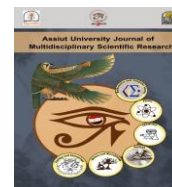


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Mycotoxicity and Embryonic Development: III- Aflatoxin B₁ Induces Hepatic and Renal Histopathological and Histomorphometric Alterations in Pregnant Mice

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ABSTRACT

Background: Aflatoxins, highly toxic compounds produced by species of *Aspergillus Flavus* and *Aspergillus parasiticus*, are known for their hepatonephric toxicity. There are no published randomized controlled trials evaluating the effect of aflatoxin exposure during pregnancy. **Aim of the work:** The current study aims to evaluate the effect of AFB₁ on hepatorenal structure of pregnant mice. **Methods:** Female mice were daily

fed on fodder contaminated with 5, 10 or 20 μg AFB₁ seven days before mating and during pregnancy. Liver and kidney specimens were processed for histopathological and histomorphometric investigations. **Results:** 5 μg AFB₁ treatments resulted in hepatonecrosis, inflammatory cells infiltration, vascular congestion and interstitial edema. 10 μg AFB₁ group exerted hydropic and ballooning degeneration of hepatic cells, increased nuclear chromatin content, presence of trinucleated cells, dilation of blood sinusoids and areas of necrosis. In liver, 20 μg AFB₁ group revealed marked vascular congestion and binucleated cells, with variable sized nuclei anisonucleosis and interstitial edema. Liver histomorphometry analyses revealed significant alterations in the central vein, nuclear and cytoplasmic area. Kidney treated with 5 μg AFB₁ showed glomerular hypertrophy, tubular necrosis, congestion and Bowman's space increase. Kidney treated with 10 μg AFB₁ exhibited necrosis, damage in corpuscles & renal cast. Examination of kidney treated with 20 μg AFB₁ revealed hemorrhage, necrosis, renal cast and glomerular hypertrophy. Kidney histomorphometry analyses revealed significant alterations in Bowman's space, glomerular and corpuscle areas. **Conclusion:** AFB₁ exerted hepatonephrotoxic negative impact in pregnant mice fed on contaminated fodder and caused many

histopathological and histomorphometric alterations including necrosis, hydropic degeneration, edema, hemorrhage, blood congestion reflecting high risk toxicity of AFB₁. It is suggested that accumulation of bioactivated AFB₁ metabolites severely induced hepatonephrotoxicity in pregnant mice. Further studies are recommended.

INTRODUCTION

Aflatoxins constitute a class of mycotoxins produced as secondary metabolites, including AFB₁, AFB₂, AFG₁, and AFG₂. These compounds are primarily synthesized by fungi belonging to the genus *Aspergillus*, particularly *Aspergillus flavus* and *A. parasiticus* [1, 2]. They commonly contaminate a wide variety of food products and animal feedstuffs. Structurally, aflatoxins are difuranocoumarin derivatives, possessing a highly conjugated coumarin core fused with a bifuran moiety, which underlies their distinct physicochemical characteristics. Among these, aflatoxin B₁ (AFB₁) is considered the most potent in terms of toxicity, mutagenicity, and carcinogenicity [3, 4].

Contamination of food crops with AFB₁ arises from both pre-harvest and post-harvest conditions, including environmental factors that promote fungal growth, such as elevated temperature, drought, and high humidity, in addition to poor practices during harvesting, transport, and storage [2, 5]. Furthermore, insect infestation and increased moisture levels after harvest enhance fungal invasion and toxin production. These stress conditions stimulate activation of the aflatoxin biosynthetic gene cluster

in *Aspergillus* species, thereby increasing secondary metabolite synthesis and elevating AFB₁ concentrations in vulnerable crops [2, 5]. Climate change has further exacerbated these contributing factors, leading to a wider geographic spread and increased incidence of aflatoxin contamination.

AFB₁ is found in numerous staple food items, particularly cereals and oilseeds, making dietary intake the principal route of human exposure, including exposure during pregnancy via maternal consumption [1, 6& 7]. After ingestion, AFB₁ undergoes metabolic activation in the liver, where cytochrome P450 enzymes convert it into a highly reactive 8,9-epoxide form capable of binding covalently to DNA and proteins. Consequently, the global impact of AFB₁ exposure is considerable, with billions of individuals estimated to be chronically exposed to levels exceeding recommended safety limits [8].

Exposure to high doses of aflatoxins can lead to acute aflatoxicosis, which is characterized by severe and potentially fatal liver toxicity [9]. This condition is mainly attributed to the highly reactive electrophilic metabolites and the instability of the lactone ring, resulting in rapid hepatocellular necrosis, coagulation defects, and liver failure. A notable example is the 2004 aflatoxicosis outbreak in Kenya, associated with consumption of highly contaminated maize, which demonstrated severe clinical outcomes such as hepatic necrosis and coagulopathy [10].

In contrast, prolonged exposure to low doses, especially AFB₁, is strongly linked to the development of hepatocellular carcinoma due to its genotoxic and mutagenic effects. Based on substantial epidemiological, experimental, and mechanistic data,

AFB₁ has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) [11].

Globally, approximately 14.7% of infants are born with low birth weight each year [12]. In addition, around 15 million preterm births and 2 million stillbirths are reported annually, while 12–15% of clinically recognized pregnancies up to 20 weeks of gestation end in spontaneous abortion [13, 14]. Collectively, these adverse pregnancy outcomes represent major contributors to maternal and fetal morbidity and mortality. Importantly, AFB₁ is capable of crossing the placental. Experimental studies in animals have consistently shown that exposure to AFB₁ during pregnancy induces a wide range of fetal toxic effects, including growth retardation [16]. In that study it was revealed that aflatoxicosis caused low birth weight and negatively affected the offspring. There is a direct relationship between low birth weight and AFB₁ induced hepatonephrotoxicity, immunotoxicity, and organ-specific teratogenic alterations [17–19]. Similarly, human epidemiological studies have demonstrated that maternal biomarkers of AFB₁ exposure are associated with decreased birth weight and impaired fetal development [6, 20 & 21].

Accordingly, the present study was designed to evaluate the effects of AFB₁ on the liver and kidney of pregnant female mice through histopathological and histomorphometric investigations to evaluate the potential negative impact of AFB₁ on liver and kidney of pregnant dams and consequently might affect the offspring.

MATERIALS AND METHODS

2.1. Production of AFB₁:

Aflatoxin B₁ was produced in Botany & Microbiology Department, Faculty of Science, Assiut University using a special strain of *Aspergillus flavus* (*A. flavus* AUMC 16585). This strain was isolated from popcorn, identified and characterized as a highly producer of aflatoxin B₁ in a recent study [22]. AFB₁ produced by this strain *via* fermentation of uncontaminated feed was used for feeding albino mice in the present study by the method described by Shotwell *et al.* [23] with minor modification by Oguz [24]. Briefly, broken uncontaminated feed (each 300 g) was placed in each 1000 mL Erlenmeyer flask at 20% moisture content and was sterilized in an autoclave at 121°C for 15 min. The flasks were cooled and inoculated with spore suspension of one-week old culture of *Aspergillus flavus* on Czapek's liquid medium. The flasks were incubated at 28°C for 10 days shaken vigorously by hand six to ten times a day to avoid clump formation. At the end of fermentation period, moldy feed was dried and the aflatoxin content was estimated by the thin layer chromatography [25] and quantitative analysis of aflatoxin was detected by ultra-performance liquid chromatography (UPLC) according to the method described by Benvenuti and Burgess [26] in the central laboratory of the Faculty of Veterinary Medicine at Assiut University.

2.1.1. Detection of aflatoxin B₁

25 gm of contaminated fodder was analyzed with High Performance Liquid Chromatography [27] as follows:

2.2. Preparation for AFB₁:

2.2.1. Standard preparation:

Two stock solutions were purchased from Supelco; 46319-U and 46304-U. From these, intermediate stocks were prepared in methanol and diluted with 1% acetic acid (aq) to produce six standard mixes with concentrations. These concentrations bracket the concentration of the final extract following the sample preparation procedure described below. From each standard, 20 µL was injected in triplicate.

2.2.2. Sample preparation:

Contaminated fodder with AFB₁ sample was divided into two 25 g portions. One portion was kept as a blank. The other was spiked with aflatoxin standards at EU levels as described below. Both portions were then carried through the VICAM AflaTest cleanup procedure.

2.2.3. Spiking Procedure:

Each sample was spiked at 4 µg/kg total B and G (1.54 µg/kg B₁). This was achieved by adding 384.6 µL of 0.26 mg/L solution to the blender for the sample preparation. In addition, the fodder containing AFB₁ was spiked at 0.05 µg/kg by adding 125.0 µL of a 0.01 mg/L solution of M1.

2.2.4. AflaTest sample preparation procedure:

- Using a blender, blend 25 g sample, 5 g sodium chloride, and 100 ml of a 80:20 methanol: water (HPLC grade) mix at high speed for 1 min.
- Filter this mixture through fluted Whatman filter paper (filtrate 1).
- Mix 10 mL of filtrate 1 and 40 mL of water.
- Load 10 mL of filtrate 2 onto a VICAM AflaTest Affinity Column, part no. G1024.
- Wash with two 10 mL portions of HPLC-grade water.

- Elute with 1 mL HPLC grade methanol.
- Dilute 1:1 with 1% aqueous acetic acid and inject.

The result solution, assuming 100% recovery, had a calculated concentration of 1000 ng/L total aflatoxins B, G and 12.5 ng/L M1 (spiked fodder). A total of 20 μ L was injected for UPLC-FLR analysis.

2.2.5. Separation of AFB₁:

The separation of the aflatoxins analyzed in this work was achieved in a run time of less than 4 min. figure below shows a representative overlay of the six different concentrations of the standard mixes.

2.3. Preparation of fodder contaminated with aflatoxin B₁ in different concentrations:

After determination of AFB₁ concentration, three different concentrations of fodder were prepared as follows:

5 μ g/kg concentration: Was prepared by mixing 49.5 g of free fodder and 5.5 g of contaminated one with AFB₁.

10 μ g/kg concentration: Was prepared by mixing 49.5 g of free fodder and 11.1 g of contaminated one with AFB₁.

20 μ g/kg concentration: Was prepared by mixing 49.5 g of free fodder and 22.1 g of contaminated one with AFB₁.

2.4. Experimental Animals:

40 Adult females and 20 males of albino mice (CD1) of an average body weight of 22-25 gm. were obtained from the animal house of the Egyptian Organization for Biological Products and Vaccines (VACSERA), Helwan, Cairo, Egypt.

2.5. Ethical approval:

The study was approved by the Ethics Committee of the Faculty of Medicine, Assuit University (Approval No. 04-2022-20004, dated January 18, 2023)

2.6. Experimental design:

After acclimatization for 10 days, females were randomly divided into 4 groups each group consisted of 10 females as follows:

*Group (1): Mice were kept as a control and received only uncontaminated fodder and drinking water *ad libitum*.

*Group (2): Mice were fed on 5 µg AFB₁/kg of contaminated fodder *ad libitum*.

*Group (3): Mice were fed on 10 µg AFB₁/kg of contaminated fodder *ad libitum*.

*Group (4): Mice were fed on 20 µg AFB₁/kg of contaminated fodder *ad libitum*.

2.6.1. After the mice had adapted to their environment, control and treated females were administered daily doses of tap water and different concentrations of AFB₁ for seven days before mating and continued during pregnancy.

2.6.2. Females were mated at night using apparently healthy males in a ratio of two female/ one male/ cage.

2.6.3. Early next morning (before 9 am), copulation was checked by examining the outer surface of the vagina for the presence of a vaginal plug formed by coagulation of semen (white clotting, sperm clot). When such a white to grayish clot, blocking the mouth of vagina, was detected, this day was considered as half a day of gestation.

2.6.4. The gradual increase in maternal body weight was considered a sign of pregnancy. The gestation period in mice was about 21 days.

Body weights of pregnant mice of both control and treated groups were recorded every day, also body weight of female mice before pregnancy as well as the infants at (neonatal and 21 postnatal days).

Pregnancy rate was calculated by dividing the number of pregnancies for all females in the group throughout the experimental period/the number of females in the group (10 females).

2.7 Histopathological examinations:

Histological studies were carried out on liver and kidney of mothers. Specimens were dehydrated in ascending series of alcohols, cleared in xylene substitutes and embedded in paraffin wax. Harris hematoxylin and eosin stains were utilized for general histological studies [28]. Photomicrographs were prepared at known magnifications using a (Research Microscope & Image analysis system, Olympus microscope equipped with an Olympus EP50 digital camera, Epview software version 1.2, Central Laboratory of Zoology & Entomology Department, Faculty of Science, Assiut University). Images were acquired at a resolution of 2592×1944 pixels.

2.8. Histomorphometric analysis:

2.8.1. Liver histomorphometric analysis:

Liver sections were examined using ImageJ software for quantitative morphometric assessment. The following parameters were evaluated:

2.8.1.1. Hepatocyte Area:

The area of hepatocytes was measured by manually outlining individual hepatocytes using the freehand selection tool in ImageJ. The cell boundaries were carefully identified,

and the area was automatically calculated in μm^2 . An average of five hepatocytes per field was recorded.

2.8.1.2. Nuclear Area:

The nuclear area was measured by selecting clearly visible nuclei within hepatocytes using the freehand or oval selection tool. The measured values were expressed in μm^2 , and the mean nuclear area per field was calculated.

2.8.1.3. Nuclear/Cytoplasmic Ratio (N/C Ratio):

The N/C ratio was calculated by dividing the nuclear area by the cytoplasmic area for the same hepatocyte using the formula:

$$\text{N/C ratio} = \text{Nuclear area} / (\text{Cell area} - \text{Nuclear area})$$

The mean N/C ratio was calculated for each field.

2.8.1.4. Nuclear Count:

The total number of hepatocyte nuclei was counted manually or using the cell counters plugin in ImageJ. The results were expressed as the number of nuclei per field.

2.8.1.5. Inflammatory Cell Count:

Inflammatory cells were identified based on their morphological characteristics (small, dark-stained nuclei with minimal cytoplasm). The number of inflammatory cells was manually counted in each field and expressed as the number of cells per field (cells/field).

2.8.1.6. Kupffer Cell Count:

Kupffer cells were identified as irregularly shaped cells lining the hepatic sinusoids with dark elongated nuclei. These cells were manually counted in each field and expressed as the number of Kupffer cells per field.

2.8.1.7. Central vein area:

The area of central veins was measured by manually outlining individual hepatocytes using the freehand selection tool in ImageJ. The central vein boundaries were carefully identified, and the area was automatically calculated in μm^2 .

2.8.2. Kidney histomorphometric analysis:

Kidney sections were examined using ImageJ software for quantitative morphometric assessment. The following parameters were evaluated:

2.8.2.1. Total Renal Corpuscle Area:

The total area of the renal corpuscle (glomerulus + Bowman's capsule) was measured by outlining the entire corpuscle using the freehand selection tool in ImageJ. The measured area was expressed in μm^2 , and the mean value per field was calculated.

2.8.2.2. Glomerular Area:

The glomerular tuft area was measured by carefully outlining only the glomerular capillary tuft, excluding Bowman's space, using the freehand selection tool. The area was recorded in μm^2 , and the mean glomerular area per field was calculated.

2.8.2.3. Bowman's Space Area:

Bowman's space was calculated indirectly by subtracting the glomerular area from the total renal corpuscle area using the formula:

Bowman's space area = Total corpuscle area – Glomerular area

The mean value per field was then calculated.

2.8.2.4. Glomerular Cellularity:

Glomerular cellularity was assessed by counting the total number of nuclei within the glomerular tuft (including mesangial, endothelial, and podocyte nuclei). Counting was performed manually or using the cell counter plugin in ImageJ. Results were expressed as the number of cells per glomerulus.

2.8.2.5. Cell Density:

Cell density was calculated as the number of cells per unit area within the glomerulus using the formula:

Cell density = Number of cells / Glomerular area

The results were expressed as cells/ μm^2 , and the mean value per field was calculated.

2.8.2.6. Inflammatory Cell Infiltration Assessment:

Inflammatory cells were identified based on their characteristic morphology, including small, densely stained hyperchromatic nuclei with minimal cytoplasm, typically located within the interstitial spaces and around renal tubules and glomeruli.

The number of inflammatory cells was manually counted in randomly selected non-overlapping fields using the cell counter plugin in ImageJ. Results were expressed as the number of inflammatory cells per field (cells/field).

To minimize bias, counting was performed in a blinded manner.

For each specimen, multiple non-overlapping fields were analyzed, and measurements were recorded and averaged to obtain representative values for each group.

2.9. Statistical analysis:

All quantitative data were statistically analyzed using SPSS software. Results were expressed as mean $\pm$ standard deviation (Mean $\pm$ SD). Descriptive statistics were initially performed using case summaries to calculate mean and standard deviation for each parameter. Comparisons between groups were conducted using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey post hoc test was applied to determine pairwise group comparisons for all parameters. However, hepatocyte area, nuclear area and inflammatory cell count for kidney data, which did not follow a normal distribution, were analyzed using the Kruskal-Wallis test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

3.1. Histological examination histomorphometric analysis:

3.1.1. Liver histology:

The control group showed normal structure of the hepatic tissue and its intact vasculature. The central veins are lined by endothelial cells surrounded by hepatic strands. The hepatic cells arranged in a series of branching and anastomosing perforated plates separated by blood sinusoids (Fig. 1 a). The 5 μ g AFB₁ group revealed areas of necrosis and inflammatory cell infiltration, along with vascular congestion and interstitial edema, increase in Kupffer cells number and Anisonucleosis (Fig. 1 b - d). The 10 μ g AFB₁ group revealed hydropic and ballooning degeneration of cells, increased nuclear chromatin content, and the presence of trinucleated cells. Additionally, dilatation of blood

sinusoids, increase in Kupffer cells number and areas of necrosis were observed (Fig. 2 a, b). The 20 μ g AFB₁ treated group revealed marked vascular congestion and binucleated and trinucleated cells, with nuclei varying from small to enlarged forms. Interstitial edema which is associated with enlarged blood sinusoids, accompanied by disruption of the hepatic architecture manifested as hepatocellular dissociation, increase in Kupffer cells number, Anisonucleosis and focal areas of necrosis were also observed (Fig. 2 c – f).

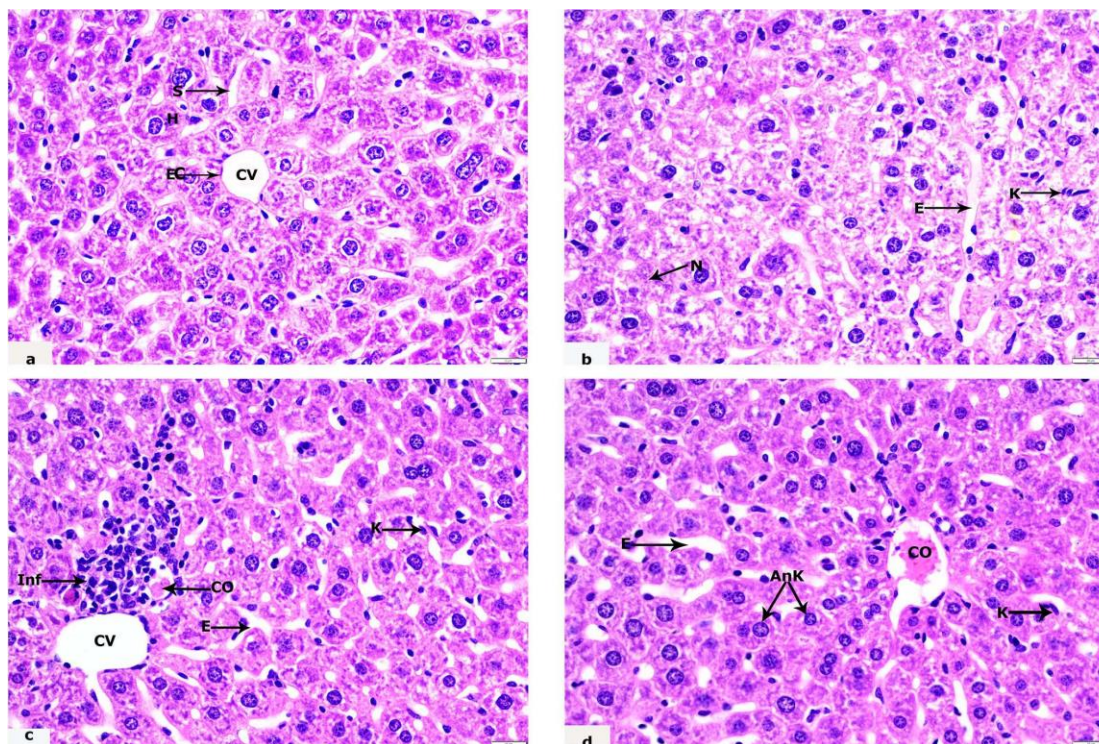


Fig. (1): Photomicrographs of liver sections of mother mice showing (a) control group showing the central veins (CV) are lined by endothelial cells (EC) surrounded by hepatic strands (H), while in (b - d) 5 μ g AFB₁ group showing areas of necrosis (N) and inflammatory cell infiltration (Inf), along with vascular congestion(CO) and interstitial

edema (E), increase in Kupffer cells number (K) and Anisonucleosis (Ank) (H & E stain scale bar: 20 μ m).

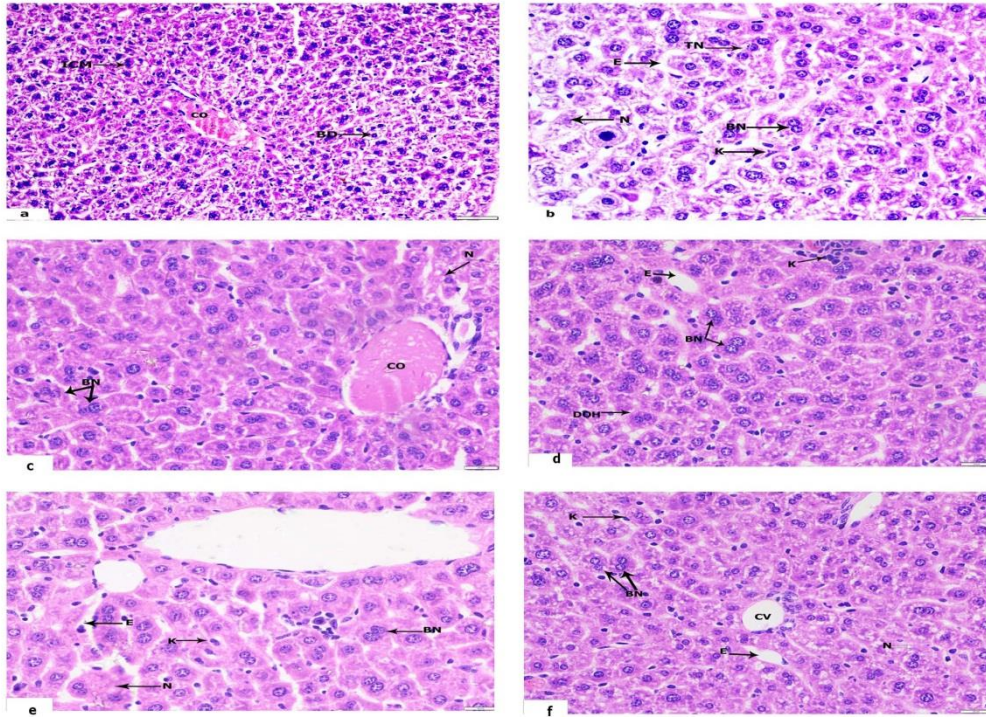


Fig. (2): Photomicrographs of liver sections of mother mice showing (a & b) 10 μ g AFB₁ group showing hydropic and ballooning degeneration (BD), increased nuclear chromatin content (ICM), binucleated cell (BC), trinucleated cells (TN). Additionally, increase in Kupffer cells number (K) and areas of necrosis (N) were observed, while (c - f) showing 20 μ g AFB₁ treated group revealed marked vascular congestion (CO) and binucleated (BN) and trinucleated (TN) cells, Anisonucleosis (Ank), edema (E), increase in Kupffer cells number (K) and necrosis (N) were also observed (H & E stain scale bar: (a) 50 μ m, (b – f) 20 μ m).

3.1.2. Liver histomorphometric analysis:

3.1.2.1. Hepatocyte area:

Statistical analysis of the Hepatocyte area revealed that there was an insignificant increase in the total area of the lowest treated group compared to control. The two higher treatments showed significant decrease compared to control (Fig. 3).

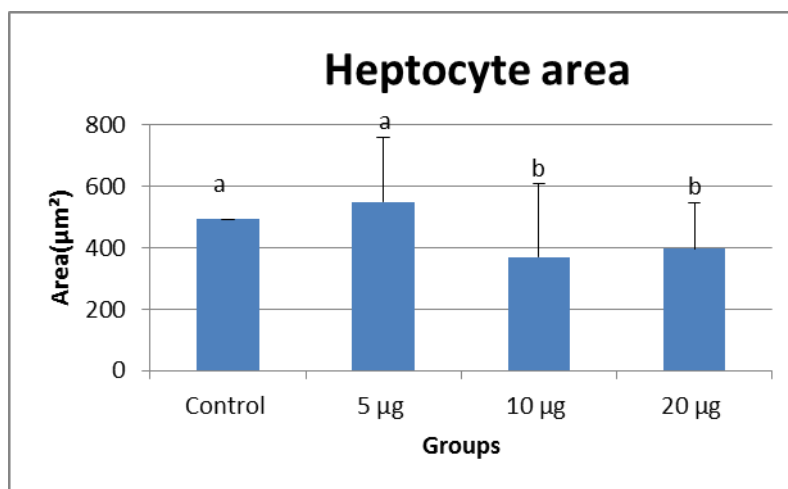


Fig. (3): A comparison of hepatocyte area of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.2.2. Nuclear area:

Statistical analysis of the hepatocyte nuclear area revealed that there was an insignificant decrease in 5µg and 10µg AFB₁ treated groups compared to control ($p > 0.05$), while there was a significant decrease in the highest treated group compared to control ($P = 0.045$) (Fig. 4).

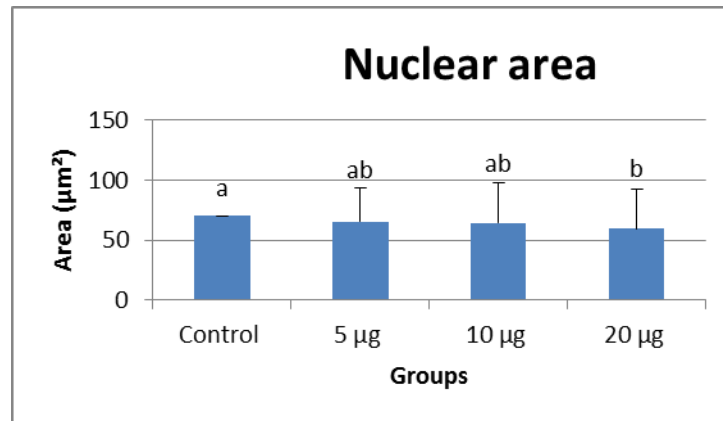


Fig. (4): A comparison of hepatocyte nuclear area of control and AFB₁ treated groups.

Values are presented as means $\pm$ SD.

3.1.2.3. Nuclear cytoplasmic ratio (N/C):

Statistical analysis of the nuclear cytoplasmic ratio (N/C) revealed that there was an insignificant difference in all treatments compared to control ($p = 0.184$) (Fig. 5).

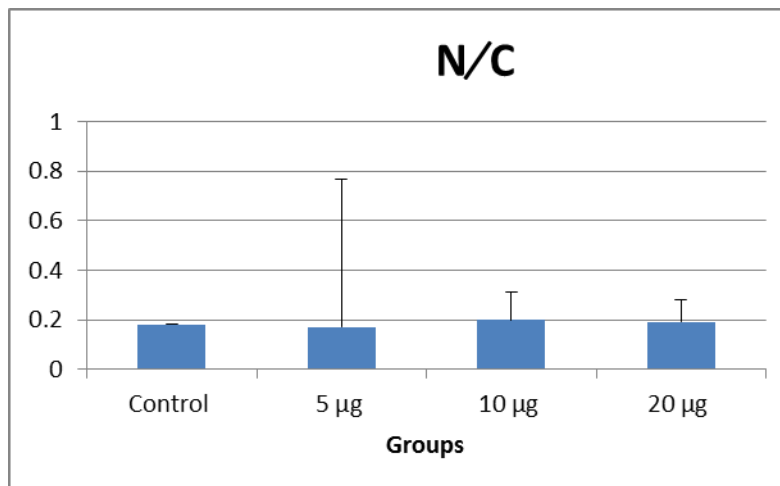


Fig. (5): A comparison of nuclear cytoplasmic ratio (N/C) of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.2.4. Nuclear count:

Statistical analysis of the nuclear count revealed that there was a significant increase in all treatments compared to control ($p = 0.000$) (Fig. 6).

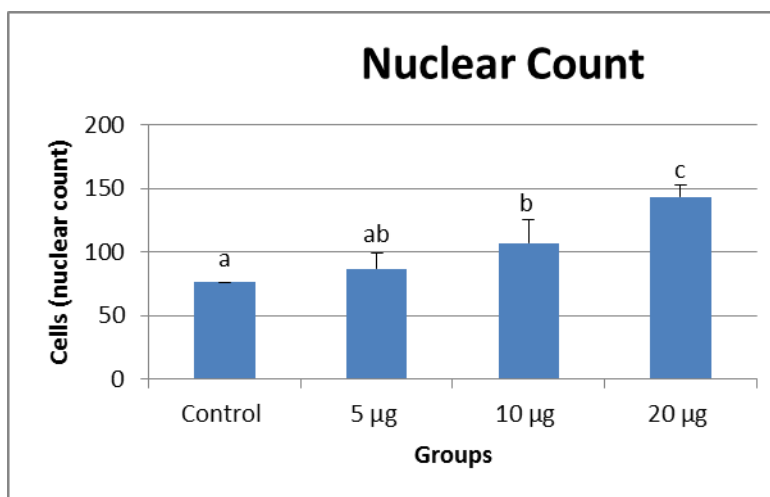


Fig. (6): A comparison of the nuclear count of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.2.5. Inflammatory Cell Count:

Statistical analysis of the inflammatory cell count revealed that there was a significant difference in the lowest treatment compared to control ($p = 0.005$), while other treatment were insignificantly different (Fig. 7).

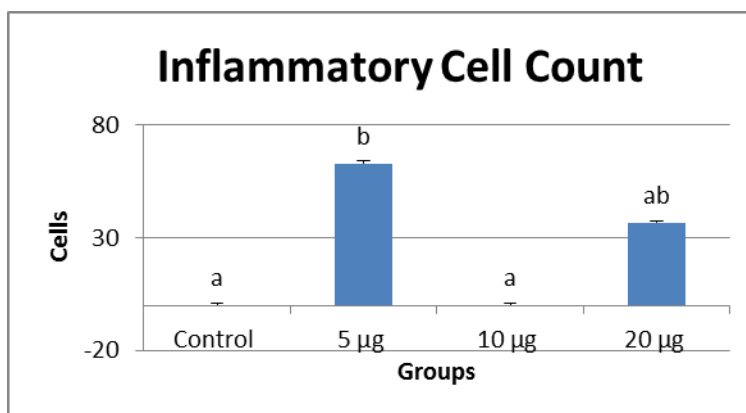


Fig. (7): A comparison of the inflammatory cell count of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.2.6. Kupffer Cell Count:

Statistical analysis of Kupffer cell count revealed that there was an insignificant variation in all treatments compared to control ($p = 0.876$) (Fig. 8).

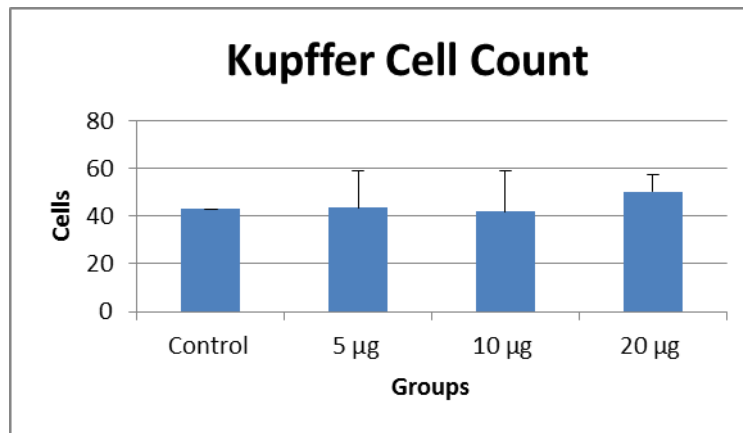


Fig. (8): A comparison of Kupffer cell count of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.2.7. Central vein area:

Statistical analysis of the central vein area revealed that there was an insignificant increase in all treatments compared to control ($p = 0.074$) (Fig. 9).

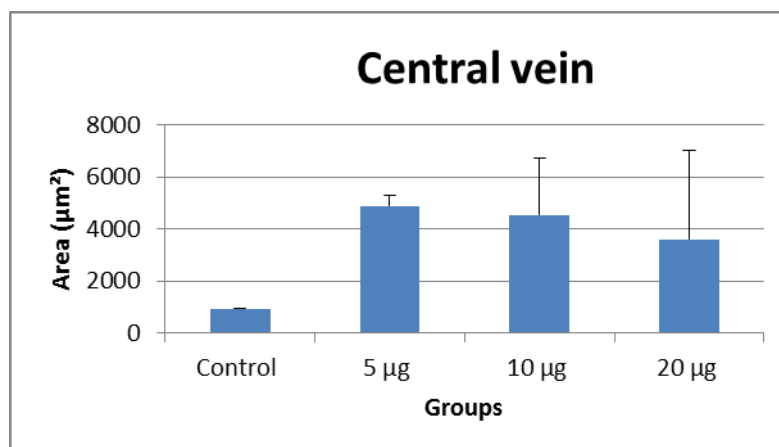


Fig. (9): A comparison of central vein area of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.3. Kidney:

The control group showed normal arrangement of the renal tubules, normal histological structure of the renal corpuscles and the renal tubular cells (Fig. 10 a). 5 μ g AFB₁ group showed hypertrophy of the glomeruli, necrotic changes in the renal tubules, marked vascular congestion, an increased Bowman's space, shrinkage of glomeruli and inflammation (Fig. 10 b – d). 10 μ g AFB₁ group showed necrotic changes, nuclear degeneration represented in pyknosis and karyolysis, cloudy swelling, hypercellularity in glomeruli, hydropic degeneration, dilation in glomeruli and increase in Bowman's space (Fig. 11 a – c). 20 μ g AFB₁ group showed Hemorrhage, blood congestion, necrosis, hypertrophy and shrinkage of glomeruli, lobular accentuation and hypercellularity of glomeruli, granular appearance of cytoplasm, accumulation of cellular debris in the tubular lumen and inflammation (Fig. 11 d – f).

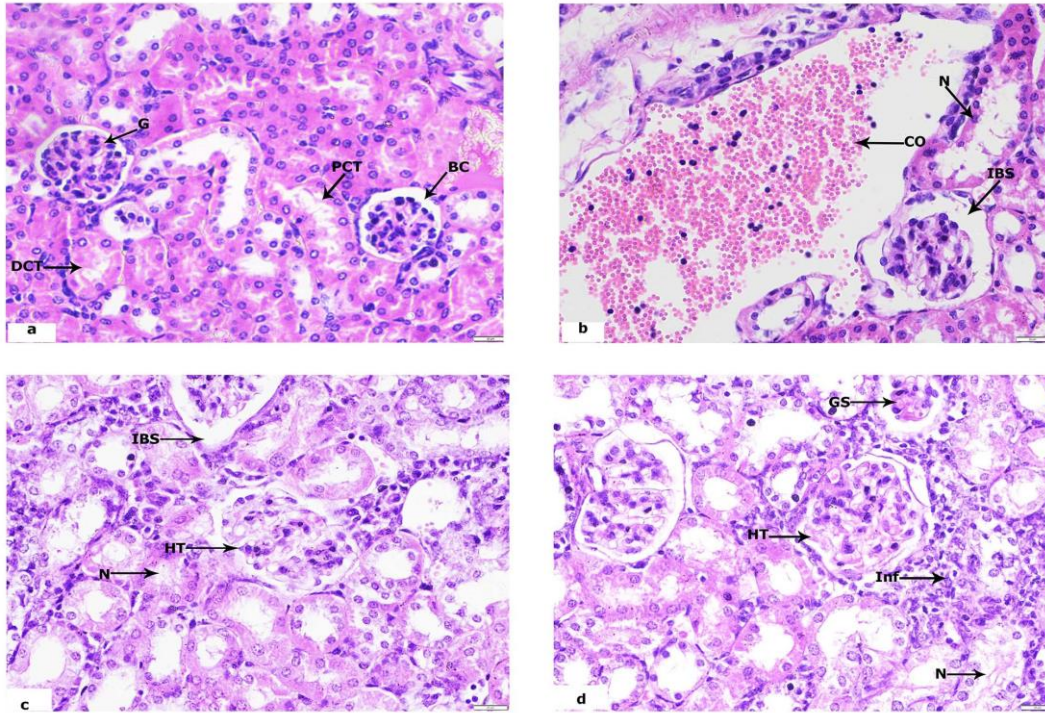


Fig. (10): Photomicrographs of transverse sections of kidney of mother mice showing: (a) control group, normal architecture of renal corpuscles with their glomeruli and renal tubules. Bowman's capsule (BC), distal tubules (DCT), proximal tubules (PCT) and glomeruli (G). (b - d) 5 µg AFB₁ treated group showing hypertrophy of the glomeruli (HT), necrotic changes in the renal tubules (N), marked vascular congestion (CO), an increased Bowman's space (IBS), shrinkage of glomeruli (GS) and inflammation (Inf) (H&E stain scale bar: 20 µm).

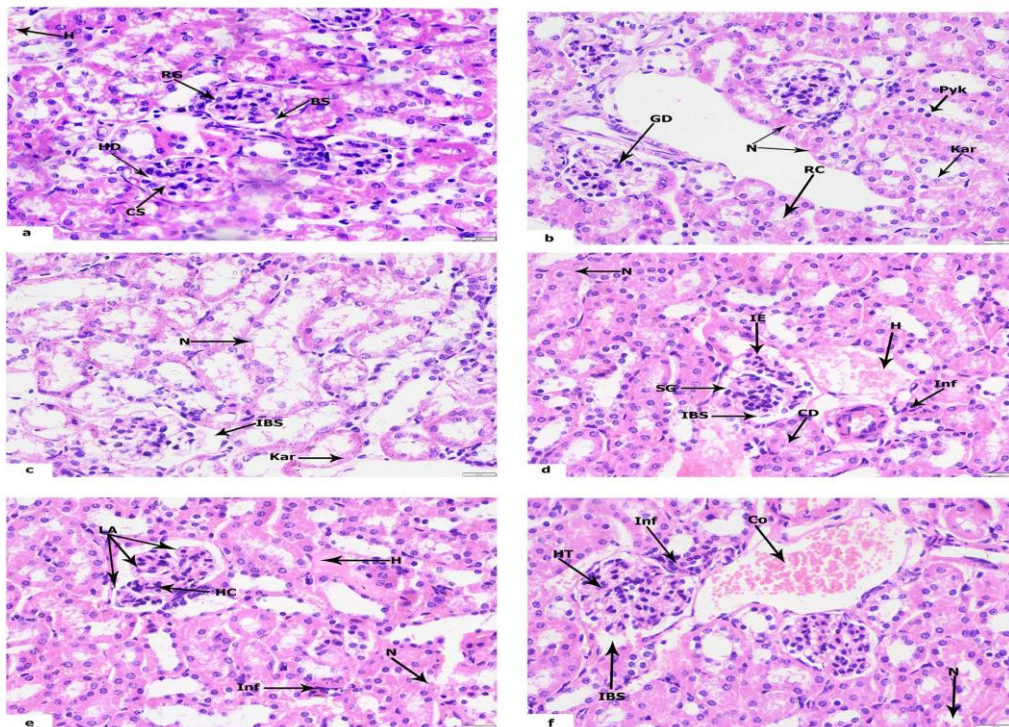


Fig. (11): Photomicrographs of transverse sections of kidney of mother mice showing: (a - c) 10 µg AFB₁ treated group showing necrotic changes (N), nuclear degeneration represented in pyknosis (Pyk) and karyolysis (Kar), cloudy swelling (CS), hypercellularity in glomeruli (HC), hydropic degeneration (HD), dialation in glomeruli (GD) and increase in Bowmn's space (IBS). (d - f) 20 µg AFB₁ treated group showed Hemorrhage (H), blood congestion (CO), necrosis(N), Hypertrophy (HT) and shrinkage of glomeruli (SG), lobular accentuation (LA) and hypercellularity of glomeruli (HC), accumulation of cellular debris in the tubular lumen (CD) and inflammation (Inf) (H&E stain scale bar: 20 µm).

3.1.4. Kidney histomorphometric analysis:

3.1.4.1. Malpighian corpuscule total area:

Statistical analysis of the total Malpighian corpuscle total area revealed that there was an increase in the total area of all treated groups compared to control. This increase was significant in the lowest AFB₁ concentration only ($p < 0.05$), while it was insignificant in the other two concentrations ($p = 0.000$) (Fig. 12).

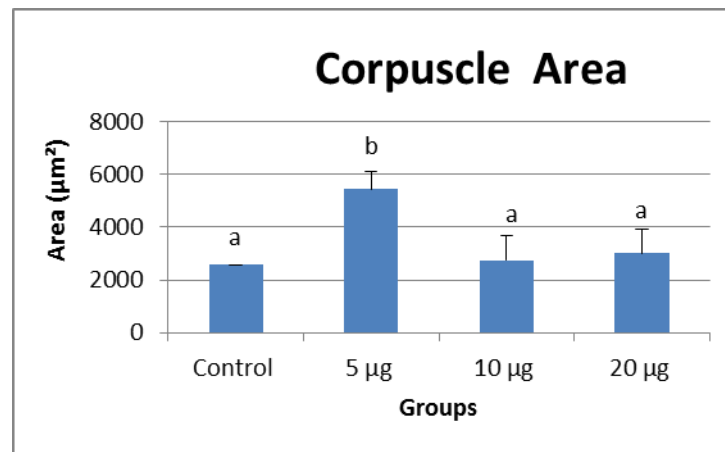


Fig. (12): A comparison of Renal Corpuscle total area of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.4.2. Glomerular area:

Statistical analysis of the glomerular area revealed that there was an increase in the glomerular area of all treated groups compared to control. This increase was significant in the lowest AFB₁ concentration only ($p < 0.05$), while it was insignificant in the other two concentrations ($p = 0.00$) (Fig. 13).

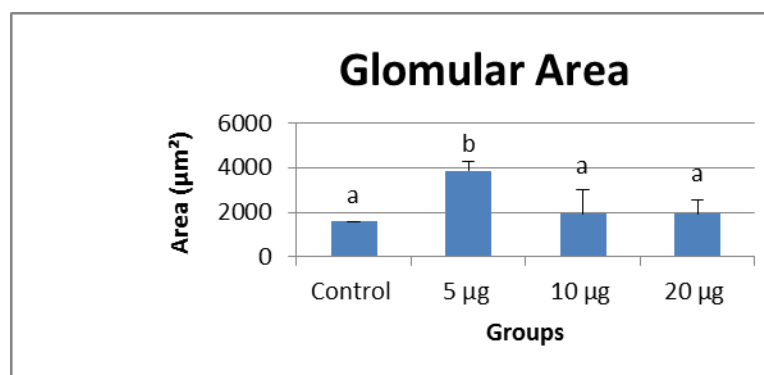


Fig. (13): A comparison of Glomerular area of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.4.3. Bowman's space area:

Statistical analysis of the Bowman's space area revealed that there was an increase in the glomerular area of all treated groups compared to control except 10 μ g AFB₁ treated group which revealed a decrease in the glomerular area compared to control. This increase was significant in the lowest AFB₁ concentration only ($p = 0.007$), while it was insignificant in the other two concentrations (Fig. 14).

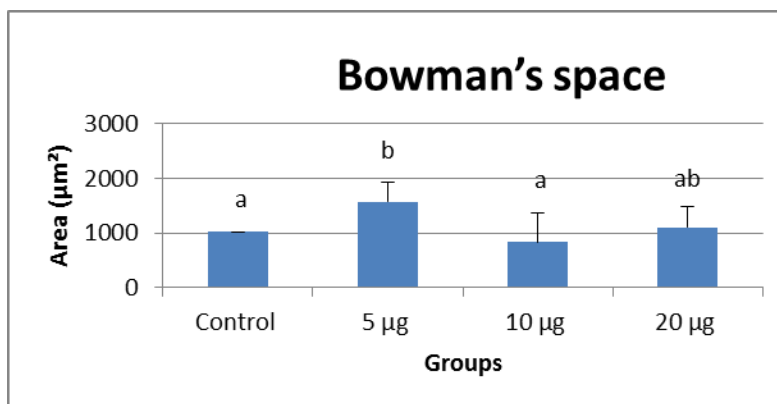


Fig. (14): A comparison of Bowman's space area of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

3.1.4.4. Glomerular cellularity:

Statistical analysis of the glomerular cellularity revealed that there was an increase in the glomerular cellularity of all treated groups compared to control. This increase was significant in the lowest and highest AFB₁ concentration ($p = 0.002$), while it was insignificant in the 10 μ g AFB₁ concentration ($p > 0.05$) (Fig. 15).

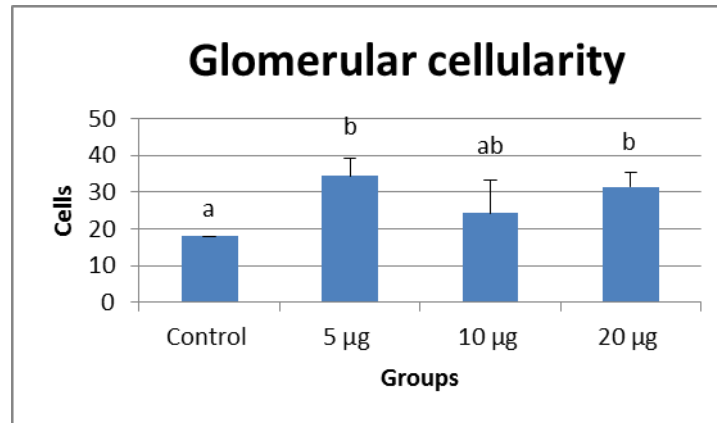


Fig. (15): A comparison of Glomerular cellularity of control and AFB₁ treated groups.

Values are presented as means ± SD.

3.1.4.5. Glomerular cell density:

Statistical analysis of the glomerular cell density revealed that there was an increase in the glomerular area of the two highest treated groups compared to control. This increase was significant in the highest AFB₁ concentration only ($p < 0.05$), while the lowest AFB₁ concentration showed a significant decrease compared to control ($p = 0.00$) (Fig. 16).

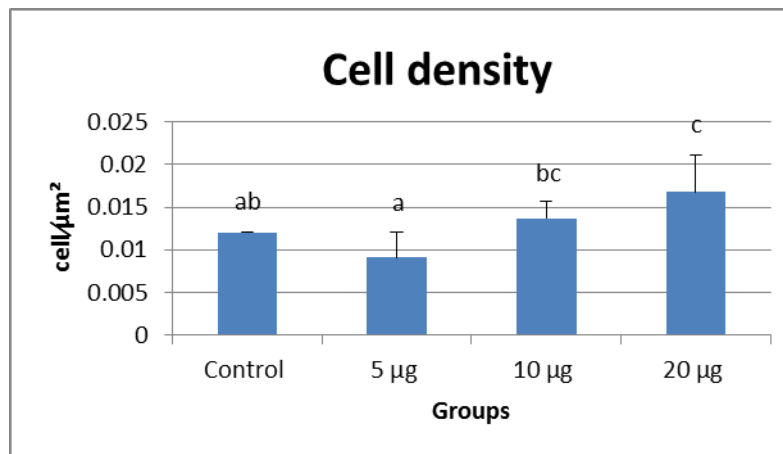


Fig. (16): A comparison of cell density of control and AFB₁ treated groups. Values are presented as means ± SD.

3.1.4.6. Inflammatory Cell Count:

Statistical analysis of Inflammatory Cell Count revealed that there was an increase in the count of inflammatory cells of all AFB₁ treated groups compared to control. This increase was insignificant in all AFB₁ treated groups ($p = 0.235$) (Fig. 17).

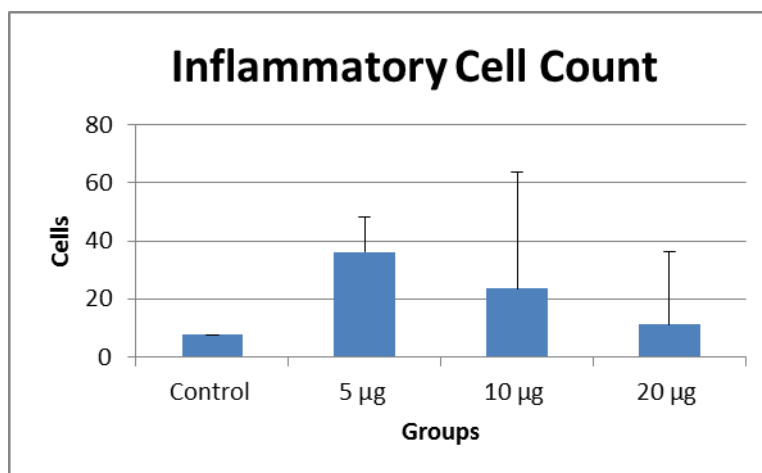


Fig. (17): A comparison of inflammatory cell count of control and AFB₁ treated groups. Values are presented as means $\pm$ SD.

DISCUSSION

The present study demonstrated that maternal exposure to AFB₁ induced various histopathological alterations and necrotic changes in the liver. These changes included inflammatory cell infiltration, vascular congestion, interstitial edema, an increased number of Kupffer cells, and anisokaryosis. Additionally, hepatocytes exhibited hydropic and ballooning degeneration, elevated nuclear chromatin content, the presence of trinucleated cells, and dilatation of hepatic sinusoids. Consistent with these findings, Orsi et al. [29], Wang et al. [30] and Mo et al. [31] reported that prolonged oral administration of AFB₁ (30 µg/kg body weight/day for 21 days) caused hepatic damage in male New Zealand rabbits. Histological examination revealed hydropic and ballooning degeneration of hepatocytes, disruption of hepatic trabecular architecture, and subsequent biliary duct

hyperplasia. Similarly, Shyamal et al. [32] and Wu et al. [33] showed that intraperitoneal administration of AFB₁ at a dose of 1.5 mg/kg induced acute liver injury, characterized by distorted hepatic lobular architecture, ballooned hepatocytes, bile duct hyperplasia in the portal region, and inflammatory cell infiltration.

The current findings are also consistent with those of Fetaih et al. [34], who demonstrated that administration of AFB₁ at 1 mg/kg body weight to pregnant rats between gestational days 6 and 15 resulted in marked hepatic damage. Histopathological changes included hepatomegaly, enlarged hepatocytes containing numerous cytoplasmic vacuoles, pyknotic nuclei, focal areas of necrosis, disorganized hepatic cord arrangement, and congestion of central veins. Additionally, newly formed bile ducts were observed within portal areas. Mughal et al. [35] further reported that dietary exposure to AFB₁ in chickens for three weeks induced progressive histological alterations from day 7 to day 21, including hepatic congestion, vacuolar and fatty degeneration, bile duct proliferation, nuclear fragmentation, and increased apoptosis in hepatocytes. Zandi et al. [36] found that AFB₁ administered to broilers in a concentration of 500 ppb induced a significant increase in the central vein diameter, in addition to decrease in the hepatocyte diameter and hepatocyte nuclear diameter. In Wistar rats, AFB₁ was found to induce central vein congestion and dilation, vacuolar degeneration, necrotic foci, bile duct hyperplasia, and portal congestion [37]. The explanation of how AFB₁ was able to induce such injuries depended on hepatic bioactivation of AFB₁ to the 8, 9-epoxide which generates DNA/protein adducts and oxidative injury, and consequently contributes to sinusoidal stasis and venous dilation [38 & 39]. The current study is siding with and confirms the earlier studies in this aspect.

In line with previous studies, the present investigation also revealed significant renal alterations in the AFB₁-treated group. These included glomerular hypertrophy, shrinkage of glomeruli, expansion of Bowman's space, necrotic changes in renal tubules, marked vascular congestion, and inflammatory infiltration. Nuclear degeneration was evident in the form of pyknosis and karyolysis. Additional findings included cloudy swelling, hydropic degeneration, glomerular hypercellularity, dilation of glomerular structures, hemorrhage, lobular accentuation, granular cytoplasmic appearance, and accumulation of cellular debris within tubular lumina.

These findings are in agreement with Fetaih et al. [34], who reported that administration of AFB₁ (1 mg/kg body weight from gestational days 6–15) in pregnant rats induced interstitial hemorrhage and necrosis in both proximal and distal convoluted tubules, along with the formation of renal casts due to tubular degeneration. Furthermore, Abou-Zeid et al. [40] observed that birds fed aflatoxin-contaminated diets exhibited severe renal lesions, including marked vascular congestion, dilated blood vessels with inflammatory reactions, glomerular atrophy, hydropic degeneration of tubular epithelial cells, and thickening of the Bowman's capsule basement membrane.

The present results are also comparable to those reported by Liang et al. [41], who investigated the nephrotoxic effects of dietary AFB₁ (0.3 mg/kg) in newly hatched chickens. Histopathological examination revealed swelling and necrosis of renal epithelial cells. Cellular swelling, including granular and hydropic degeneration, was observed as early as day 7, while tubular epithelial necrosis became evident by day 21. The authors suggested that the progressive severity of these lesions may be attributed to the accumulation of AFB₁ metabolites over time.

Nephrotoxicity of AFB₁ in the present study did not involve only degeneration and necrosis of renal tubules but also extended to involve the renal corpuscles. Histomorphometric analysis revealed widening of the Bowman's space accompanied with the decrease in the cellular density of glomeruli reflecting the degenerative and nephrotoxic effect of AFB₁. Similar results were reported by Abdel-Hamid and Firgany [42], who noticed a significant widening of the urinary space within Malpighian corpuscles of rats after AFB₁ treatment. The present study is in parallel with and confirms the findings of Abdel-Hamid and Firgany [42]. In a contradictory finding of an earlier study, Abdu et al. [43] reported urinary spaces narrowing of cortical and juxtamedullary corpuscles and swollen glomeruli due to proliferation of the mesangial tissue of rat kidney after ochratoxin A four weeks of treatment. It is suggested here that the conflict with the earlier study of Abdu et al. [43] might be explained based on different mode of action among different mycotoxins, species differences or the duration of treatment.

CONCLUSION

AFB₁ exerted hepatotoxic and nephrotoxic impact on liver and kidney histological structure of pregnant mice fed on contaminated fodder and caused many histopathological and histomorphometric alterations including necrosis, hydropic degeneration, edema, hemorrhage and blood congestion reflecting high risk toxicity of AFB₁.

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