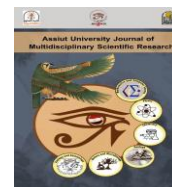


Assiut University Journal of Multidisciplinary Scientific Research (AUNJMSR)
Faculty of Science, Assiut University, Assiut, Egypt.
Printed ISSN 2812-5029
Online ISSN 2812-5037
Vol. 55 (3): 646- 685 (2026)
<https://aunj.journals.ekb.eg>



Mycotoxicity and Embryonic Development: II- Aflatoxin B₁ Triggers Agonistic Aggressive Behaviour and Cannibalism in Pregnant Mothers and Induces Morphological and Morphometric Alterations in Mice Infants

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ARTICLE INFO

Article History:

Received: 2026-05-24

Accepted: 2026-06-16

Online: 2026-08-31

Keywords:

Mortality Percentage,
Resorption, Pregnancy
Percentage, Maternal
Toxicity, Fetal
Retardation

ABSTRACT

Background: Aflatoxins are harmful mycotoxins. Among the various types of aflatoxins, aflatoxin B₁ (AFB₁) is the predominant form with the most toxic effect. AFB₁ is produced by *Aspergillus*, which usually contaminates a wide range of agricultural products, such as grain, nuts, or spices. The problem of aflatoxin contamination of the food products is a common problem in tropical and subtropical regions of the world, especially in the developing countries where the environmental conditions of warm temperatures and humidity favor the growth of fungi. AFB₁ is known to interfere with embryonic development. **Aim of the work** is to evaluate the

effect of AFB₁ on pregnant mice and their offspring. **Methods:** 40 pregnant female mice were divided into groups and fed fodder contaminated with 5, 10 and 20 µg AFB₁ / kg of fodder. Administration started ten days before mating and continued during the whole period of pregnancy. Treated mothers and offspring were morphologically examined and weighed daily. **Results:** The treated mothers showed body weight loss, in utero embryo resorption, reduced pregnancy percentage (90.9% and 83.3%), and low number of embryos per mother (3.53, 5.18 & 5.42 compared to 8.23 in controls). Mortality percentage increased (3.77, 5.26 & 16.92 compared to 2% in control). Neonatal infants exhibited insignificant reduction in body weight and altered morphometric measurements including crown rump and head circumference, ear pinna, tail length fore and hind limb lengths, which were significantly reduced. 21 postnatal day-old infant restored normal body weight. Exposed mothers showed aggressive agonistic behavior and cannibalism. Conclusion: AFB₁ has a negative impact on embryonic development, altered and reduced infant morphological parameters. 21 postnatal day-old infant restoration of normal weight might be attributed to early disappearance of AFB₁ from mothers' milk. AFB₁ induced cognitive neurotoxicity leading to aggression. Further studies are recommended.

INTRODUCTION

Aflatoxins are harmful mycotoxins [1]. Among the various types of aflatoxins, aflatoxin B₁ (AFB₁) is the predominant form with the most toxic effect [2]. AFB₁ is produced by *Aspergillus*, which usually contaminates a wide range of agricultural products, such as grain, nuts, or spices, especially in tropical and subtropical climatic regions [3]. AFB₁ is not only known as one of pathogenic factors for liver cancer, but also it is associated with

other chronically adverse effects [4], including immune suppression, nutrient absorption, and metabolic disturbance.

Notably, AFB₁ exposure is ubiquitous in the natural environment throughout the world, and disconcertingly it has been detectable in pregnant women [5-7]. Accumulating evidence indicates a link between prenatal AFB₁ exposure and adverse pregnancy and birth outcomes, including maternal anemia and intrauterine growth restriction [8, 9]. Anemia is confirmed to be connected with fetal growth restriction [10], premature birth, and low birth weight [11]. Animal studies have revealed that AFB₁ has impaired the morphology of erythrocytes by inducing oxidant stress and caused a decrease in hemoglobin concentration [12, 13]. However, the effect of AFB₁ has not been extensively studied in humans. Only one cross-sectional research study reported the relationship between AFB₁ exposure and anemia among pregnant women in Ghana, Africa [14].

Aflatoxicosis has been reported to cause lymphocytopenia and monocytopenia and increased percentage of neutrophil counts [15]. Generally, aflatoxins have been reported to depress growth and alter many aspects of humoral and cellular immunity and thus affect the hematological parameters [16, 17]. The exposure of pregnant females or birds to aflatoxins can affect embryos in utero or in fertilized eggs, respectively, producing various adverse health effects and different pathological gestation/incubation outcomes [9].

Pregnant rats that were administered AFB₁ between the sixth and fifteenth days of pregnancy, were harmed at a dosage of one mg/kg body weight [18]. Also, pregnant women or birds that were exposed to aflatoxins produced a variety of negative health effects and pathological pregnancy/incubation outcomes [9]. Pregnant women exposed to

increased risk of experiencing a range of consequences, such as preterm birth, fetal loss and retarded growth. There has been evidence of growth limitation in both people and animals, where there is a negative correlation between birth weight and the concentrations of the right biomarkers in the cord blood [19].

This study aims to explore the adverse morphological effects of AFB₁ on the developing mice embryo, to determine whether a small dose of AFB₁ could induce anomalies and the effect of AFB₁ on the pregnant female mice that were fed on a contaminated fodder.

MATERIALS AND METHODS

2.1. Production of AFB₁:

Aflatoxin B₁ was produced in Botany & Microbiology Department, Faculty of Science, Assiut University using special strain of *Aspergillus flavus* (*A. flavus* AUMC 16585). This strain was isolated from popcorn, identified and characterized as highly producer of aflatoxin B₁ in a recent study [20]. 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.* [21] with minor modification by Oguz [22]. Briefly, broken uncontaminated feed (each 300 g) was placed in each 1000 mL Erlenmeyer flask at 20% moisture content and was sterilized in 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 with hand shaken vigorously 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 [23] and quantitative analysis of aflatoxin was detected

by ultra-performance liquid chromatography (UPLC) according to the method described by Benvenuti and Burgess [24] 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 [25] 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 an 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 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 resulting 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 dose: Was prepared by mixing 49.5 g of free fodder and 5.5 g of contaminated fodder with AFB₁.

10 µg/kg dose: Was prepared by mixing 49.5 g of free fodder and 11.1 g of contaminated fodder with AFB₁.

20 µg/kg dose: Was prepared by mixing 49.5 g of free fodder and 22.1 g of contaminated fodder with AFB₁.

2.4. Experimental Animals:

40 Adult females and 20 males albino mice (CD1) of an average body weight 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:

2.4. 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*.

The dose selection was achieved according to the reported permissible limits of aflatoxin in some countries such as South Korea (10–20 µg/kg). A lower dose (5 µg/kg) was also included to assess the effects of sub-threshold levels. The dose selection was further supported by a previous study of Ali et al. [26].

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 a half a day of gestation.

2.6.4. The gradual increase in motherly body weight was considered as a sign for 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).

Also, mortality rate of infants and embryo number per mother were calculated.

2.7. Specimens preparation:

2.7.1. Neonatal and 21-postnatal day mice infants were considered for investigation.

2.7.2. Pregnant female mice and embryos at 10.5 and 15.5 days of gestation were also subjected to investigation. Mice infants (neonatal and 21 postnatal days) were fixed in

10% formalin for morphological studies. After fixation, the specimens were preserved in 70% ethanol.

2.8. Morphological investigations:

Weight and morphological abnormalities were recorded for infants at the ages of (neonatal and 21 postnatal days). Morphometric measurements were recorded for infants at the age of 21 postnatal days, such as crown-rump length, head length, head circumference, head height, tail length, ear length, fore limb and all parts of hind limb lengths.

2.9. Statistical analysis:

The obtained data were subjected to one-way ANOVA analysis followed by Duncan post hoc-test at $p \leq 0.05$. Correlation coefficient was also applied to the present data using SPSS package program.

RESULTS

3.1. Aflatoxin B₁ Concentration:

Quantitative analysis of aflatoxin B₁ using HPLC revealed that its concentration was 8.401 ppm while AFB₂ was 0.68 ppm, as determined from the corresponding calibration curve (fig. 1)

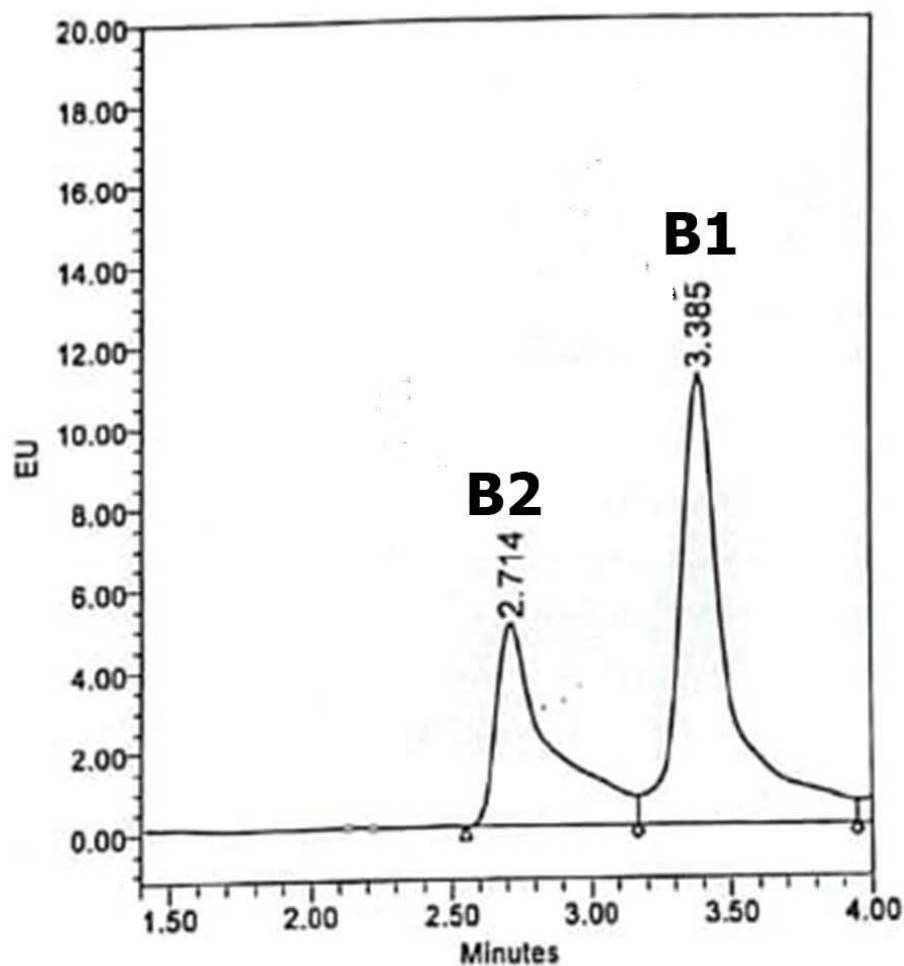


Fig. (1): A histogram representing the absorbance of Aflatoxin B₁ and B₂ (EU/min)

3.2. Morphological Observations on female mice

3.2.1. Effect of AFB₁ on female mice weight before pregnancy:

Statistical analysis of the body weight of female mice before pregnancy indicated that body weight was changed due to AFB₁ treatment. AFB₁ 5 & 20 µg treatments showed significant decrease compared to control mothers ($p = 0.032$), while this decrease was insignificant in case of 10 µg treatment ($p > 0.05$) (Fig. 2).

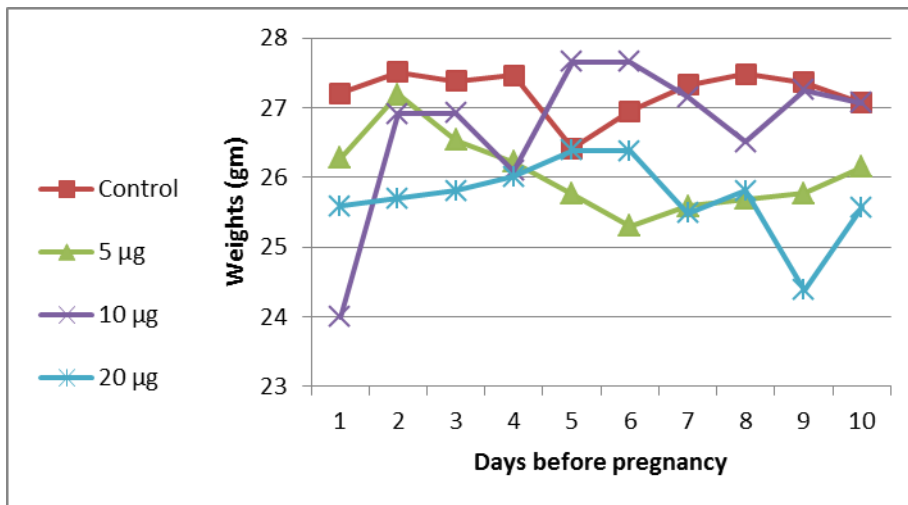


Fig. 2: Effect of AFB₁ on female mice weight before pregnancy.

3.2.2. Effect of AFB₁ on female mice during pregnancy days:

Statistical analysis of the body weight of female mice during pregnancy indicated that all treated groups showed insignificant decrease compared to control mothers ($p > 0.05$) (Fig. 3).

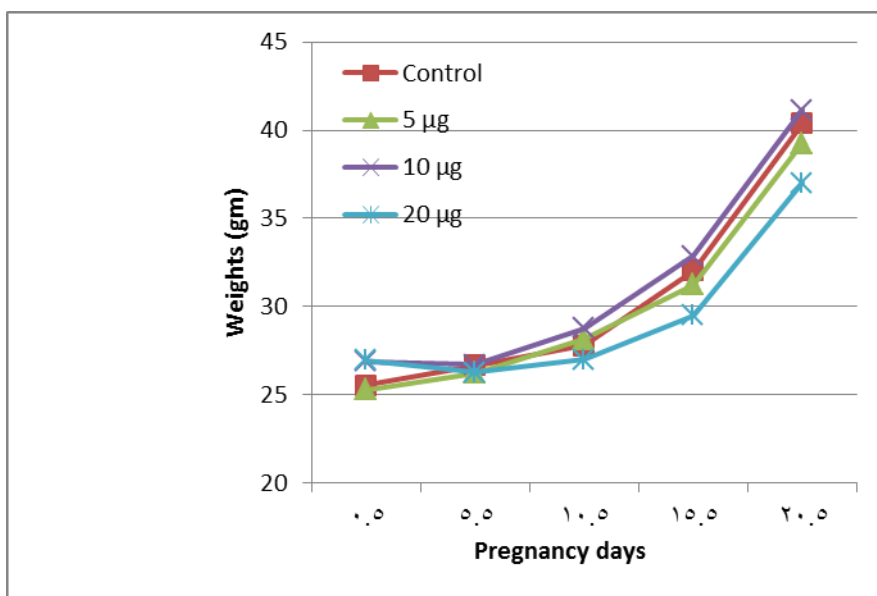


Fig. 3: Effect of AFB₁ on female mice weight during pregnancy.

3.2.3. Effect of AFB₁ on mother mice during lactation:

Statistical analysis of the body weight of female mice during lactation indicated that all treated groups were greatly affected and showed significant decrease compared to control mothers ($p = 0.00$). The median AFB₁ concentration (10 µg) was less effective than the lowest and highest AFB₁ concentrations (5 & 20 µg) where the most reducing effect was obtained (Fig. 4)

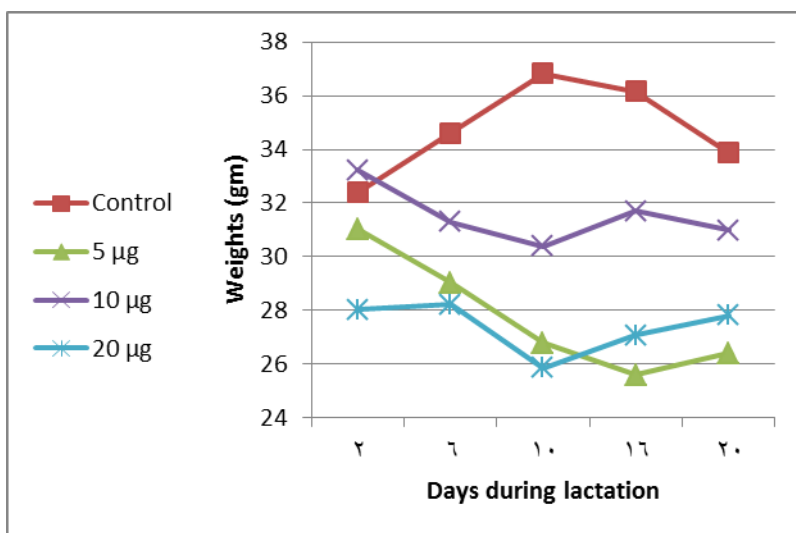


Fig. 4: Effect of AFB₁ on female mice weight during lactation period.

3.2.4. Effect of AFB₁ on pregnancy outcomes:

From each group, three pregnant females were sacrificed by cervical dislocation on days 10.5 and 15.5 of gestation days (GD). The uteri of the control pregnant mice at 10.5 gestation days showed a normal distribution of the embryos among the two horns, with an average size of implantation sites (Fig. 5 a). However, 5 & 10 µg AFB₁ treated groups showed abnormal distribution of the embryos between the two horn parts, with delay in the development of embryos which was indicated by small size of implanted embryos, also there was a decrease in the number of implantation sites compared to control indicating that some embryos may be resorbed (Fig 5 b, c & d). Moreover, there were clearly visible resorbed embryos in 10 µg AFB₁ treated groups (Fig. 2 d). In 20 µg AFB₁ group the abnormalities were less than the two lower doses of AFB₁, the size of embryos and number of implantation sites were less than control which indicates that miscarriage may not occurred in these cases, also in another case, growth retarded embryos were indicated by small size, compared to control group (Fig. 5 e).

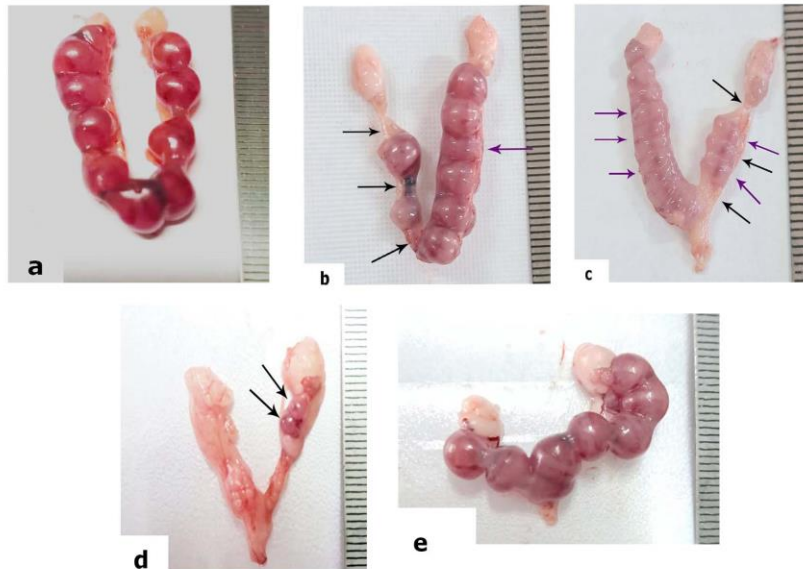


Fig. (5): Photographs of the mice uteri at GD 10.5 in control and AFB₁treated groups, (a) control, (b & c) 5 µg AFB₁, (d) 10 µg AFB₁ and (e) 20 µg AFB₁. Abnormal size of implantation sites (purple arrows) and resorbed embryos (black arrows).

The uteri of the control pregnant mice at 15.5 GD showed normal distribution of the embryos among the two horns, with an increase in the size of developing embryos (Fig. 6 a). However, 5 µg AFB₁ treated group showed a decrease in the number of implantation sites compared to control, and uterus also showed a marked cystic dilatation of one uterine horn filled with clear to yellowish fluid (Fig. 6 b). In 10 µg & 20 µg AFB₁ treated groups, the uterine horns showed growth retardation of embryos and there was also a decrease in the number of implantation sites in 10 µg AFB₁ treated groups compared to control group (Fig. 6 c & d).

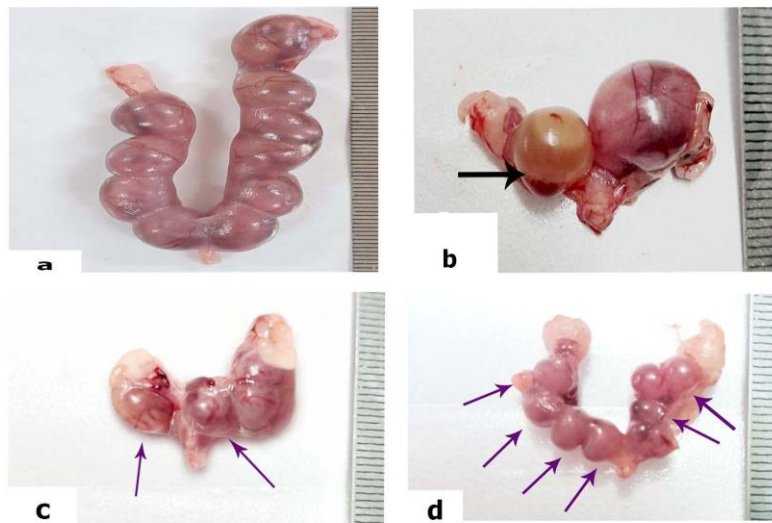


Fig. (6): Photographs of the mice uteri at GD 15.5 in control and AFB₁ treated groups, (a) control, (b) 5 µg AFB₁, (c) 10 µg AFB₁ and (d) 20 µg AFB₁. Abnormal size of implantation sites (purple arrows) and resorbed embryos (black arrows).

Pregnancy percentage: daily observations during the experiment revealed that pregnancy percentage was 100% in control mothers and those fed on the lowest concentration of AFB₁. The pregnancy percentage was decreased to 90.9% upon treatment with the median concentration of AFB₁ and reached the highest percentage of about 83.33% upon feeding on the highest concentration of AFB₁. This means that pregnancy rate is concentration dependent (Fig. 7).

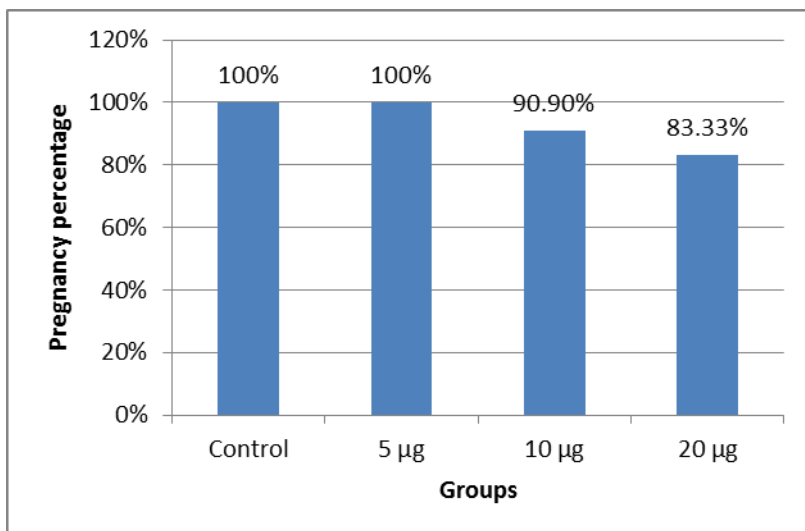


Fig. 7: A comparison of pregnancy percentage of control and experimental female mice fed on different concentrations of AFB₁.

Mortality percentage of the infants: visual observations of the newly born infants during lactation period revealed that the lowest mortality percentage was recorded in the control group, where only 2% of infants died without any clear cause. The percentage of mortality was 3.77% in case of the lowest concentration of AFB₁. This percentage increased to 5.26% in the median concentration of AFB₁ and reached the highest percentage of about 16.92% in after treatment with the highest concentration of AFB₁. These observations clearly indicate that the mortality rate was concentration dependent (Fig. 8).

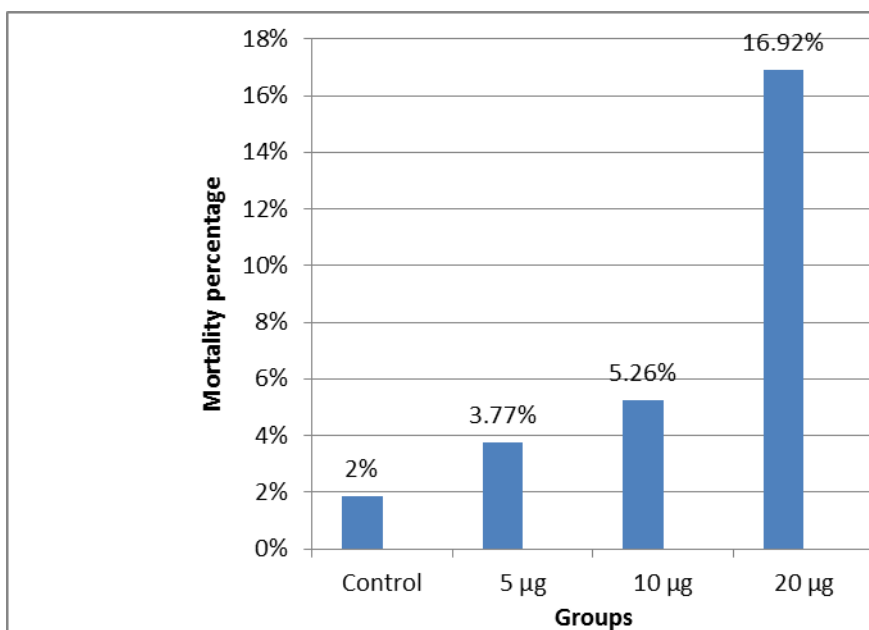


Fig. 8: A comparison of mortality percentage of newly born infants of control and lactating mothers fed on different concentrations of AFB₁.

Embryo number per mother: counting the number of embryos or newly born babies revealed that the average number of embryos per mother was 8.23 embryos per control mother. The lowest concentration of AFB₁ produced the lowest average of 3.53 embryos per mother. The median and highest concentrations of AFB₁ gave the averages of 5.18 and 5.42 embryos per mother respectively (Fig. 9).

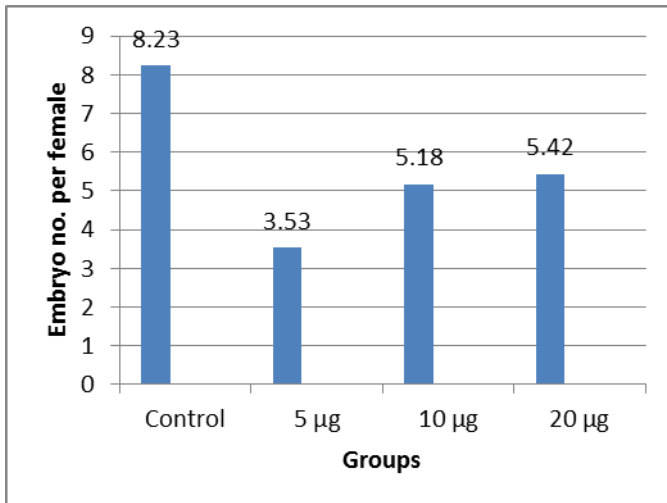


Fig. 9: A comparison of number of embryos per mother of control treated mothers fed on different concentrations of AFB₁.

3.3. Morphometric measurements of the offspring:

3.3.1. Measurements of neonatal infant body weight:

Statistical analysis of measurements of the neonatal infants of control and experimental groups revealed insignificant differences among all treatments, where $p > 0.05$ (Fig.10).

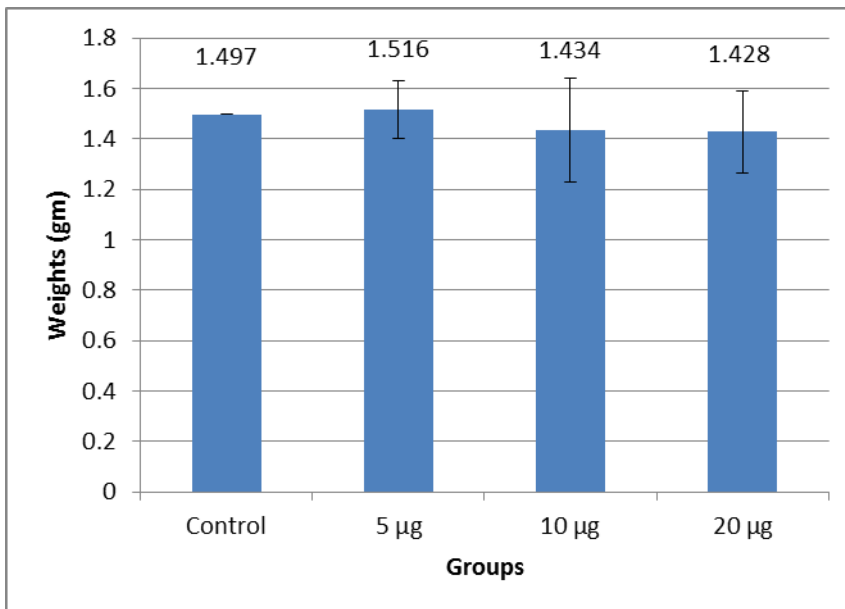


Fig. 10: A comparison of weight of control and experimental neonatal infants after treatment with different concentrations of AFB₁. Data are presented as Mean $\pm$ SD.

3.3.2. Measurements of 21 postnatal day-old infant body weight:

All treated groups demonstrated higher mean body weights compared to control group, with a statistically significant difference ($p = 0.028$). Notably, the 20 μg AFB₁ treated group showed the highest increase in mean body weight compared to control (Fig. 11)

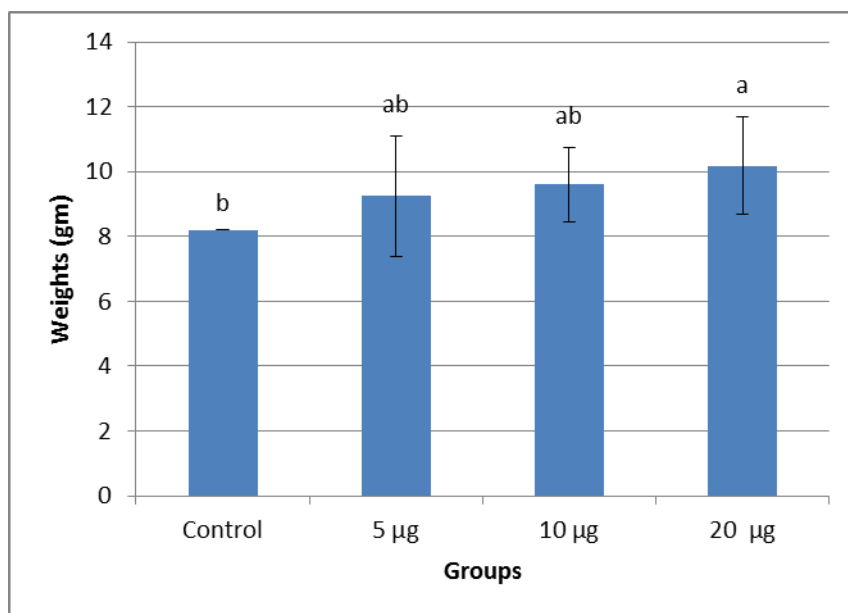


Fig. 11: A comparison of weight of control and experimental infants at the age of 21 postnatal days after treatment with different concentrations of AFB₁. Data are presented as Mean $\pm$ SD.

3.3.3. Morphometric parameters of infants at 21 postnatal days:

Statistical analysis of the crown rump length measurements in AFB₁ treated groups showed insignificant increase in the two lower concentrations compared to control groups, while there was a significant increase in the highest concentration of AFB₁, where $P = 0.034$ (Fig. 12).

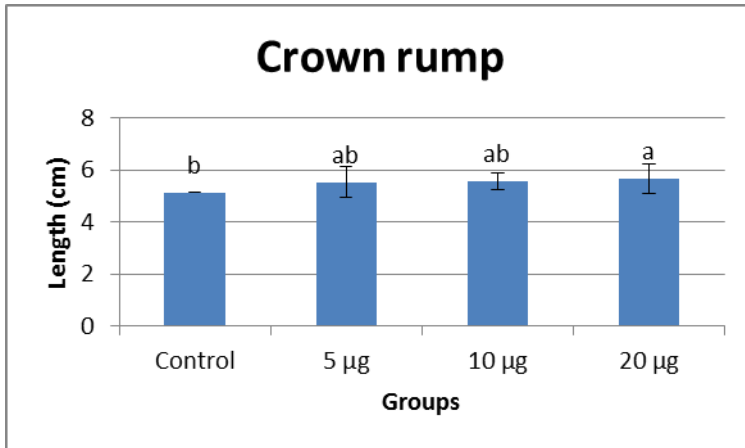


Fig. 12: A comparison of crown-rump length (cm) at 21 days old infant mice between different treated groups. Data are presented as Mean $\pm$ SD.

Statistical analysis of the head height measurements revealed insignificant decrease in 10 & 20 µg AFB₁ treated group compared to control, where $P > 0.05$ (Fig. 13).

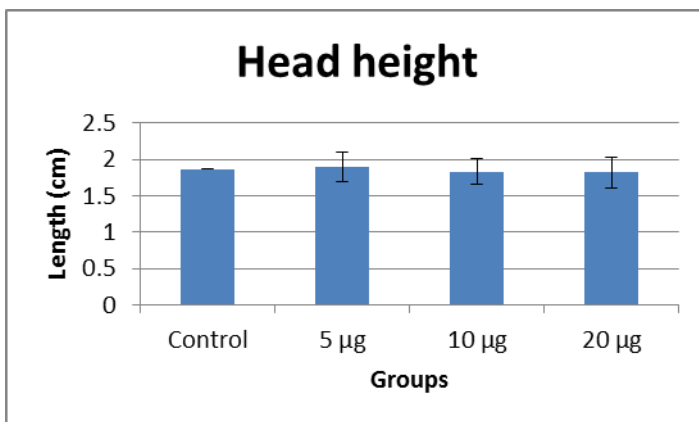


Fig. 13: A comparison of head height length (cm) at 21 days old infant mice between different treated groups, showing insignificant differences with little decrease in the highest two concentrations of AFB₁. Data are presented as Mean $\pm$ SD.

Statistical analysis of the head circumference measurements revealed decrease in measurements in all treated groups. The decrease was significant in the lowest and highest two concentrations of AFB₁ where $p = 0.049$, while the median concentration exhibited insignificant decrease (Fig. 14).

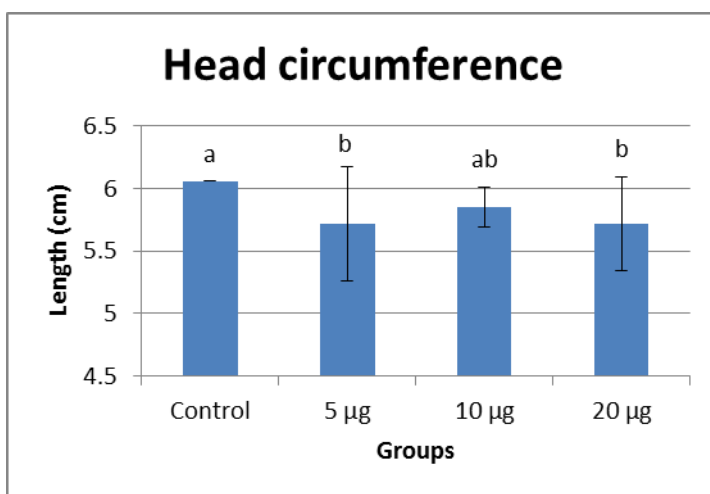


Fig. 14: A comparison of head circumference length (cm) at 21 days old infant mice between different treated groups, showing decrease in measurements in all treated groups. The decrease was significant in the lowest and highest two concentrations of AFB₁. Data are presented as Mean $\pm$ SD.

Statistical analysis of the head length measurements revealed insignificant decrease in 10 & 20 μg AFB₁ treated group compared to control, where $P > 0.05$ (Fig. 15).

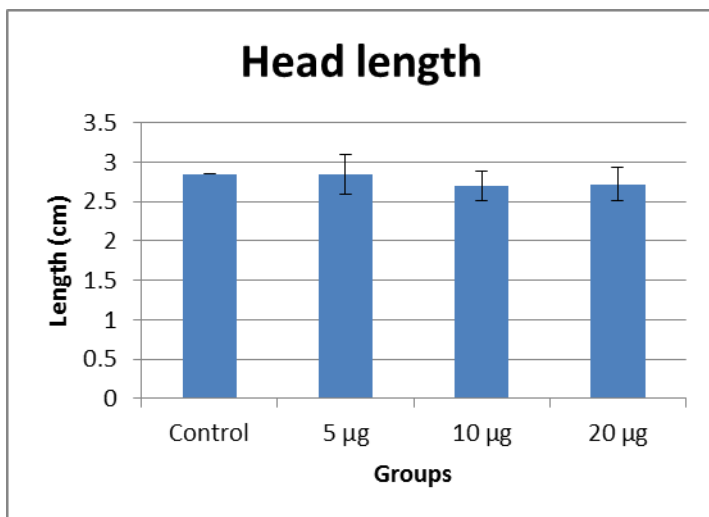


Fig. 15: A comparison of head height length (cm) at 21 days old infant mice between different treated groups, showing insignificant decreases compared to control. Data are presented as Mean $\pm$ SD.

Statistical analysis of the ear pinnae lengths revealed increase lengths in all treatments. This increase was significant in the two lower concentrations of AFB₁ treatments compared to control group, where $P = 0.002$. The increase in length was insignificant in the highest concentration of AFB₁ (Fig. 16).

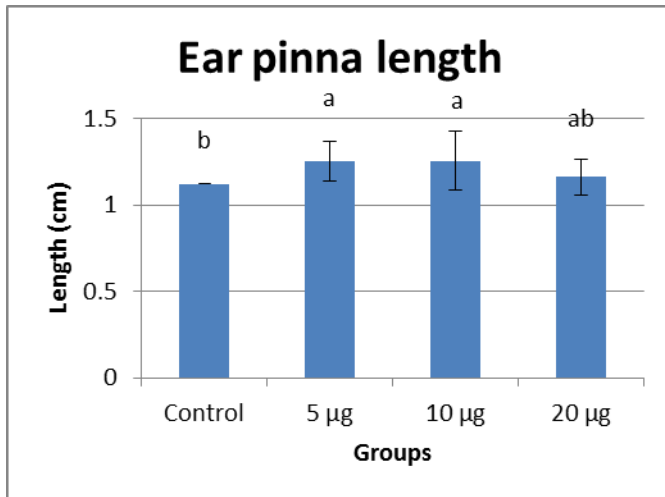
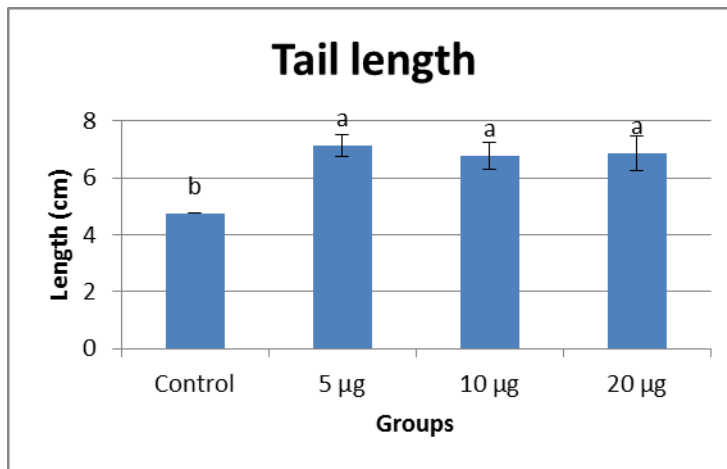


Fig. 16: A comparison of ear pinnae lengths (cm) at 21 days old infant mice between different treated groups, showing increase in measurements in all treated groups. The decrease was significant in the lowest two concentrations of AFB₁, while the highest concentration of AFB₁ was insignificantly increased. Data are presented as Mean $\pm$ SD.

Statistical analysis of the tail length revealed a highly significant increase in all treated groups compared to control, where $P = 0.00$ (Fig.



17).

Fig. 17: A comparison of tail height length (cm) at 21 days old infant mice between different treated groups, showing highly significant increases compared to control ($P = 0.00$). Data are presented as Mean $\pm$ SD.

Statistical analysis of the fore-limb length revealed insignificant differences among all treatments compared to control. Such differences ranged from insignificant decreases in the two lower concentrations to an insignificant increase in the highest concentration of AFB₁. Among treatments, the lowest and highest concentrations exhibited a significant difference, where $P = 0.058$ (Fig. 18).

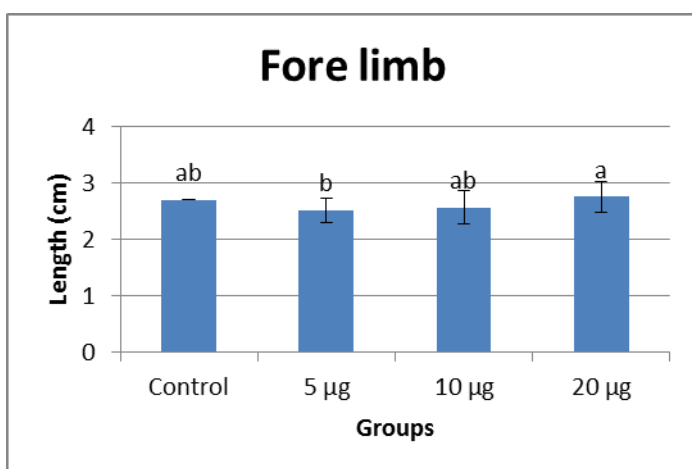


Fig. 18: A comparison of fore-limb lengths (cm) at 21 days old infant mice between different treated groups, showing insignificant differences compared to control. The lowest concentration of AFB₁ was significantly different from the highest one, where $P = 0.058$. Data are presented as Mean $\pm$ SD.

Statistical analysis of the hind limb length revealed an increase in all AFB₁ treated group compared to control. This increase was significant in case of the median concentration of AFB₁, where $P = 0.031$ (Fig.19).

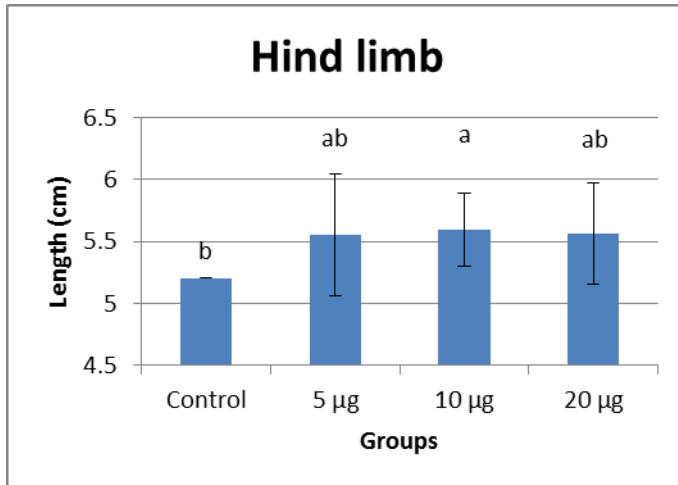


Fig. 19: A comparison of hind limb lengths (cm) at 21 days old infant mice between different treated groups, showing increases in all treated groups compared to control. The median concentration of AFB₁ was significantly different from control, where $P = 0.031$. Data are presented as Mean $\pm$ SD.

Statistical analysis of the hind limb parts, thigh and foot, exhibited insignificant increases in length compared to control in all treated groups. The shank was the only limb part that showed a significant increase in all AFB₁ treated groups compared to control, where $P < 0.05$ (Fig. 20).

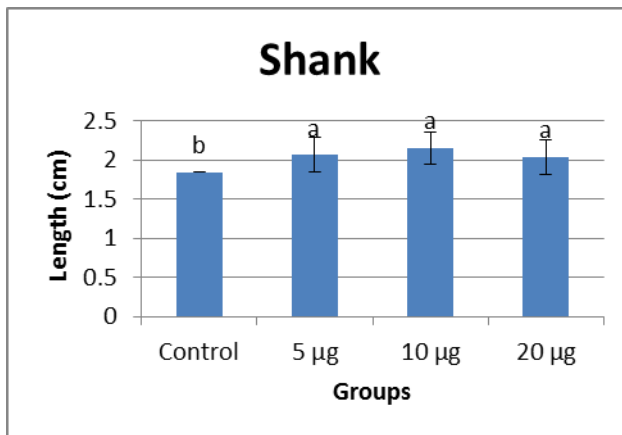


Fig. 20: A comparison of shank lengths (cm) at 21 days old infant mice between different treated groups, showing significant increases in all treated groups compared to control. $P < 0.05$. Data are presented as Mean $\pm$ SD.

Collectively, feeding female mice on a fodder contaminated with different concentrations (5, 10 & 20 μg) of AFB₁ resulted in variable morphometric and morphological alterations in size, lengths of crown rump, head, ear pinna, limbs and tail of the experimental offspring compared to control (Fig. 21).



Fig. 21: A photograph showing a comparison between infants of control and treated offspring showing differences in lengths of crown rump, head, ear pinnae, tail, and limbs at the age of 21 postnatal days.

3.3.4. Aggressive Behavior:

The aggressive behavior was recorded in this study, where some females of the treated groups, especially mothers fed on the fodder containing the highest two concentration of AFB₁ (10 & 20 µg), behaved aggressively against other females or males in the same cage. Such kind of fighting resulted in killing a female victim, destroying its skull and eating the brain (Fig. 19 a). Another sort of aggression was cannibalizing the offspring (Fig. 19 b & c).

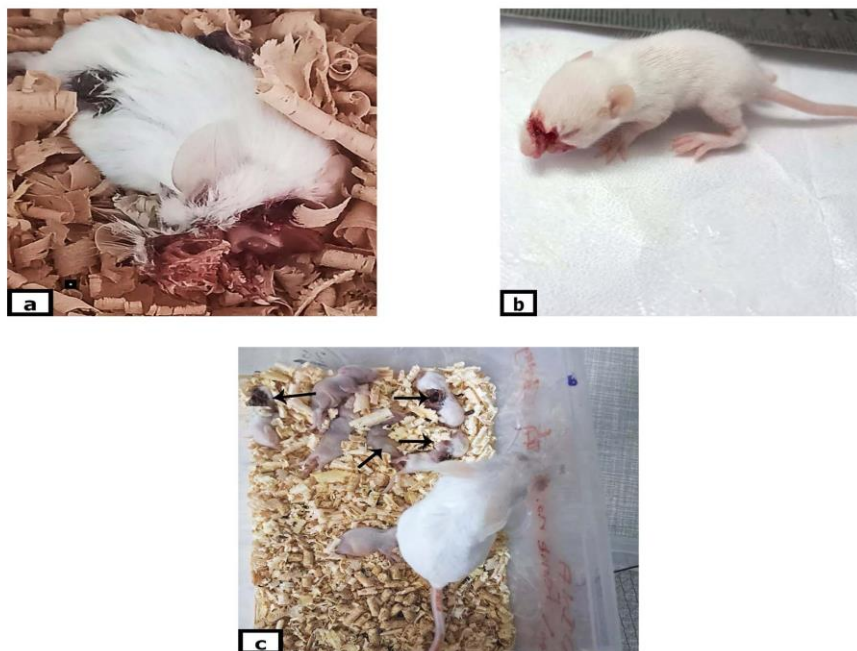


Fig. 19: Aggressive behavior of female mice treated with AFB₁. a) Fighting against another female resulted in killing it, destroying the skull and eating its brain. b & c) Cannibalism of the offspring (black arrows).

DISCUSSION

The current study demonstrated that exposure of pregnant mice to AFB₁ has a negative impact on pregnant mothers, birth quality and induced morphological and morphometric alterations in the offspring. The present results demonstrated that the body weight of mothers decreased before pregnancy due to AFB₁ administration. These results confirm previous studies carried out by Fetaih *et al.* [18] who demonstrated that AFB₁ caused harm in pregnant rats when given at a level of 1 mg/kg body weight. Xu *et al.* [27] reported mice liver injury involved mitochondrial dysfunction mediated by mitochondrial biogenesis inhibition and hepatocyte membrane permeability dysfunction after AFB₁ exposure. Tsouloufi [28] reported weight loss and anorexia after AFB₁ exposure in rabbits. It is suggested here that liver is the main protein, lipid and carbohydrate manufacturer and hence, its harm due to AFB₁ exposure might result in weight loss. In addition, pregnant mice fed on AFB₁-contaminated fodder might suffer anorexia and fatigue leading to weight loss. The present study is in agreement with and confirms the earlier studies in this respect.

The present results revealed that the mortality percentage of infants increased while pregnancy percentage and average embryo number per female decreased in mothers treated with AFB₁ compared to control. This coincides with the results of the study achieved by Khan *et al.* [29] who stated that *in ovo* administration of AFB₁ resulted in significant mortalities, embryonic malformations and hatchability of chicks with deficient immune system. Also, Ali *et al.* [26] recorded decreased percentage of pregnancy, number of infants per mother and increased mortalities in mice offspring after gastric administration of AFB₁. Such results are similar to what is reported in the current study.

The current study demonstrated that the body weight of mothers decreased. These results confirm previous studies done by Fetaih et al. [18] who demonstrated that AFB₁ caused harm in pregnant rats when given at a level of 1 mg/kg body weight. The body weights of mothers showed a considerable reduction and teratogenic changes in the fetuses representing the impacts and damage. Also, Ivanovics et al. [30] found that in domesticated animals and humans, AFB₁ contaminated feeds and meals caused a variety of health concerns, including tumor formation and hepatotoxicity. Exposure to AFs may result in negative pregnancy outcomes such as baby growth impairment, preterm delivery, and pregnancy loss [31]. According to Xu et al. [27], AFB₁ addition reduced body weight of mice. Wangikar et al. [32] found that the body weight of pregnant Wistar rats significantly decreased at 1.00 mg/kg dosage. Cases of abortion were observed in 0.50 and 1.00 mg/kg dose groups. One rat each died from treated groups 0.25, 0.50, and 1.00. In the highest (1.00 mg/kg) dose group, the percent implants resorbed was significantly higher whereas the percent live fetuses significantly decreased as compared with those in controls. The only dead fetus observed was in the 1.00 mg/kg dose group. When male rats were treated with 50 µg/kg AFB₁ for 28 consecutive days, AFB₁ reduced the mean body weight of rats [33].

Also, Smith et al. [9] demonstrated that Aflatoxin exposure during pregnancy may affect fetal growth and aflatoxin exposure may raise the chance of preterm delivery and pregnancy loss. The fetus might be harmed by maternal aflatoxin exposure by direct and indirect toxicity, such as maternal systemic inflammation, reduced placental growth, or increased placental cytokines. Aflatoxin's cytotoxic and systemic actions may have a role

in maternal anemia, intrauterine growth restriction, fetal death, premature birth and low birth weight.

The present findings indicate that prenatal exposure to AFB₁ induced alterations in several morphometric parameters of the offspring, suggesting an adverse effect on normal fetal growth and development. Although crown–rump length was significantly increased only in the highest-dose group, this increase was accompanied by reductions in several cranial measurements, including head circumference, head height, and head length. These findings suggest that AFB₁ exposure may disrupt the proportional growth of different body regions rather than causing uniform growth retardation.

The reduction in head circumference observed in all treated groups, particularly in the low- and high-dose groups, may indicate impaired cranial development and altered brain growth. Similar observations have been reported in studies demonstrating that AFB₁ can cross the placental barrier and interfere with fetal development through oxidative stress, DNA damage, and disruption of cellular proliferation. The observed decreases in head dimensions may therefore reflect the vulnerability of developing neural tissues to AFB₁ toxicity.

Interestingly, ear pinna and tail lengths were increased in AFB₁-exposed offspring, with tail length showing a highly significant increase in all treated groups. These findings suggest that different embryonic tissues may respond differently to AFB₁ exposure. Such disproportionate growth could result from disturbances in developmental signaling pathways regulating skeletal patterning and morphogenesis. The differential sensitivity of cranial and appendicular structures to toxic insult has been documented in developmental

toxicology studies and may explain the variations observed among morphometric parameters.

In contrast, forelimb length showed only minor, non-significant changes, indicating that limb development was less affected than cranial structures. This variation in susceptibility may be related to differences in the timing of tissue differentiation and growth during embryogenesis.

Overall, the morphometric alterations observed in the present study support the notion that maternal AFB₁ exposure can adversely affect fetal growth and body proportions. The absence of a consistent dose-dependent pattern for some parameters may reflect the complexity of developmental responses to AFB₁ and suggests the involvement of multiple mechanisms, including oxidative stress, altered cell proliferation, and impaired placental function. Further histological and molecular investigations are required to elucidate the precise pathways underlying these developmental abnormalities.

The hind limb length revealed an increase in all AFB₁ treated group compared to control. This increase was significant in case of the median concentration of AFB₁, thigh and foot, exhibited insignificant increases in length compared to control in all treated groups. The shank was the only limb part that showed a significant increase in all AFB₁ treated groups compared to control. The present findings are not consistent with those reported by the studies of [32, 34] who reported that the mean weights and crown-rump lengths significantly decreased and the percent gross anomalies significantly increased in fetuses of rabbits and rats fed diet containing 0.05 to 0.1 and 1.00 mg/kg AFB₁ respectively compared to controls.

Ali et al. [35] demonstrated that AFB₁ causes developmental abnormalities in the chick embryos, such as decreased hatchability, increased mortality, and severe congenital deformities. Weight and all morphometric parameters were significantly lower in treated groups. Mortality rates in the treated groups increased. Growth retardation, limb deformities such as limb buds or loss of limbs and poor ossification were all found in treated chick embryos with doses (5 ng, 10 ng, 20 ng) of AFB₁.

The present study is partially consistent with the findings of [32, 34, 35], showing agreement in certain aspects while differing in others.

Neonatal infant's weight showed reduction in the treated animals compared to control. This is consistent with earlier studies, while 21 postnatal day-old infants restored normal weight compared to control. Açar & Akbulut [36] stated that AFB₁ is transformed into AFM₁ can be detected in mother's milk 12–24 h after administering contaminated food. It rapidly fades with time and is undetectable after 3 days of the last administration of contaminated food. In this aspect, such an explanation might be a reasonable interpretation for restoring normal weight of 21 postnatal day old infants. It is suggested here that 21 postnatal day old infants were at normal weight compared to control due to disappearance of the AFB₁ or AFM₁ from the mother milk and hence there was no toxic effect and normal weight was easily restored. The present study confirms and supports the statement of [36].

The present study showed increased aggressive agonistic behavior of females towards conspecifics and cannibalism against their offspring in the 10µg & 20 µg AFB₁ treated groups. This is in agreement with a study conducted by Ishola [37], who stated that

aggressive behavior of resident Wistar rats in resident/ intruder encounters was significantly influenced by prolonged time of exposure to aflatoxin. [37, 38] noticed that the number and duration of aggression attacks increased over time. The neurotoxic effect of aflatoxin impacted on the emotion regulating center in the hypothalamus and brain centers that modulate aggression. They suggested that the ability to evaluate the situation appropriately and react accordingly when confronted with an intruder was deeply impaired by neuro-cognitive disruptions of the brain bio-chemicals leading to excessive aggression. Based on the conclusions of Ishola [37] and Stagkourakis [38], it is suggested here that the agonistic aggressive behavior and cannibalism reported in the present study is attributed to the aflatoxin induced neurotoxic damage neuro-cognitive disruptions of the brain. The observed behavioral changes, including aggression and cannibalism, as well as the suggested cognitive neurotoxicity, were not the primary focus of the present study. Rather, these findings were incidental observations recorded during the experimental period. Therefore, no standardized behavioral or cognitive assessments were performed to substantiate these effects. These observations may, however, provide a basis for future investigations specifically designed to evaluate the neurobehavioral impact of AFB₁ exposure.

CONCLUSION

Aflatoxin B₁ has a negative impact on embryonic development, birth quality, reduced body weight of mothers and offspring, in addition to alterations in morphological parameters. Also, AFB₁ triggered agonistic aggressive behavior and cannibalism.

Funding:

This research was supported by the Science and Technology Development Fund (STDF), Egypt (Project ID: 48863).

Conflict of interest: The authors declare that there is not any kind of conflict of interest.

REFERENCES

1. International Agency for Research on Cancer, Some traditional herbal medicines, some mycotoxins, naphthalene and styrene, IARC Monogr Eval Carcinog Risks Hum, 82 (2002) 1.
2. J.W. Bennett, M. Klich, Mycotoxins, Clin. Micro. Rev., 16 (2003) 497.
<https://doi.org/10.1128/cmr.16.3.497-516.2003>
3. P. Battilani, P. Toscano, H.J. Van Der Fels-Klerx, A. Moretti, M.C. Leggieri, C. Brera, A. Rortais, T. Goumperis, T. Robinson, Aflatoxin B1 contamination in maize in Europe increases due to climate change, Sci. Rep., 6 (2016) 24328.
<https://doi.org/10.1038/srep24328>
4. J.H. Williams, T.D. Phillips, P.E. Jolly, J.K. Stiles, C.M. Jolly, D. Aggarwal, Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. 80(2004) 1106.
<https://doi.org/10.1093/ajcn/80.5.1106>
5. J.Y. Andrews-Trevino, P. Webb, G. Shively, B.L. Rogers, K. Baral, D. Davis, K. Paudel, A. Pokharel, R. Shrestha, J.S. Wang, S. Ghosh, Relatively Low Maternal Aflatoxin Exposure Is Associated with Small-for-Gestational-Age but Not with Other

Birth Outcomes in a Prospective Birth Cohort Study of Nepalese Infants, *J. Nutr.*, 149 (2019) 1818. <https://doi.org/10.1093/jn/nxz122>

6. J.M. Lauer, C.P. Duggan, L.M. Ausman, J.K. Griffiths, P. Webb, J.S. Wang, K.S. Xue, E. Agaba, N. Nshakira, S. Ghosh, Maternal aflatoxin exposure during pregnancy and adverse birth outcomes in Uganda, *Matern. Child Nutr.*, 15 (2019) e12701. <https://doi.org/10.1111/mcn.12701>

7. J.D. Groopman, P.A. Egner, K.J. Schulze, L.S.F. Wu, R. Merrill, S. Mehra, A.A. Shamim, H. Ali, S. Shaikh, A. Gernand, S.K. Khatry, S.C. LeClerq, K.P. West Jr, P. Christian, Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B1-lysine albumin biomarkers, *Food Chem. Toxicol.*, 74 (2014) 184. <https://doi.org/10.1016/j.fct.2014.09.016>

8. N.N.A. Kyei, D. Boakye, S. Gabrysch, Maternal mycotoxin exposure and adverse pregnancy outcomes: A systematic review, *Mycotox. Res.*, 36(2020) 243. <https://doi.org/10.1007/s12550-019-00384-6>

9. L.E. Smith, A.J. Prendergast, P.C. Turner, J.H. Humphrey, R.J. Stoltzfus, Aflatoxin exposure during pregnancy, maternal anemia, and adverse birth outcomes, *Am. J. Trop. Med. Hyg.*, 96 (2017) 770. <https://doi.org/10.4269/ajtmh.16-0730>

10. N. Mitsuda, J.-P. Naw Awn, M. Eitoku, N. Maeda, M. Fujieda, N. Suganuma, Association between maternal hemoglobin concentration and placental weight to birthweight ratio: The Japan Environment and Children's Study (JECS), *Placenta*, 101 (2020) 132. <https://doi.org/10.22541/au.158645560.02907152>

11. M.M. Rahman, S.K. Abe, M.S. Rahman, M. Kanda, S. Narita, V. Bilano, E. Ota, S. Gilmour, K. Shibuya, Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: Systematic review and meta-analysis, *Am. J. Clin. Nutr.*, 103(2016) 495. <https://doi.org/10.3945/ajcn.115.107896>
12. R.J. Verma, P.J. Raval, Cytotoxicity of aflatoxin on red blood corpuscles, *Bull. Environ. Contam. Toxicol.*, 47(1991) 428. <https://doi.org/10.1007/bf01702206>
13. M.I. Yousef, M.H. Salem, K.I. Kamel, G.A. Hassan, F.D. El-Nouty, Influence of ascorbic acid supplementation on the haematological and clinical biochemistry parameters of male rabbits exposed to aflatoxin B1, *J. Environ. Sci. Health B*, 38(2003) 193. <https://doi.org/10.1081/pfc-120018449>
14. F.M.B. Shuaib, P.E. Jolly, J.E. Ehiri, Y. Jiang, W.O. Ellis, J.K. Stiles, N.J. Yatich, E. Funkhouser, S.D. Person, C. Wilson, J.H. Williams, Association between Anemia and Aflatoxin B1 Biomarker Levels among Pregnant Women in Kumasi, Ghana, *Am. J. Trop. Med. Hyg.*, 83 (2010) 1077. <https://doi.org/10.4269/ajtmh.2010.09-0772>
15. N. Dönmez, H.H. Dönmez, E. Keskin, I. Kisadere, Effects of aflatoxin on some haematological parameters and protective effectiveness of esterified glucomannan in Merino rams, *ScientificWorldJournal*, 2012 (2012) 342468. <https://doi.org/10.1100/2012/342468>
16. M. Tuzcu, E. Sur, I. Çeuk, Y. Öznurlu, Effects of Aflatoxin on the Proportions of Peripheral Blood Leukocytes and Alpha-Naphthyl Acetate Esterase (ANAE) Positive

Lymphocytes in the Mouse, Kafkas Univ Vet Fak Derg, 16 (2010) 337.

<https://doi.org/10.9775/kvfd.2009.1194>

17. D.E. Marin, I. Taranu, R.P. Bunaciu, F. Pascale, D.S. Tudor, N. Avram, M. Sarca, I. Cureu, R.D. Criste, V. Suta, I.P. Oswald, Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin B1, J. Anim. Sci., 80 (2002) 1250. <https://doi.org/10.2527/2002.8051250x>

18. H.A. Fetaih, A.A. Dessouki, A.A. Hassanin, A.S. Tahan, Toxopathological and cytogenetic effects of aflatoxin B1 (AFB1) on pregnant rats, Pathol. Res. Pract., 210 (2014) 1079. <https://doi.org/10.1016/j.prp.2014.06.001>

19. Y.M. Abdulrazzaq, N. Osman, A. Ibrahim, Fetal exposure to aflatoxins in the United Arab Emirates. Ann. Trop. Paediatr, 22 (2002) 3.

<https://doi.org/10.1179/027249302125000094>

20. M. Hamdy, A.M. Moharram, A.A. Zohri, Control of mycobiota and mycotoxins contaminating popcorn grains, Assiut University Journal of Multidisciplinary Scientific Research (AUNJMSR), 54 (2025) 99.

<https://doi.org/10.21608/aunj.2024.316559.1090>

21. O.L. Shotwell, C.W. Hesseltine, R.D. Stubblefield, W.G. Sorenson, Production of aflatoxin on rice, Appl. Microbial, 14 (1966) 425.

<https://doi.org/10.1128/am.14.3.425-428.1966>

22. H. Oğuz, Ö. Demet, Single and Combination Effects of Dietary Aflatoxin and Adsorbents (*polyvinylpyrrolidone* and *bentonite*) on Some Performance Parameters of Broiler chickens, *Vet. Bil. Derg.* 13 (1997) 61.

23. T.R. Romer, Screening method for the detection of aflatoxins in mixed feeds and other agricultural commodities with subsequent confirmation and quantitative measurement of aflatoxins in positive samples, *J. Ass. Offic. Analyt. Chem.*, 58 (1975) 500. <https://doi.org/10.1093/jaoac/58.3.500>

24. M.E. Benvenuti, J.A. Burgess, Rapid analysis of aflatoxins in corn, cereals, and almonds using ACQUITY UPLC H-Class System with fluorescence detection, Corporation W, editor Milford, MA2010.

25. J.-P. Suomela, R. Jarvinen, M. Lassila, Derivatization. In *First Dice Your Dill* (*Anethum graveolens* L.) New Methods and Techniques in Sample Handling, University of Turku FI-20014: Turku, Finland, 2010.

26. R.A. Ali, F. Khalil, H.A. Hassan, I.M. Amer, Z. Kamal, Mycotoxicity and embryonic development: I- Aflatoxin B1 reduces quality and birth rate during mice embryonic

development, SVU- International Journal of Veterinary Sciences, 6 (2023) 1.

<https://doi.org/10.21608/svu.2023.183342.1248>

27. F. Xu, Y. Li, Z. Cao, J. Zhang, W. Huang, AFB1-induced mice liver injury involves mitochondrial dysfunction mediated by mitochondrial biogenesis inhibition, *Ecotoxicol. Environ. Saf.*, 216 (2021) 112213. <https://doi.org/10.1016/j.ecoenv.2021.112213>

28. T.K. Tsouloufi, An overview of mycotoxicoses in rabbits, *J Vet Diagn Invest.*, 36 (2024) 638. <https://doi.org/10.1177/10406387241255945>

29. W.A. Khan, M.Z. Khan, A. Khan, Z.U. Hassan, S. Rafique, M.K. Saleemi, A. Ahad, Dietary vitamin E in White Leghorn layer breeder hens: a strategy to combat aflatoxin B1-induced damage, *Avian Pathology*, 43 (2014) 389. <https://doi.org/10.1080/03079457.2014.943691>

30. B. Ivanovics, G. Gazsi, M. Reining, I. Berta, S. Poliska, M. Toth, A. Domokos, B. Nagy, A. Staszny, M. Cserhati, E. Csosz, A. Bacsi, Z. Csenki-Bakos, A. Acs, B. Urbanyi, Z. Czimmerer, Embryonic exposure to low concentrations of aflatoxin B1 triggers global transcriptomic changes, defective yolk lipid mobilization, abnormal gastrointestinal tract development and inflammation in zebrafish, *J. Hazard. Mater.*, 416 (2021) 125788. <https://doi.org/10.1016/j.jhazmat.2021.125788>

31. F.M. Shuaib, P.E. Jolly, J.E. Ehiri, N. Yatich, Y. Jiang, E. Funkhouser, S.D. Person, C. Wilson, W.O. Ellis, J.S. Wang, [J.H. Williams](#), Association between birth outcomes

and aflatoxin B1 biomarker blood levels in pregnant women in Kumasi, Ghana, *Trop. Med. Int. Health*, 15 (2010) 160. <https://doi.org/10.1111/j.1365-3156.2009.02435.x>

32. P.B. Wangikar, P. Dwivedi, N. Sinha, Effect in rats of simultaneous prenatal exposure to ochratoxin A and aflatoxin B1. I. Maternal toxicity and fetal malformations, *Birth Defects Research Part B-Developmental and Reproductive Toxicology*, 71 (2004)

343. <https://doi.org/10.1002/bdrb.20021>

33. S.E. Owumi, C.E. Irozuru, U.O. Arunsi, A.K. Oyelere, Caffeic acid protects against DNA damage, oxidative and inflammatory mediated toxicities, and upregulated caspases activation in the hepatorenal system of rats treated with aflatoxin B1, *Toxicon*, 207 (2022) 1. <https://doi.org/10.1016/j.toxicon.2021.12.021>

34. P.B. Wangikar, P. Dwivedi, N. Sinha, A.K. Sharma, A.G. Telang, Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects, *Toxicology*, 215 (2005) 37. <https://doi.org/10.1016/j.tox.2005.06.022>

35. R.A. Ali, E.T. Wassif, M.A. Al-Mokhtar, D.E.F. Mostafa, D.A. Abdel-Raoof, Aflatoxin B1 induces morphological anomalies during chick embryo development, *Assiut University Journal of Zoology*, 1 (2019) 31. <https://doi.org/10.21608/aunj.2018.221318>

36. Y. Açar, G. Akbulut, Evaluation of Aflatoxins Occurrence and Exposure in Cereal-Based Baby Foods: An Update Review, *Current Nutrition Reports*, 13 (2024)

59. <https://doi.org/10.1007/s13668-024-00519-x>

-
37. A. A. Ishola, Exposure to aflatoxin B1 among Wistar albino rats, JNBS, 7 (2020) 70.
https://doi.org/10.4103/jnbs.jnbs_9_20
38. S. Stagkourakis, G. Spigolon, P. Williams, J. Protzmann, G. Fisone, C. Broberger, A neural network for intermale aggression to establish social hierarchy, Nat. Neurosci., 21 (2018) 834. <https://doi.org/10.1038/s41593-018-0153-x>