvic acid. The resulting pyruvic acid reacts with 2,4-dinitrophenylhydrazine to form a reddish-brown compound. The absorbance was measured at a wavelength of 505 nm using a spectrophotometer, depending on Kogbe and Adediran(2003).

2.4.6. Evaluation of Alkaline Phosphates (ALP)

ALP enzyme activity was evaluated in the blood serum using standard kits supplied by SIGMA (UK) .The measurements were performed with a Reflotron device, according to the instructions of the supplying companies as referred to (Liu et al., 2020).

2.4.7. Evaluation of urea

Urea concentration in blood serum was measured using standard kits supplied by Linear Chemicals (Spain). The analysis was performed with a spectrophotometer, following the manufacturer's instructions, as described by (Moreau et al., 2010).

2.4.8. Evaluation of creatinine

Creatinine concentration was evaluated in the blood serum using custom test kits from BIOLABO (France).The absorbance of the samples was measured at a wavelength of 490 nm and creatinine levels were calcu-

lated according to (Owumi et al., 2020).

2.5. Histological Changes

Histological examinations were conducted at the Biological Research Facility, Forensic and Scientific Services Laboratory, Archerfield, Queensland, Australia). At the end of the study period, the rats were euthanized and representative samples of liver and kidney tissues were collected. Tissue samples were constant in buffered formalin 10% for 3 days. Samples have been subsequently embedded in paraffin, and processed using sequential dehydration techniques. Afterward, microtome sliced tissue sections (4–5 µm) had been constant on charges of microscopic glass slides before Hematoxylin and Eosin (H&E) for microscopic evaluation (Bancroft and Gamble, 2008). Histopathological examination of hepatic and renal tissues was performed using a Leica DM 500 light microscope (Germany). A pathologist, blinded to the experimental groups, evaluated and scored the pathological abnormalities. Images were captured using a Leica ICC50 E digital camera (Germany) attached to the microscope.

2.6. Statistical analysis

Data were expressed as mean ± S.D. of 10 animals per group. The values were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. Values were considered statistically significant at the 0.05 probability level. Using GraphPad Prism version 8.3.0 for Mac, GraphPad Software. The data obtained with the proposed method for quantification of Aflatoxins in wheat were processed by the SAS program (Spector and Cohen-Charash, 2001).

3. Results and Discussion

3.1. AFB1 quantities in wheat grain samples

Table (1) showed the results of ELISA analysis revealed varying quantities of AFB1 across wheat grain samples of wheat which collected from markets and stores in Brisbane city, Queensland, Australia. The results confirmed that the grain samples contained with AFB1 between 30.0 to 40.5 µg/kg. The consequences aligned with (Ramamurthy and Rajakumar, 2016), who determinedAFB1 ingrains with a range of 60 to 70 µg/kg in Sri Lanka. The presence of AFB1 in grains may be production due to infected with the fungus *Aspragillus flavus* in the field or in the store (Rodrigues and Chin, 2012).

The environmental situations which includes temperature and humidity when grains are growing within the field or in store can be consider as the principle reason of generating fungi of *A. flavus* and *A. parasiticus*. Quantities of toxin are metabolic processes in the production of these Toxins (Rotimi et al., 2016).The U.S. Food and Drug Administration (FDA) has established that the permissible levels of AFB1 in grains intended for direct human consumption must not exceed 20 µg/kg. The same limit applies to AFB1 concentration in animal feed, as reported by (Mengyun et al., 2023).

Table 1. Aflatoxin B1 quantities in collected wheat grain samples.

Sample Name	Grain samples	AFB1 Quantities (µg/kg)
Wheat	Goodna shops	40. 5
	Goodna Markets	33.7
	Dara Shops	38. 9
	Dara Markets	30. 0

3.2. Biochemical parameters

3.2.1. The concentrations of blood Parameters

The results in Table (2) showed the effect of feeding rats on contaminated wheat grain samples with different levels of Aflatoxin B1 for 3 weeks on the blood Parameters: RBC, WBC, and HC. The results indicated a significant decrease in both HC and RBC count in the T1 group, with values of 10.433 mg/L and 6.520 (10^6/mm³), respectively, compared to the control group, which recorded values of 11.532 mg/L and 7.532 (10^6/mm³), respectively. In the T2, T3, and T4 groups, the RBC counts were 6.225, 6.355, and 6.312 (10^6/mm³), while HC levels were 10.375, 10.405, and 10.320 mg/L, respectively. These values were lower than those of the control group animals, which had RBC counts and HC levels of 7.532 (10^6/mm³) and 11.532 mg/L, respectively. Additionally, the results showed a significant increase in the total WBC count in animals fed with AFB1 across all concentrations. In the T1 group, the WBC count was 12.875 (10^6/mm³), compared to 11.050 (10^6/mm³) in the control group. The results aligned with (Ramamurthy and Rajakumar, 2016) who found that in India , feeding laboratory animals with AFB1 led to a decrease the number of red blood cells RBC and HC, but increase the number of WBC compared with their values in control group animals. The decrease in the level of the RBC in the rats treated with AFB1may be due to the effectiveness of the toxins in causing anemia, a down-regulation of the activity of erythropoietin, through its ability to destroy RBC causing reducing the number of RBC in the blood. A low HC may be linked to a reduction in erythrocyte volume. Conversely, an elevated WBC count, primarily consisting of neutrophils, could indicate an inflammatory response triggered by these cells, as noted by (Mengyun et al., 2023).

Table 2. The concentrations of blood parameters.

Blood Parameters	Control	Treatments			
		T1	T2	T3	T4
HC (mg/L)	11.532 ±0.20 a	10.433 ±0.10 b	10.375 ±0.15 ab	10.405 ±0.22 ab	10.320 ±0.20 ab
RBC (10^6/mm^3)	7.532 ±0.23 a	6.520 ±0.20b	6.225 ±0.12 ab	6.355 ±0.26 ab	6.312 ±0.22 ab
WBC (10^6/mm^3)	11.050 ±0.53 b	13.175 ±0.46 a	12.450 ±0.30 ab	12.900 ±0.67 a	12.850 ±0.51 a

Different letters within the same column indicate a significant difference at the 0.05 probability level : T1: AFB1 concentration 40.5 µg/kg - T2: AFB1 concentration 33.7 µg/kg - T3: AFB1 concentration 38. 9 µg/kg - T4: AFB1 concentration 30.0 µg/kg.

3.2.2. The activity of liver enzymes

Table (3) showed that the effect of feeding rats on wheat grain samples contaminated with AFB1 for 3 weeks in laboratory for each of groups T1, T2, T3, and T4 on the activity of liver enzymes. The results showed that a significant increase in the activity of liver enzymes for AST, ALT and ALP in treatments T1, T2, T3 and T4. were 50.75, 47.25 , 49.75 and 46.50 mg/L for AST and were 46.25, 41.25, 44.00 and 38.00 mg/L for ALT and were 70.01, 53.31, 58.24 and 51.91mg/L for ALP, respectively compared with in the control group animals which were 45.75 mg/L of AST enzyme, 37.75 mg/L of ALT enzyme, and 49.41 mg/L of ALP enzyme.

The obtained results agreed with (Xiaoqinget al., 2023) in Germany, who indicated that feeding rats with wheat contaminated with AFB1 had an increase in AST, ALT and ALP enzyme activities. The results also agreed with (Waliyar et al., 2008) who showed that feeding chicken on corn contaminated with AFB1 had an increase in enzyme activity in China. The increase in liver enzyme activity may be attributed to liver exposure to AFB1, which causes hepatocyte damage and enhances the permeability of their membranes. This damage leads to the release of liver enzymes into the bloodstream, resulting in elevated enzyme concentrations in the blood serum, as explained by Wang et al. (2020).

Table 3. The activity of liver enzymes.

Liver Enzymes	Control	Treatments			
		T1	T2	T3	T4
AST/GPT (mg/L)	45.75 ±1.43 b	50.75 ±1.88 a	47.25 ±1.75 ab	49.75 ±1.96 a	46.50 ±1.93 b
ALT/GPT (mg/L)	37.75 ±3.03 b	46.25 ±1.11 a	41.25 ±1.65 ab	44.00 ±1.12 ab	38.00 ±1.19 b
ALP/GPT (mg/L)	50.41 ±1.11 b	70.01 ±1.79 a	53.31 ±1.76 ab	58.24 ±1.91 ab	51.91 ±1.88 b

Different letters within the same column indicate a significant difference at the 0.05 probability level : T1: AFB1 concentration 40.5 µg/kg - T2: AFB1 concentration 33.7 µg/kg - T3: AFB1 concentration 38. 9 µg/kg - T4: AFB1 concentration 30.0 µg/kg.

3.2.3. The activity of Kidney function markers

Table (4) illustrated the effect of feeding rats on wheat grain samples contaminated with AFB1 for 3 weeks in laboratory for each of groups T1, T2, T3, and T4 on the activity of kidney function. The results showed that the level of creatinine and urea in the blood serum significantly increased. The values of urea were at 47,50, 48,50, 44,50, and 43.25 mg/ml, respectively and creatinine values were at 0.43, 0.34, 0.38, and 0.32 mg/ml compared with their values in control group animals, which were 40.75 and 0.30 mg/ml, respectively. The results are agreed with (Yilmaz et al., 2018), who indicated that feeding rats by contaminated grains by AFB1 concentration of 76 µg/kg for 29 days were increased urea concentrations from 4.49 to 8.05 mg/ml and creatinine from 17.92 to 35.37 mg/ml. The effect of AFB1 on kidney function could be duo to the cells damage, which caused a decrease in their ability to filter, which caused the excretion of creatinine and Urea in a greater amount than normal levels into the blood (Mengyunet al., 2023). The toxic effects of AFB1 on renal function could be in the increase in plasma creatinine concentrations through an increase in its secretion from the muscle or a decrease in its excretion from the kidneys in general (Owumi et al., 2020).

Table 4. The activity of Kidney functions markers.

Kidney function markers	Control	Treatments			
		T1	T2	T3	T4
Urea (mg/ml)	40.75 ±1.79 b	47.50 ±1.32 a	48.50 ±1.55 a	44.50 ±1.10 ab	43.25 ±1.65 ab
Creatinine (mg/ml)	0.30 ±0.55 b	0.43 ±0.13a	0.34 ±0.16 ab	0.38 ±0.12 a	0.32 ±0.35 ab

Different letters within the same column indicate a significant difference at the 0.05 probability level : T1: AFB1 concentration 40.5 µg/kg - T2: AFB1 concentration 33.7 µg/kg - T3: AFB1 concentration 38. 9 µg/kg - T4: AFB1 concentration 30.0 µg/kg.

3.3. Histopathologicalchanges in rat's kidney and liver

3.3.1. Histopathological changes in rat's liver

Figure (1) showed the histopathological changes in rat's liver which fed on wheat grain samples contaminated AFB1 for 3 weeks in laboratory for each of groups T1, T2, T3, and T4compared with control. The Photomicrographs of liver from rats treated with the light microscopic examination of fixed, H&E-stained section, showed that liver of control rats display typical liver morphology. While liver of treatments rats shown marked disseminated portal fibrosis and congestion, infiltration by inflammatory cells, and necrosis (black arrow).

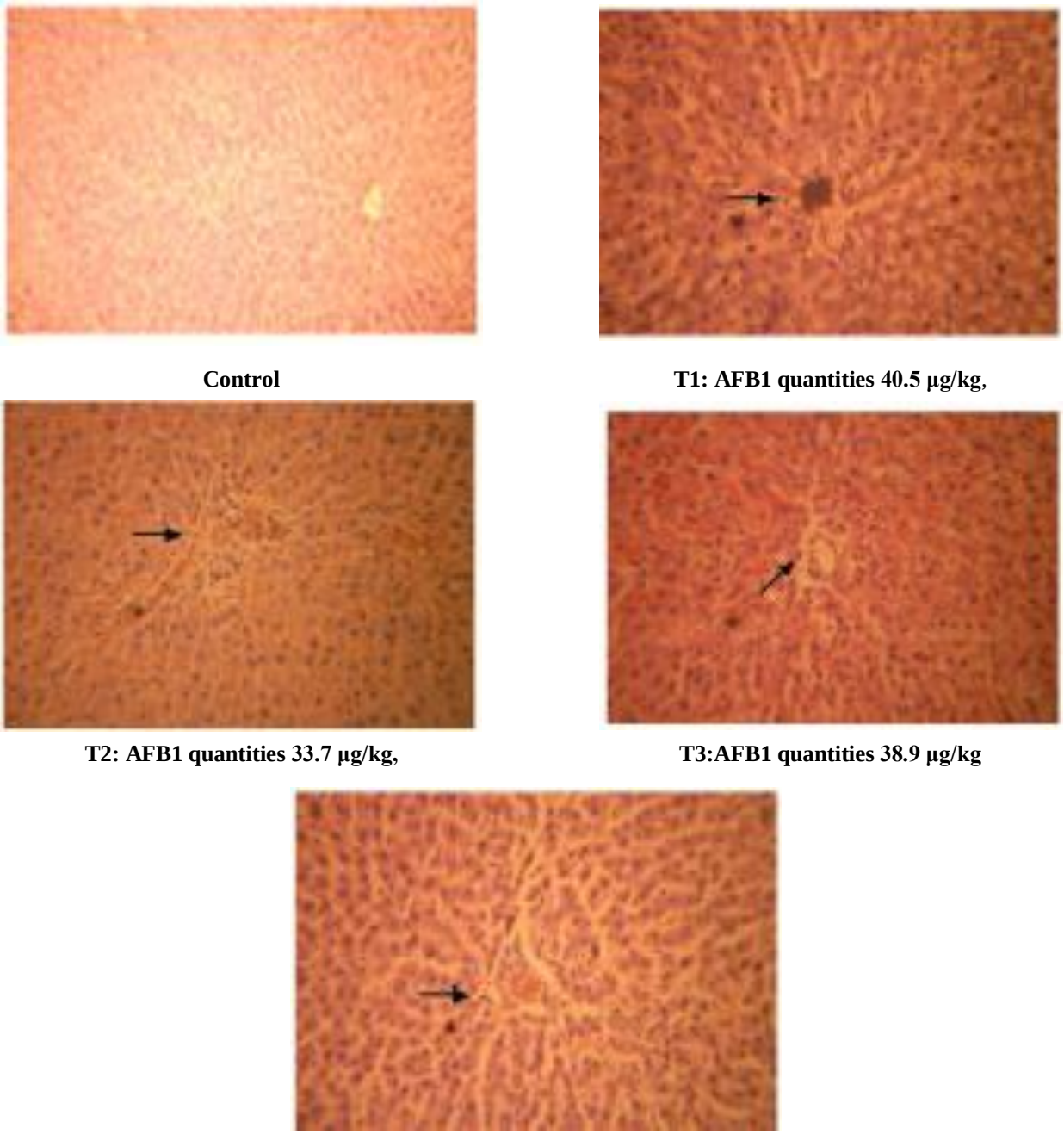
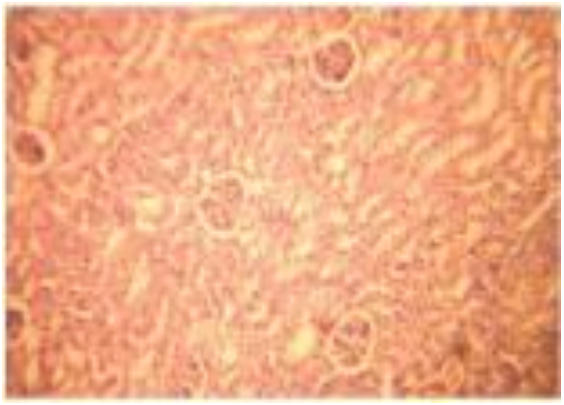


Figure 1. Histopathological changes in liver. Black arrows indicate marked disseminated portal fibrosis, congestion, infiltration by inflammatory cells, and necrosis.

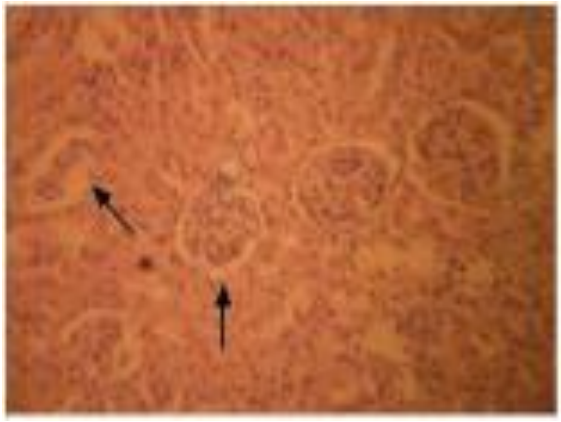
3.3. 2. Histopathological changes in rat's kidney

Figure (2) showed the histopathological changes in rat's kidney which fed on wheat grain samples contaminated AFB1 for 3 weeks in laboratory for each of groups T2, T3, T4, and T5compared with control. The Photomicrographs of kidney from rats treated with the light microscopic examination of fixed, H&E-stained section showed that kidney of control rats show typical kidney morphology. While kidney of treated rats showed severe disseminated tubular necrosis, infiltration by inflammatory cells, and disseminated segmental glomerular necrosis (black arrow).

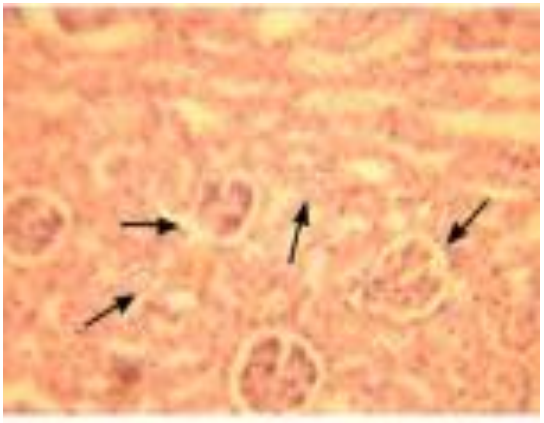


Control

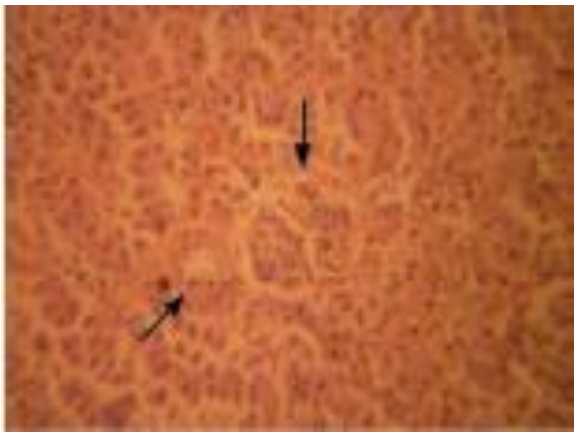
T1: AFB1 quantities40.5 µg/kg,



T2: AFB1 quantities 33.7 µg/kg,



T3:AFB1 quantities 38.9 µg/kg



T4: AFB1 quantities 30.0 µg/kg

Figure 2. Histopathological changes in kidney. Black arrows indicate severe disseminated tubular necrosis, infiltration by inflammatory cells, and disseminated segmental glomerular necrosis.

4. Conclusion

The results confirmed that AFB1 quantities in grains affected seriously on health and made risks through biochemical and histopathological changes in vital organs, liver and kidneys. Effective management strategies and strict regulations should be taken to control Aflatoxin B1 levels in agricultural grains for avoiding risks in food and feed, in addition ignoring bad effects on health.

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