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# Comparative impacts of Saudi Arabian and Egyptian *Aspergillus flavus*-associated Aflatoxin B1 on rats

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**Abstract**

Aflatoxin B1 (AFB1) is a carcinogenic secondary metabolite of filamentous *Aspergillus* species. This study investigated the effects of Saudi Arabian (local) and Egyptian (imported) *Aspergillus flavus*-associated AFB1 on rats (hematological parameters, liver, and kidneys). Saudi Arabian and Egyptian *A. flavus*-associated AFB1 (0.5 mg/kg) were separately fed to two groups of five rats each for 21 days. The control group (5 rats) was fed water and a basal pellet diet for 21 days (a total of 15 rats, 5 rats in each treated group and 5 rats as control). The blood samples from the rats in all the groups were subsequently examined for complete blood count (CBC) data [red blood cell (RBC), neutrophil, and platelet counts; basophil counts; eosinophil, hemoglobin (Hb) content; monocyte, lymphocyte, and mean corpuscular hemoglobin (MCH) counts; mean corpuscular volume (MCV); mean corpuscular hemoglobin concentration (MCHC); and white blood cell (WBC)]. Liver function was assessed by examining the serum levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and albumin in all groups. The kidney function test was based on the levels of uric acid, urea, creatinine, and urea. AFB1-induced histopathological changes were observed under a light microscope. One-way ANOVA coupled with Tukey's LSD test revealed that AFB1 in both isolates (Saudi Arabian and Egyptian) significantly decreased the RBC count, whereas a significant increase in the platelet, WBC, neutrophil, and monocyte counts was noted. Compared with the control treatment, Saudi Arabian AFB1 treatment significantly reduced the MCH and MCHC values. The serum levels of AST, ALP, urea, and creatinine were significantly greater in the Saudi Arabian AFB1-treated group than in the other two groups, whereas the level of ALB was significantly lower in this group than in the

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Egyptian AFB1-treated group and controls. Histological examination of the liver revealed central vein dilatation and congestion of the portal area with leucocyte infiltration in both AFB1-treated groups, which led to substantial cell mortality. Both AFB1 treatments caused hypertrophied hepatocytes with pyknotic nuclei and granular vacuolated cytoplasm. The AFB1-treated groups presented marked renal damage characterized by cupping of Bowman's capsule, glomerular membrane disruption, and tubular damage. However, adverse effects were more severe in the Saudi Arabian AFB1-treated group. Both AFB1 treatments induced hematological and organ toxicity in rats. However, the toxicity of Saudi Arabian *A. flavus*-associated AFB1 was more pronounced than that of Egyptian *A. flavus*-associated AFB1. The findings of the current study may help improve hygiene measures to lower mycotoxin contamination in commercial food products, as well as emphasise the health hazards posed by A. flavus-contaminated household foodstuffs.

**Keywords** – Aflatoxin B1 – *Aspergillus flavus* – Hematology – Kidney – Liver – Rats – Toxicity

## Introduction

It is thought that excessive levels of aflatoxins, particularly aflatoxin B1 in food of non-industrialized countries are a major cause of disease outbreaks due to a lack of knowledge and the consumption of aflatoxin-contaminated food and feed worldwide. Aflatoxin B1 (AFB1) is a carcinogenic secondary metabolite of filamentous *Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus minisclerotigenes*. AFB1 is associated with serious fungal contamination, particularly in subtropical and tropical regions (Rajarajan et al. 2021). *Aspergillus* species grow on agricultural produce and secrete AFB1 during harvesting, storage, handling, and shipment. A hot and humid climate further promotes fungal and mycotoxin growth in food products. Improper storage below optimal conditions during marketing and transportation contributes to mycotoxin and fungal growth as well (Rajarajan et al. 2021). The opportunistic *A. flavus* particularly affects immunocompromised individuals, and its infection is a major source of human aspergillosis after *Aspergillus fumigatus* infection (Amaike & Keller 2011). The viability of *A. flavus* at relatively high temperatures makes it a predominant pathogen in arid climates such as the Middle East, Southeast Asia, and Africa, where it accounts for 50–80% of sinusitis, cutaneous, and keratitis cases in humans (Krishnan et al. 2009).

AFB1, B2, B1, G2, G1, M2, and M1 are major types among a total of 18 identified aflatoxins (Rajarajan et al. 2021). AFB1's association with liver cancer and genetic alterations make it more detrimental. Liver enzymes break AFB1 into AFB1-8,9 epoxide (AFBO), which binds with liver cell DNA to mutate the tumor suppressor *p53* gene, leading to the occurrence of liver cancer (Owumi et al. 2022a,b). AFB1 can promote liver cell mortality and disrupt enzyme function to cause other liver diseases (Yilmaz & Bag 2022). AFB1 is also hazardous for the reproductive system, kidneys, proteins, RNA, and DNA (Owumi et al. 2022a,b, Ashi et al. 2023a). AFB1 is known to initiate interferon-γ (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) mRNA expression in rats (Aleissa et al. 2020, Ashi et al. 2023b). Multiple studies have documented AFB1-linked enhanced rat kidney function and the activities of liver enzymes such as aspartate (AST), alanine aminotransferases (ALT), gamma glutamate transferase (GGT), and alkaline phosphatase (ALP) (Owumi et al. 2020, Owumi et al. 2022a,b, Ashi et al. 2023a). This study investigated and compared the toxicity of a Saudi Arabian *A. flavus* isolate obtained from contaminated bread and a reference AFB1-producing isolate imported from a reference laboratory in Egypt to determine the effects of *A. flavus*-associated AFB1 on hematological parameters and organs (liver and kidneys). This study aimed to investigate the ability and potency of *A. flavus* growing on spoiled bread from local households to produce AFB1 and the possible health hazards associated with these strains when other edible commodities are contaminated. Understanding the differential toxicity of these *A. flavus* strains is crucial for informing food safety policies and public health interventions in affected regions.

## Materials & Methods

### Animals

Fifteen adult male Wistar albino rats (180–200 g and 7–8 weeks old) were obtained from King Fahd Medical Research Center at King Abdulaziz University, Jeddah, Saudi Arabia. During the experimental period (21 days), the rats were kept in sterile and clean polypropylene cages under a 12-hour light/dark cycle at 21–23°C and 60–65% humidity. The rats were fed water and a basic chow diet for two weeks before the start of the experimental process, which facilitated their normal growth, behavior, and adaptation to laboratory conditions. The study was approved by the Ethical Committee of King Fahd Medical Research Center. Jeddah, Saudi Arabia (Approval #163-19). The experimental animals were handled according to the guidelines of ARRIVE.

### Egyptian *Aspergillus flavus* isolate

The AFB1-producing Egyptian *A. flavus* CYA reference strain (AUMC 9779) was provided by Prof. Ahmed Y. Abdelmalek, Moubasher Mycological Center, Assiut University, Egypt. The isolate was supplied on potato dextrose agar (PDA) slants, which were stored at cryogenic temperatures of -80°C and 5°C.

### Saudi Arabian *Aspergillus flavus* isolate

To obtain a Saudi Arabian *A. flavus* isolate, a loopful of spores was scraped from a moldy bread and cultivated on potato dextrose agar (PDA). PDA is composed of potato extract (4.0 g), glucose (20 g) (BDH Chemicals Ltd., England), agar (20 g) (MOLEQULE-ON, New Zealand), and distilled water (1000 ml) (Anon 1988, Ashi et al. 2023a). The agar was dissolved by boiling the mixture followed by sterilization, after which it was poured into plates. The isolate was also cultured on Sabouraud dextrose agar (SDA) (HiMedia, India), and both media were incubated for 5–7 days at 25±2°C (Fakruddin et al. 2015). After the incubation period, a fungal disk (inoculated on *Aspergillus* differential agar) was added to the SDA and PDA plates via a cork borer. *Aspergillus* differential agar was composed of tryptone (15 g) (HiMedia), yeast extract (10 g) (HiMedia), ferric citrate (0.5 g) (British Drug House, England), agar (15 g) (MOLEQULE-ON), and distilled water (1000 ml). The media was boiled and autoclaved before *Aspergillus* inoculation, and the inoculated plates were incubated for 5–7 days at 25±2 °C (Sreekanth et al. 2011).

### Isolate storage

The long-term storage involved culturing *A. flavus* isolates on SDA plates and incubating them for 5–7 days at 25±2°C. A sterile glycerol solution (15% v/v) was subsequently used to inoculate SDA-grown *A. flavus* colonies in sterile microfuge tubes (250 µl) to maintain fungal spores at -80°C (Nielsen & Smedsgaard 2003).

### Preparation of Broth Media

Sabouraud dextrose broth (500 ml, HiMedia) was prepared as per the manufacturer's guidelines, and media aliquots (150 ml) were autoclaved (121°C and 1.5 atmospheric pressure) for 15 min in flasks (250 ml). The flasks were kept in the refrigerator until use.

### AFB1 production, extraction, and confirmation

Aflatoxin B1 was produced by *A. flavus* cultivation (5–14 days, 25±2°C) in SDB under shaking aerobic conditions. A sterile funnel, flask, and filter papers (MN 615-∅ 150 mm) (Macherey-Nagel, Germany) were subsequently used to obtain the culture filtrate, which was collected in conical tubes (15 ml) (Plastilab, Lebanon). Fungal cell walls were weakened by placing the filtrates overnight at cryogenic -80°C, which facilitated solvent penetration into the fungal cells for secondary product extraction (Schmitz et al. 2010). The filtrates were transferred to a 15 ml methanol-containing hydrophilic bottle and subjected to ultrasonic vibration for 30 minutes (Nielsen & Smedsgaard 2003). Then, an orbital shaker (100 rpm) was used to shake the contents of

the hydrophilic bottle for 30 minutes, and the steps were repeated several times. The extract was further filtered through micropore filter paper (0.45 µm) (Bedford, USA) in a sterile glass funnel and concentrated through evaporation under a nitrogen flow. Finally, the extract was dissolved in methanol (1.5 ml) and subjected to GC (Shimadzu, Kyoto, Japan) and HPLC (Shimadzu) analyses (Bertrand et al. 2013). The extracted toxin was compared with a standard crude aflatoxin B1 extract (courtesy of Prof. Ahmed Abdelmalek, Moubasher Mycological Center, Assiut University, Assiut, Egypt).

**Confirmation of AFB1 production**

The ammonia vapour method was adopted to confirm aflatoxin production in both *A. flavus* isolates (Saudi Arabian and Egyptian). This method turns toxin-secreting colonies pink on SDA and PDA culture plates. An ammonia solution (25%) was prepared by adding ammonia (25 ml) to distilled water (75 ml). Ammonia solution (0.2 ml) was placed inside a Petri dish cover and incubated for 24 hours at 25°C, after which it developed a red color at the bottom of the fungal colonies (Saito & Machida 1999).

**Experimental Design**

Fifteen rats were randomly divided into 3 groups (5 each). The control group was fed a basal pellet diet and water, whereas AFB1 (0.5 mg/kg) was orally administered to the other two groups (Saudi Arabian and Egyptian AFB1) for 21 days via a gastric tube and fed a basal diet (Yilmaz et al. 2018).

**Biochemical analysis**

The rats were sacrificed, and blood samples were collected through cardiac puncture after the completion of the experimental period. Blood samples were collected in plain tubes and centrifuged (6000xg, 4°C) for 10 minutes to obtain serum, which was immediately aliquoted and stored at -80°C. Rat-specific ELISA kits (Bender MedSystems Gmbh, Austria) were used to examine the functioning of liver enzymes [aspartate aminotransferase (AST), alkaline phosphatase (ALP), and albumin] and kidneys (creatinine, urea, and uric acid). The plasma from EDTA-treated blood samples was used for complete blood count (CBC), which included red blood cells (RBCs), hemoglobin content, platelets, basophils, eosinophils, monocytes, lymphocytes, neutrophils, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and white blood cells (WBCs).

**Histological examination**

The rats were killed by cervical dislocation after the completion of the experimental period (21 days). The kidneys and liver were immediately isolated, rinsed with water, and incubated in formalin (10%) at room temperature for 24 hours. Then, the organs (kidneys and liver) were removed and subjected to treatment with a graded series of ethanol and xylene followed by paraffin embedding. A rotary microtome was used to separate a section (5 µm) from each paraffin-embedded sample, which was mounted on a slide and stained with hematoxylin-eosin. A light microscope (Olympus) was used to examine the slides for AFB1-induced histopathological changes in the kidneys and liver.

**High-performance liquid chromatography (HPLC) of aflatoxin B1**

Aflatoxin B1 ($C_{17}H_{12}O_6$, MW≈312.27) can be a white powder or colourless to pale yellow crystal. The Beckman system (goldTM) HPLC is composed of solvent modules (127 pumps) and a programmable UV detector. The isocratic mobile chromatography phase was composed of $H_2O$ and MeOH (80%:20% v/v). The mobile phase was filtered through a guard column-protected reversed-phase column (5 µm) (LiChrosphere® C18; 250 mm × 4 mm nonpolar) and pumped to waste (1 ml/min). The injection loop volume was 10 µl, whereas the wavelength was adjusted to 375 nm. The liquid mobile phase was pushed through the solid stationary phase column by frequently

applying pressures ranging from 50–350 bar. The pumps were primed, and the first run was performed with methanol to remove impurities from the injection loop and column. Then, freshly prepared aflatoxin B1 samples (100 µl) were injected for HPLC analysis.

**Gas chromatography (GC) analysis**

GC analysis was performed with a Shimadzu Chromatopac C-R4A chromatogram integrator-fitted Shimadzu model GC15 (Japan) containing a fused silica capillary column with a chemically bonded phenylmethyl silicon liquid phase (5%) (J & W DB-5, 0.25 mm, USA). The program settings were as follows: initial temperature, 50°C; final temperature, 250°C; heating rate, 15°C/min; and carrier gas flow rate, 2 ml/min. An OC-9 capillary on a column injector was used for sample injection, and AFB1 UV absorbance was monitored at 272 nm.

**Statistical analysis**

The experimental data are expressed as the means ± standard errors. SPSS version 23 (IBM SPSS, USA) was used for the statistical analysis, and the normality of the data distribution was evaluated via the Shapiro–Wilk test. One-way ANOVA coupled with Tukey's LSD test significantly differentiated the values at $P < 0.05$.

## Results

**GC analysis of aflatoxins**

GC analysis revealed that the AFB1 produced by the local Saudi Arabian *A. flavus* isolate was slightly different from the AFB1 produced by the Egyptian *A. flavus* isolate (Fig. 1A - B). A small peak appeared in the Saudi Arabian AFB1 sample at 1.6 min, followed by a sharp peak at 1.7 min and a tiny peak at 1.8 min (Fig. 1 A). These peaks demonstrated the presence of three compounds in the AFB1 mixture. Egyptian AFB1 was characterized by the presence of two peaks. The first peak appeared at 1.6 min, followed by the second peak at 1.7 min (Fig. 1 B). Thus, the GC results revealed more separation in the local AFB1 toxin than in the Egyptian AFB1 toxin.

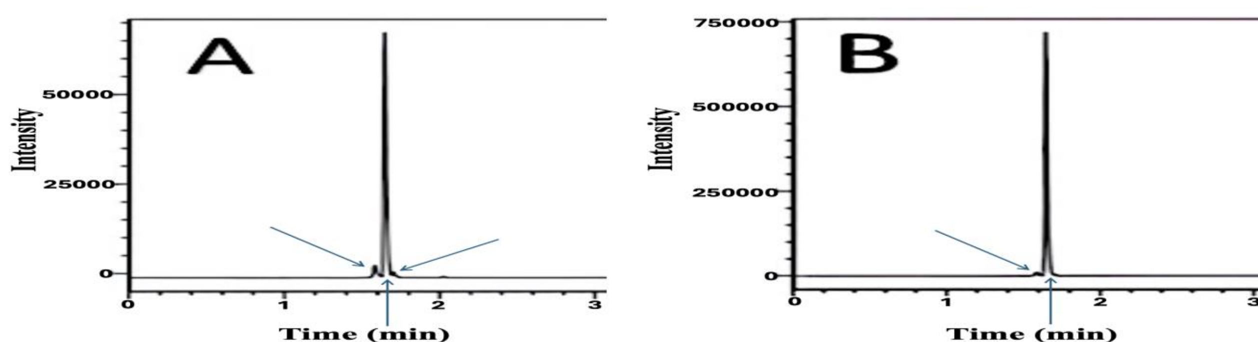


**Fig. 1** – Gas chromatography of aflatoxins (A) AFB1 produced by Saudi Arabian *A. flavus* isolate and (B) AFB1 produced by Egyptian *A. flavus* isolate.

**HPLC analysis of aflatoxins**

The HPLC data revealed that AFB1 from the Saudi Arabian *A. flavus* isolate presented four well-defined peaks at Rt1=6.8 min, Rt2=7.0 min, Rt3=7.8 min, and Rt4=8.2 min (Fig. 2A). AFB1 from Egyptian *A. flavus* isolates presented three peaks at Rt1=5.0 min, Rt2=6.0 min, and Rt3=6.8 min. A one-minute difference was noted in the second peak (Rt2=7.0) of Saudi Arabian AFB1 and Egyptian AFB1 (Rt2=6.0 min). The RT2 peaks of both aflatoxins were sharp, which indicated the separation of 80% of the molecules. The second peak (RT2) in Egyptian AFB1 was wider than that in Saudi Arabian AFB1, which could be due to the overlap of the two peaks. The RT2 peak of Saudi Arabian AFB1 was 10 times greater than that of Egyptian AFB1. The three small peaks in Saudi Arabian AFB1 could be attributed to the AFB1 compound. Thus, Saudi Arabian AFB1

contains more toxins than Egyptian AFB1 does. The two peaks in the Egyptian AFB1 samples at Rt= 6.8 min and Rt=5 min revealed the production of AFB1 and AFB2 in the Egyptian *A. flavus* isolate. However, the Saudi Arabian *A. flavus* isolate produced more toxins (Rt=7, Rt=7.8, and Rt=8.2) in addition to AFB1 (Rt=6.8). Therefore, compared with Egyptian AFB1, the toxins in Saudi Arabian AFB1 exerted greater effects/toxic effects on rat hepatic and renal tissues (Fig. 3C and Fig. 4C). Future studies should also involve mass spectrometry (MS) to define the exact nature of toxins/compounds in Saudi Arabian AFB1.

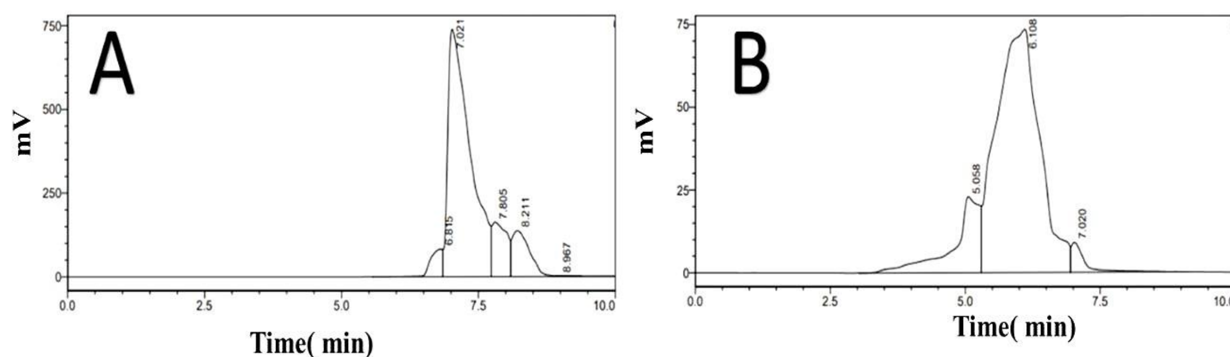


**Fig. 2** – HPLC chromatograms of aflatoxins at 375 nm (A) AFB1 produced by the Saudi Arabian *A. flavus* isolate and (B) AFB1 produced by the Egyptian *A. flavus* isolate.

**Blood parameters**

Red blood cell (RBC) counts were significantly lower in both AFB1-treated groups than in the control group ($p < 0.050$). Similarly, the values of mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were significantly lower in the Saudi Arabian AFB1-treated group than in the control group ($p < 0.050$, $p < 0.010$) and Egyptian AFB1-treated group ($p < 0.001$, $p < 0.001$). In contrast, a significant increase in the platelet count was noted in both AFB1-treated groups compared with the control group ($p < 0.001$) (Table 1).

**Table 1** Effects of AFB1 administration on the RBC count, hemoglobin level, MCV, MCH, MCHC, and platelet count in rats.

| Variables | Control | Egyptian AFB1 | Saudi Arabian AFB1 |
|---|---|---|---|
| **RBCs** ($X10^6/\mu L$) | 9.66±0.22 | $8.70 \pm 0.30^{\dagger *}$ | $8.59 \pm 0.06^{\dagger *}$ |
| **Hemoglobin** (g/dL) | 16.10±0.03 | 16.44±0.13 | 16.32±0.37 |
| **MCV** (fL) | 52.32±1.26 | 52.72±0.70 | 51.26±0.84 |
| **MCH** (pg/dL) | 18.76±1.34 | 18.94±0.13 | $17.10 \pm 0.08^{\dagger **, \ddagger **}$ |
| **MCHC** (g/dL) | 35.84±0.50 | 35.94±0.30 | $33.00 \pm 0.28^{\dagger ***, \ddagger ***}$ |
| **Platelets** ($X10^3/\mu L$) | 530.40±22.45 | $778.60 \pm 17.41^{\dagger ***}$ | $812.56 \pm 36.57^{\dagger ***}$ |

**†:** Significance versus control; **‡:** significance versus Egyptian AFB1.
***:** $P < 0.05$; ****:** $P < 0.01$; *****:** $P < 0.001$.

Compared with the control treatment, AFB1 treatment (Saudi Arabian and Egyptian) resulted in significant increases in white blood cell (WBC) counts ($p < 0.010$ and $p < 0.001$), neutrophil counts ($p < 0.010$ and $p < 0.050$), and monocyte counts ($p < 0.001$). The counts of WBCs and monocytes were significantly greater in the Saudi Arabian AFB1-treated group than in the Egyptian AFB1-treated group ($p < 0.010$), whereas the level of eosinophils was significantly greater in the Saudi Arabian AFB1-treated group than in the control and Egyptian AFB1-treated groups ($p < 0.050$ and $p < 0.050$) (Table 2).

**Table 2** Effects of AFB1 administration on white blood cell, lymphocytic, neutrophil, monocyte, eosinophil, and basophil counts in rats.

| Variables | Control | Egyptian AFB1 | Saudi Arabian AFB1 |
|---|---|---|---|
| **WBCs** (X$10^3$/µL) | 13.21±0.48 | 15.80±0.48$^{\dagger **}$ | 18.71±0.45$^{\dagger ***, \ddagger **}$ |
| **Lymphocytic count** (X$10^3$/µL) | 11.48±0.50 | 11.24±0.55 | 12.80±0.68 |
| **Neutrophil count** (X$10^3$/µL) | 1.22±0.07 | 3.30±0.52$^{\dagger **}$ | 3.13±0.46$^{\dagger *}$ |
| **Monocyte count** (X$10^3$/µL) | 0.36±0.02 | 2.48±0.12$^{\dagger ***}$ | 3.33±0.23$^{\dagger ***, \ddagger **}$ |
| **Eosinophil count** (X$10^3$/µL) | 0.50±0.31 | 0.67±0.03 | 1.42±0.10$^{\dagger *, \ddagger *}$ |
| **Basophil count** (X$10^3$/µL) | 0.36±0.02 | 0.32±0.01 | 0.34±0.02 |

**†:** Significance versus control; **‡:** significance versus Egyptian AFB1.
***:** *P* <0.05; ****:** *P* <0.01; *****:** *P* <0.001.

Saudi Arabian AFB1 treatment caused a significant increase in aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels in the control and Egyptian AFB1-treated groups (*p* <0.001). AST and ALP values also remained significantly greater in the Egyptian AFB1-treated group than in the control group (*p* <0.001). Moreover, the serum ALB concentration was significantly lower in both AFB1-treated groups than in the control group (*p* <0.001) (Table 3).

**Table 3** Effects of AFB1 treatment on liver parameters (AST, ALP, and ALB).

| Variables | Control | Egyptian AFB1 | Saudi Arabian AFB1 |
|---|---|---|---|
| **AST** (U/L) | 113.00±3.42 | 140.60±3.08$^{\dagger ***}$ | 156.60±1.27$^{\dagger ***, \ddagger **}$ |
| **ALP** (U/L) | 76.00±0.55 | 127.00±1.90$^{\dagger ***}$ | 179.80±6.64$^{\dagger ***, \ddagger ***}$ |
| **Albumin** (g/dL) | 38.20±0.58 | 27.60±0.68$^{\dagger ***}$ | 23.31±0.09$^{\dagger ***, \ddagger ***}$ |

**†:** Significance versus control; **#:** significance versus Egyptian AFB1.
*****:** *P* <0.001.

AFB1 treatment (Saudi Arabian and Egyptian) led to a significant increase in the serum urea and creatinine levels (*p* <0.001), whereas a significant decrease in the uric acid levels was noted in both AFB1-treated groups (*p* <0.001) compared with those in the control group. Compared with those in the Egyptian AFB1-treated group, a significant increase in the serum urea level and a significant decrease in the uric acid level (*p* <0.050) were detected in the Saudi Arabian AFB1-treated group (Table 4).

**Table 4** Effects of AFB1 treatment on rat kidney parameters.

| Variables | Control | Egyptian AFB1 | Saudi Arabian AFB1 |
|---|---|---|---|
| **Urea** (mmol/L) | 6.98±0.36 | 11.84±0.43$^{\dagger ***}$ | 19.60±1.51$^{\dagger ***, \ddagger ***}$ |
| **Creatinine** (µmol/L) | 0.31±0.03 | 0.45±0.02$^{\dagger ***}$ | 0.51±0.01$^{\dagger ***}$ |
| **Uric acid** (µmol/L) | 1.74±0.07 | 1.07±0.06$^{\dagger ***}$ | 0.81±0.01$^{\dagger ***, \ddagger *}$ |

†: Significance versus control; ‡: significance versus Egypt AFB1.
*: *P* <0.050; ***: *P* <0.001.

**Histopathological features of AFB1-induced renal and hepatic damage**

H&E staining revealed normal hepatic tissues in control rats (Fig. 3a). Aflatoxin treatments are characterized by significantly enlarged central veins, massive leucocyte infiltration, and substantial cell mortality. Histopathological signs were less severe in the Egyptian AFB1-treated group than in the Saudi Arabian AFB1-treated group (Fig. 3b - 3c).

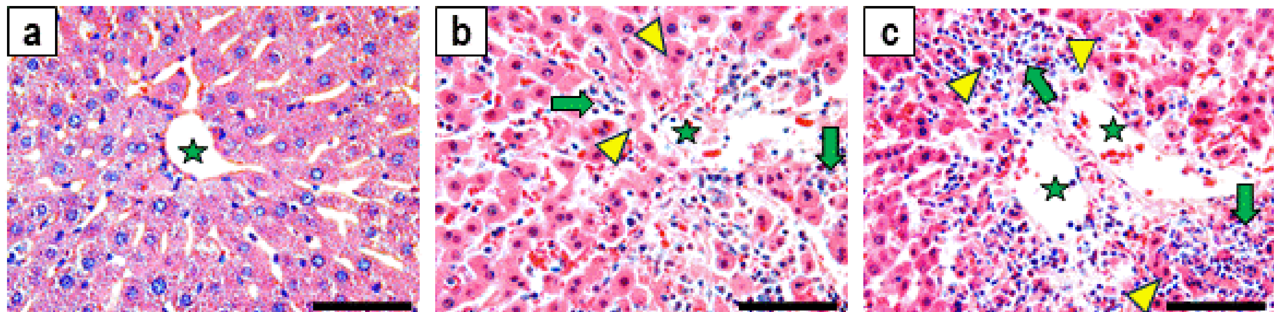


**Fig. 3** – H&E-based histological features of hepatic tissues from the a negative control. b Egyptian AFB1-treated. c Saudi Arabian AFB1-treated groups at 40× objective magnification. Scale bars=10 µm; green star=central vein; green arrow=lymphocytic infiltration; yellow arrowhead=apoptotic/necrotic cells.

Renal tissues from the control group presented normal histological features (Fig. 4a). The AFB1-treated groups presented noticeable renal damage characterized by cupping of Bowman's capsule, glomerular membrane disruption, and tubular damage. The effects were more severe in the Saudi Arabian AFB1-treated group (Fig. 4b - 4c).

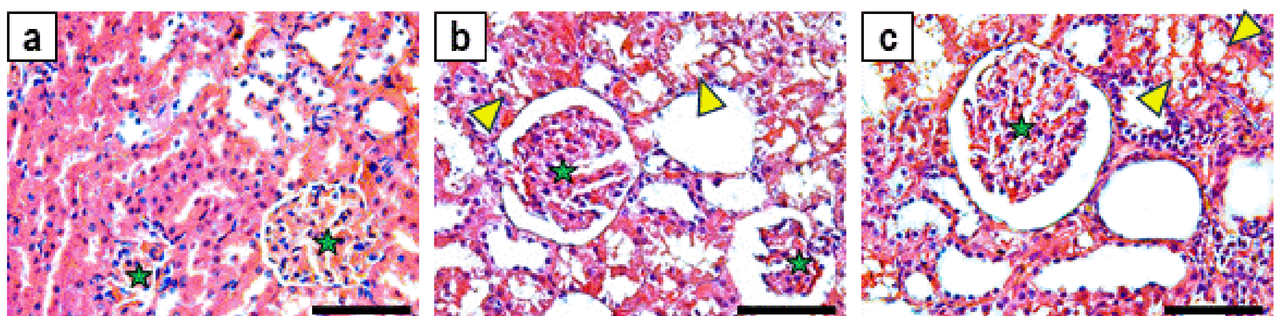


**Fig. 4** – H&E-based histological features of renal tissues from the a negative control. b Egyptian AFB1-treated. c Saudi Arabian AFB1-treated groups at 40× objective magnification. Scale bars=10 µm; green star=glomerulus; yellow arrowhead=tubular damage.

**Discussion**

This study compared the effects of Saudi Arabian and Egyptian *A. flavus*-associated AFB1s on the hematology parameters and organs (liver and kidneys) of albino rats. The results revealed a significant decrease in RBC, MCH, MCHC, albumin, and uric acid levels, whereas WBC, monocyte, eosinophil, AST, ALP, urea, and creatinine levels were significantly greater in the Saudi Arabian AFB1-treated group than in the Egyptian AFB1-treated group. The different toxicity impacts of both AFB1s can be attributed to varying metabolic enzymes (phases I and II), which can predict the comparative in vivo toxicity impacts (Klein et al. 2000, Kim et al. 2013). The digestive tract can rapidly absorb AFB1, whereas it is metabolized in the liver (Veldman et al. 1992, Weidenbörner 2001, Wolf-Hall 2009, Moody 2021). Metabolism is initiated by two phase I metabolic cytochrome P450 enzymes (CYP3A37 and CYP1A5). CYP3A37-based AFB1 metabolism features sigmoidal Hill kinetics, which produces exo-AFB1-8,9-epoxide (AFBO). It reacts with cell macromolecules (DNA) to form AFB1-N7-guanine adducts, which are the main

carcinogenic and mutagenic cellular lesions in the liver and kidneys (Wayne et al. 1990, Coulombe 1993, Iyer et al. 1994, Kumar et al. 2017 Moody 2021). Conversely, CYP1A5 metabolism features hyperbolic Michaelis kinetics, which results in the production of only AFM1 (4-hydroxy aflatoxin M1) during AFB1 exposure (Campbell et al. 1970, Johnson & Guengerich 1997, Rawal & Coulombe 2011, Moody 2021). It utilizes butylated hydroxytoluene (BHT), an hCYP1A5 inhibitor, and phase II glutathione S-transferases (GSTs) to alleviate aflatoxicosis symptoms after exposure (Zamek-Gliszczynski et al. 2006, Guarisco et al. 2008, Moody 2021). AFB1 is comparatively more cytotoxic than AFM1, as it releases lactate dehydrogenase to stimulate DNA alterations, covalent binding with macromolecules, and monolayer cell mortality (Salocks et al. 1981, Wu et al. 2020, Caceres et al. 2020, Wu et al. 2021, Gerdemann et al. 2023).

In this study, Egyptian AFB1 was found to be less toxic than Saudi Arabian AFB1; however, it exhibited greater genotoxicity than the control. Kumar et al. (2017) reported that, compared with AFB1, AFM1 has lower carcinogenicity and mutagenicity. The differential toxicity of AFB1 in Saudi Arabian and Egyptian populations may be linked to genetic differences among AFB1-producing *A. flavus* strains and varying synthesis and metabolic pathways (Johnson & Guengerich 1997, Benkerroum 2020, Moody 2021). The production of other aflatoxins (AFB2), in addition to AFB1, by Saudi Arabian AFB1 may also be another factor contributing to its increased toxicity. HPLC analysis also revealed the presence of other aflatoxins in Saudi Arabian AFB1 samples, whereas Egyptian *A. flavus* isolates produced only AFB1. Fakruddin et al. (2015) reported the production of AFB1 and AFB2 by *A. flavus* strains. The varying quantities of AFB could also have contributed to differential toxicity levels in Saudi Arabian and Egyptian isolates, as *A. flavus* from different geographic locations could vary in aflatoxin cytotoxicity and production quantities (Klich 2007).

The decreased RBC count in AFB1-treated rats might be due to toxin-based anaemia leading to decreased activity of the liver and kidney-secreted erythropoietin hormone. The reduced hemoglobin content could be attributed to defective heme biosynthesis in the bone marrow, decreased erythrocyte volume, and reduced erythropoietin formation (Khaled & Thalij 2021, Rajaura et al. 2023). AFB1-based T-cell suppression in the cellular immune system occurs via reduced complement synthesis in the liver, neutrophil activity, and macrophage phagocytosis (Abdel-Wahhab et al. 2002). It also disturbs lymphocyte receptors and other functions to induce cytotoxicity (Tran et al. 2021). Multiple studies have reported that AFB1 is associated with significant reductions in RBC and blood hemoglobin contents in rats (Abdel-Wahhab et al. 2002, Husain et al. 2014, Ramamurthy & Rajakumar 2016, Abd Allah et al. 2017, Uluişik et al. 2020, Khaled & Thalij 2021)**.**

The significant increase in the WBC and monocyte counts in AFB1-treated rats could be due to AFB1-related inflammation in different tissues and organs, which is in agreement with previous reports (Khaled & Thalij 2021). These findings are in line with the results of previous studies (Husain et al. 2014, Ramamurthy & Rajakumar 2016, Khaled & Thalij 2021, Dai et al. 2022). A significant reduction in MCH and MCHC values and increased eosinophil levels are known to be caused by AFB1-related elevated microcytic hypochromic anaemia (Abd Allah et al. 2017, Uluişik et al. 2020, Lei et al. 2021, Ashi et al. 2023a,b). The results revealed a significant increase in AST, ALP, urea, and creatinine serum levels, whereas albumin and uric acid were significantly lower in both AFB1-treated groups than in the control group, which has also been reported in previous studies (Stoev 2015, Karabacak et al. 2015, El-Nekeety et al. 2017, Abdel-Latif et al. 2017 Khaled & Thalij 2021). AFB1 toxicity to hepatocytes might increase liver enzyme activities (ALP and AST) (Ashi et al. 2023a). The increased membrane permeability after AFB1 exposure enhances the release of liver enzymes into the bloodstream, leading to increased serum concentrations, hypofunction, and liver degeneration (Lee et al. 2012, Varior & Philip 2012, El Arem et al. 2014, Owumi et al. 2019, Wang et al. 2020, Khaled & Thalij 2021, Ashi et al. 2023a,b). AFB1-exposed liver exhibited decreased albumin and total protein levels, which indicates a lack of protein metabolism and synthesis (Hussain et al. 2009, Karabacak et al. 2015).

The histological examination of the liver revealed congestion and dilatation of the central vein, high cell mortality, and leucocyte infiltration in the portal area. Most hepatocytes in both AFB1-treated groups were hypertrophied with pyknotic nuclei and granular vacuolated cytoplasm, which is in accordance with previous investigations (Husain et al. 2014, Wahab et al. 2018, Rotimi et al. 2019, Ashi et al. 2023a,b). DNA damage, inflammation, and oxidative stress are thought to mediate AFB1-induced hepatorenal injury (Aleissa et al. 2020). AFB1 is also known to interfere with homeostasis and the liver's cellular milieu (Ali et al. 2021). AFB1-induced histopathological changes might generate reactive oxygen species (ROS) that cause oxidative stress, leading to various chronic diseases and cancer due to DNA damage, cytotoxicity, lipid peroxidation, and reduced protein function (Kotan et al. 2011, Combes et al. 2018, Wu et al. 2020).

AFB1-induced pathophysiological and histological alterations in kidneys are characterized by increased serum levels of urea and creatinine and decreased uric acid levels, which is consistent with other rat studies (Abdel-Wahhab et al. 2002, Yilmaz et al. 2018, Alsayyah et al. 2019, Khaled & Thalij 2021, Wang et al. 2022, Ashi et al. 2023). The potential mechanisms of AFB1 toxicity to kidneys might include oxidative stress through alterations in proline levels and PRODH expression, leading to downstream apoptosis (Li et al. 2018). AFB1 toxicity can increase plasma creatinine concentrations in muscular secretions (Owumi et al. 2020). An increase in urea, creatinine, and uric acid levels in the serum could result in protein catabolism and reduced filtration and excretion efficiency in the kidneys (Wang et al. 2020, Khaled & Thalij 2021, Al-Shahari et al. 2022, Ashi et al. 2023a,b). Histological examination revealed different structural changes in the kidney tissue of AFB1-treated rats, including hemorrhage, tubule degeneration, epithelial swelling, granular cytoplasm appearance, glomerular membrane disruption, and Bowman's capsule (Al-Habib et al. 2007, Abdel-Hamid & Firgany Ael 2015, Yilmaz et al. 2018, Alsayyah et al. 2019, Abdel-Daim et al. 2021, Wang et al. 2022). The observed reduction in red blood cell (RBC) counts and hemoglobin levels in AFB1-treated rats reflects significant hematological toxicity, likely linked to anemia caused by decreased erythropoietin and impaired heme production, aligning with prior research (Abdel-Wahhab et al. 2002). Additionally, the rise in white blood cell (WBC) and monocyte counts indicates a heightened inflammatory response (Khaled & Thalij 2021). The differing toxicity levels between Saudi Arabian and Egyptian AFB1 may be attributed to genetic variations in local *A. flavus* strains (Johnson & Guengerich 1997). These results highlight the need for tailored public health interventions, and further research should focus on the molecular mechanisms to better address AFB1 exposure risks

## Conclusions

This study revealed that AFB1-induced (Saudi Arabian and Egyptian) significant biochemical and histopathological changes in kidney and liver tissues caused organ dysfunction. Saudi Arabian AFB1 presented greater toxicity than Egyptian AFB1. *A. flavus*-produced AFB1 might mirror its toxicity in ready-to-consume commodities. However, further studies involving mass spectrometry (MS) are needed to define the exact nature of Saudi Arabian *A. flavus*-associated aflatoxins in addition to AFB1. This study explored the potency of *A. flavus* isolates that contaminate edible food in households. Moreover, hygiene practices should be adopted to prevent mycotoxin contamination in commercial food products.

This study highlights the significant threat posed by *A. flavus* isolates contaminating household food items, underscoring the urgent need for enhanced hygiene practices to reduce mycotoxin contamination in commercial food products. Furthermore, the findings should guide agricultural practices and inform food safety regulations to better protect consumers. Future research should focus on the effects of AFB1 across diverse populations and examine the environmental factors that may influence its toxicity. By implementing proactive measures, stakeholders–including policymakers and food producers–can more effectively safeguard public health and ensure the safety of our food supply.

### Ethical Approval

The experiments were approved by the Ethical Committee of the study, which was approved by the Ethical Committee of King Fahd Medical Research Center. Jeddah, KSA (Approval #163-19). The experimental animals were handled according to the guidelines of ARRIVE.

### Author Contribution

**Research design:** HA, KE, HHA. **Conducting experiments:** HA, TFHA, SHA, OTA, FAR, EHA, FSA. **Data curation and analysis:** EAH, BR, HMA, YAA, KE, HAM, AAA, GHA, AAA **Writing-first draft:** HA, EAH, BR, HHA, **Writing-final draft and editing:** LAN, HHA

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### Conflict of interest

The authors declare that no conflicts of interest exist.

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