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EFFECTS OF AFLATOXIN B1 ON LIVER, TESTIS, AND EPIDIDYMIS OF REPRODUCTIVELY MATURE MALE PIGS: HISTOPATHOLOGICAL EVALUATION.
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# EFFECTS OF AFLATOXIN B1 ON LIVER, TESTIS, AND EPIDIDYMIS OF REPRODUCTIVELY MATURE MALE PIGS: HISTOPATHOLOGICAL EVALUATION.

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## ABSTRACT

***Background:*** **Aflatoxin is a major food contaminant, with adverse effects on the physiology of both humans and animals. Exposure to aflatoxins has been known to pose a great threat to both humans and animals particularly in the tropics, with varied pathophysiological effects. This research focused on pigs since their physiology is closely related to that of humans.**
***Objective:*** **To evaluate the histopathological changes related to AFB1 on the liver, testis, and epididymis of reproductively mature male pigs.**
***Design:*** **An experimental study**
***Subjects:*** **Mature large white pigs of the age of seven to nine months and of average body weight of 53.7 kg. They were obtained from the University of Nairobi and housed in a pig pen at Karen in Nairobi. Completely randomised design was used in the allocation of the animals to the control group and to three treatment groups, each group comprising three pigs. AFB1 was obtained from Bora Biotechnology Company in Nairobi and the doses were given in three levels in the ratio of 1:2:3. The first treated group received 80 ppb per day, second treated group 160 ppb and the third treated group 240 ppb per day for 60 days. The control group received aflatoxin-free diet for the same study period. The pigs were sacrificed following termination of the treatment, and their tissues collected, processed for histopathological examination and photomicrographs taken using a smartphone Galaxy note II.**
***Results:*** **In the liver tissue there was marked bile duct proliferation dilatation of the central vein; mononuclear cell infiltration; fatty change, fibrosis and marked congestion of the parenchyma. In the testis there was progressive decrease of spermatogenesis cells, Leydig cells and Sertoli cells. Peritubular ooedema, necrosis and atrophy of seminiferous tubules were also noted. Epididymis revealed epithelial hyperplasia and epithelial cytoplasmic vacuolation**
***Conclusion*****: Ingestion of aflatoxin caused significant histopathological changes in the liver, testis, and epididymis. The changes in the liver, and testis were dose dependent while those in the epididymis were not.**

## INTRODUCTION

Aflatoxin (AF) is one of the contaminants in foods and feeds, with varying effects on the physiology of both animal and humans. It is a mycotoxin, a secondary metabolic by-product of the toxigenic fungi mainly Aspergillus flavus and Aspergillus parasiticus during their natural metabolic processes (1). The four major groups of aflatoxin that have been described based on their fluorescence at chromatography are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). The two main Aspergillus species yield different secondary metabolites such that AFB1 and B2 are produced by A.flavus and A.parasiticus while AFG1 and G2 are produced by A.parasiticus. In this classification, "B" and "G" stand for blue and green fluorescent colors respectively (2). The subscript numbers 1 and 2 indicate major and minor compounds, based on the degree of toxicity. AFB1 is the most common, most toxic and a known carcinogen (3).

Aflatoxins are not transmissible between animals, thus, the main cause of the toxicity in humans and animals is consumption of contaminated food and feed stuff. The toxigenic fungi can contaminate various feed components like maize, rice, wheat, peanuts,

millet, oily feedstuffs, some of which form the majority of common food for both humans and animals. This contamination is dependent on the prevailing environmental conditions such as humidity of the substrate, relative humidity, temperature and oxygen availability, which favour production of aflatoxin in feeds. Contamination of animal feeds with aflatoxin can occur during the growth of the crop while in the field, at harvest and during postharvest operations as well as in storage.

Aflatoxins have been shown to disrupt the reproductive system of both humans and animals, in both sexes. Results from experimental male rats fed on aflatoxin-contaminated feeds indicate that aflatoxins cause pathological changes in the testicles and epididymis of male rats. Such alterations in the testicles as well as the epididymis has related results in decreased number of spermatogenesis, spermatocytes as well as spermatids in these animals (4). In male rabbits treated with aflatoxin for 60 days hyperplasia of the epididymal epithelial cells as well as cytoplasmic vacuolation is known to occur. These epithelial cells of the epididymis are already terminally differentiated cells and usually, they do not divided unless induced to mitosis and this is by the aflatoxin. (5)

The effects of aflatoxin B1 on the male reproductive system has been shown to be duration dependent. Histopathological analysis of testicles and seminal vesicles of Aflatoxin treated mouse reveal a duration-dependent decrease in the weights of the testicles and seminal vesicles respectively. Histological changes are also observed in both the spermatogenic and androgenic compartments of the testis. Leydig cells were shown to undergo hypertrophy and distortion of the nucleus shape following the treatment (6)

Similar testicular changes including ooedematous interstitial tissue, atrophy of spermiogenic epithelium and tissue ooedema as well as reduced number of mature spermatozoa have been observed in young rabbits treated dietary aflatoxin (7). Akbarsha *et al.*, (8) observed related histopathological changes in the testes of Swiss mouse and Wistar rat treated with aflatoxin B1 via intra-peritoneal route in chronic male reproductive toxicity testing. The seminiferous epithelium in the aflatoxin treated rats was severely disrupted hampering both mitotic and meiotic division of the germ cells. This impaired spermatogenesis resulting in decreased sperm counts. Elham and Mona (2004) (9) had observed degeneration of the lining of the epithelium of seminiferous tubules and congestion of testicular blood vessels with intertubular ooedema in the treated group.

Several changes have been shown to occur in the liver of different experimental animals. In rats such changes include hydropic and vacuolar degeneration of hepatocytes, mainly around the congested blood vessels. Inflammatory cellular infiltration of portal area mostly lymphocytes and congestion of portal vessels with presence of small newly formed bile ducts are noted (Elham and Mona, 2004)(9). Bbosa *et al*.(1) in their review article describes similar results in which case aflatoxin B1 is reported to cause pallor discoloration of liver and enlargement of liver, congestion of liver parenchyma, cytoplasmic vacuolation or fatty change of hepatocytes, necrosis of hepatocytes and newly formed bile ducts, mononuclear and heterophilic cell infiltration in broiler chicks. Marai and Asker (2008)(10) reporting on histopathological effects of aflatoxin highlight that administration of daily low AF doses over an experimental period of six days results in bile duct proliferation, perivascular oedema, fibroblastic infiltration, dilated lymphatic ducts and loss of glycogen in the liver tissue of calves.

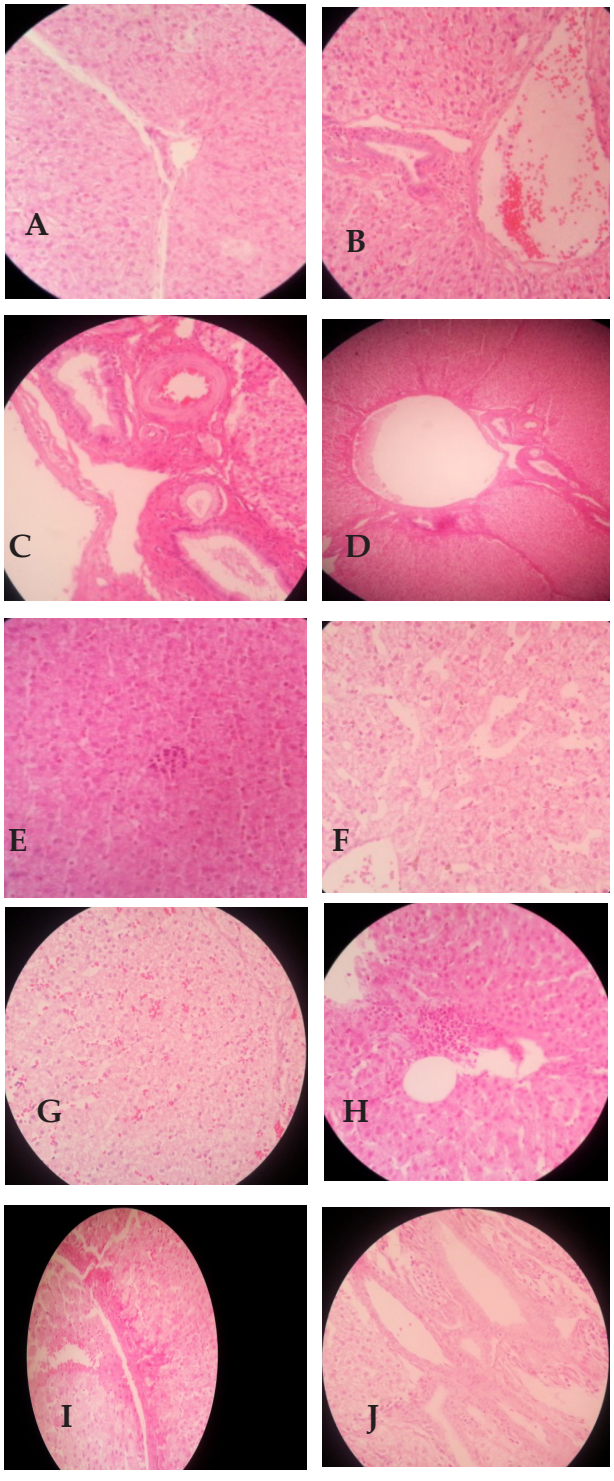
## MATERIALS AND METHODS

This research adopted an experimental design using twelve reproductively mature Large White pigs aged seven to nine months and of average body weight of 53.7kg. They were obtained from the University of Nairobi and housed in a pig pen at Karen in Nairobi. Completely randomised design was used in the allocation of the animals to the control group and to three treatment groups, each group comprising three pigs. AFB1 was obtained from Bora Biotechnology Company in Nairobi and the doses were given in three levels in the ratio of 1:2:3. The first treatment group received 80 ppb per day, second group 160 ppb and the third group 240 ppb per day for 60 days. The control group received aflatoxin-free diet for the same study period. Oral route administration of the aflatoxins was used, whereby predetermined aflatoxin concentrations were mixed with feed which was served to the pigs daily during the study period. Following the termination of the treatment, the pigs were sacrificed. The liver, testes and epididymis were harvested and 5mm thick representative tissues were trimmed and immediately fixed in 10% formalin to prevent postmortem autolysis. The tissues were left to fix for 72 hours. The tissues were then processed, examined by a pathologist and photomicrographs taken using a smartphone Galaxy note II.

## RESULTS

Ingestion of aflatoxin caused significant histopathological changes in the liver, testis, and epididymis. In the liver tissue these changes are ; marked bile duct proliferation predominantly in the 160 ppb and 240 ppb treated groups; dilatation of the central vein; mononuclear cell infiltration; fatty change; fibrosis and marked congestion of the

parenchyma.



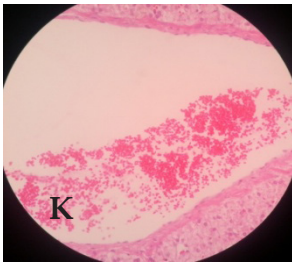
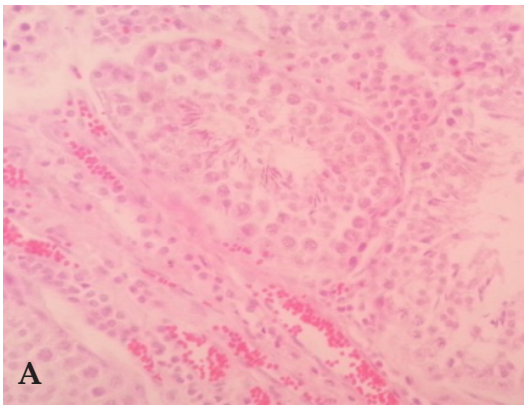




*Figure (a) shows normal histology of liver from control group (X40). Figure (b) shows liver from treatment group one (80ppb) with dilated central vein (X40). Figure (c) shows liver from treatment group two (160ppb) with moderate bile duct proliferation. Figure (d) shows liver from second treatment group (160ppb) with marked dilation of central vein (X10). Figure (e) shows liver from second treatment group (160ppb) with mononuclear cell infiltrate (X10). Figure (f) shows liver from second treatment group (160ppb) with fatty change (steatosis) (X40). Figure (g) shows liver from second treatment group (160ppb) with marked congestion of the parenchyma (X10). Figure (h) shows liver from third treatment group (240ppb) with mononuclear cell infiltrate and fatty change (X40). Figure (i) shows liver from third treatment group (240ppb) with fibrosis and fatty change (X40). Figure (j) shows liver from third treatment group (240ppb) with marked bile duct proliferation (X40). Figure (k) shows liver from third treatment group (240ppb) with marked dilation of central vein (X40).*

Histopathologic changes in the testis of the treated pigs were; progressive decrease of spermatogenesis cells, progressive reduction of Leydig cells and Sertoli cells, Progressive increase in peritubular ooedema, necrosis and atrophy of seminiferous tubules characterized by thickened basement membrane. These changes were dose-dependent.



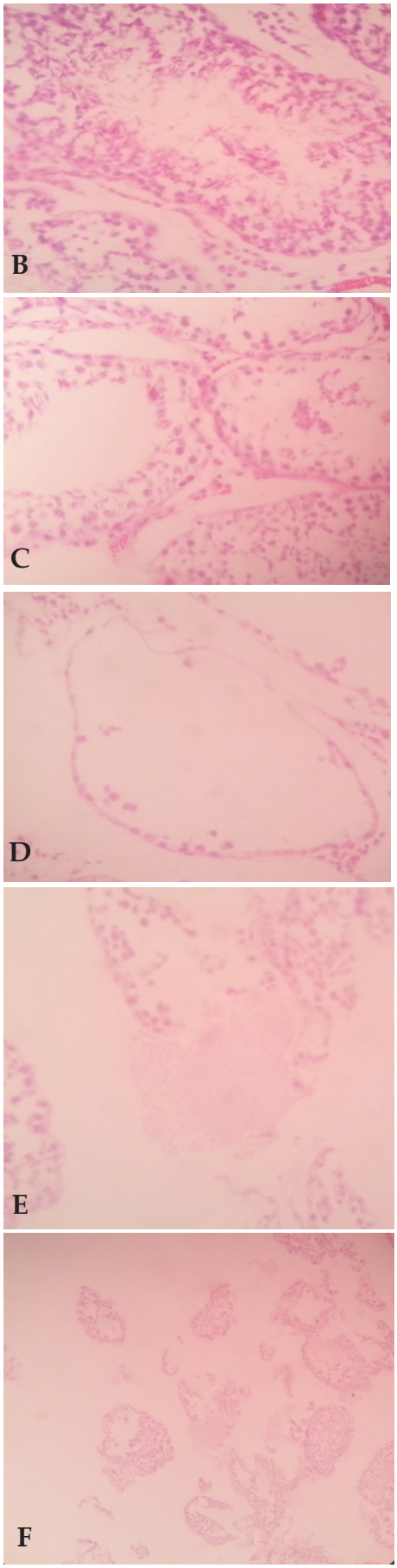



*Figure (a) shows normal testis from the control group (X40), Figure (b) shows testis from first treatment group (80ppb) with mild reduction of spermatogenesis cells and reduced number of Leydig cells (X40). Figure (c) shows testis from second treatment group (160 ppb) with reduced number of spermatogenesis cells and reduced number of Leydig cells (X40). Figures (d, e, f) show testis from third treatment group (240ppb) with marked reduction of spermatogenesis cells, Leydig cells and Sertoli cells; necrosis, and peritubular oedema respectively (X40).*

Microscopic examination of the epididymis revealed epithelial hyperplasia in all pigs treated with aflatoxin $B_1$. These findings were not dependent on the aflatoxin dosage given because even the 80 ppb group showed extensive hyperplasia and epithelial cytoplasmic vacuolation. There were no significant histopathological changes with the control group.

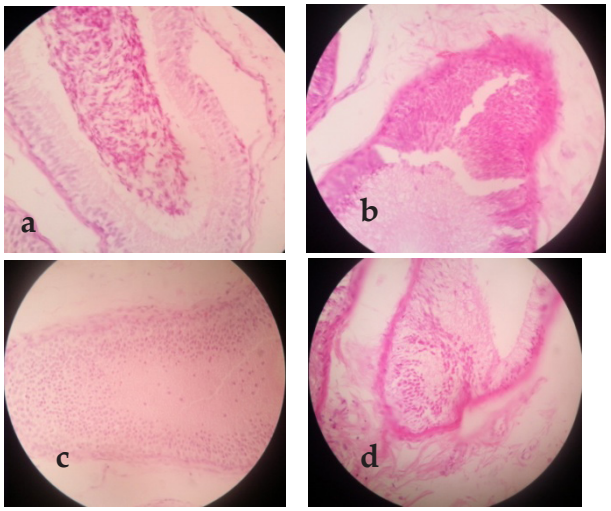



*Figure (a) shows normal epididymis from the control group (X40), Figure (b) shows epididymis from the first treatment group (80ppb) with extensive epithelial hyperplasia and epithelial cell cytoplasmic vacuolation (X40), Figure (c) shows epididymis from the second treatment group (160ppb) with extensive epithelial hyperplasia (X40), Figure (d) shows epididymis from the third treatment group (240ppb) with extensive epithelial hyperplasia and epithelial cell cytoplasmic vacuolation(X40).*

## DISCUSSION

There were significant histopathological changes seen in the the liver, testis, and epididymis. Histopathologic changes in the liver tissue included; marked bile duct proliferation predominantly in the 160 ppb and 240 ppb treated groups, dilatation of the central vein; mononuclear cell infiltration, fatty change, fibrosis and marked congestion of the parenchyma.
These findings agree with those reported by Feddern

*et al.*, (11) on the occurrence of liver diseases in swines exposed to aflatoxin. Marai & Asker (10) reports on related histopathologic changes in the liver tissue of calves where daily aflatoxin doses as low as 0.04mg/kg body weight within a period of 6 days resulted in bile duct proliferation, perivascular ooedema, and fibrosis. Similar observations were made by Elham and Mona (5) on the liver of rats fed with 7.5mg/200grams body weight of aflatoxin for three weeks. These included inflammatory cellular infiltration of portal area mostly lymphocytes, congested portal vessels, dilatation of the central vein as well as bile duct proliferation. Bbosa *et al.*, (1) report similar AFB1 related changes on the liver of broiler chicks. The control group in this study showed normal histology of the liver with no significant changes as observed in the aflatoxin challenged groups.

The study revealed varying results on the testicular tissues which ranged from moderate to severe in the 160 ppb group and 240 ppb group respectively. The observed changes of the pigs challenged by aflatoxin included progressive decrease of spermatogenesis cells and progressive reduction of Leydig cells and Sertoli cells in a dose-dependent manner. Although there is no existing data on the effect of aflatoxin on the testis of swines reports on from other experimental animals do exist. The findings in the current study agree with those of Murad *et al.*, (4) who observed similar effects on the reproductive system of Albino male rats given three AFB1 doses of 15, 30 and 45 ppm respectively, where the number of Leydig cells, Sertoli cells including spermatogenesis cells were significantly reduced with increased toxin concentration. Other observations of the testes in the current study were progressive increase in peritubular ooedema, necrosis of seminiferous tubules and atrophy of the seminiferous tubules characterized by thickened basement membrane. These findings agree with those of Lakkawa *et al.*, (7) who observed similar testicular changes in young rabbits treated with 0.5 ppm/kg of feed for 50 days. Ahmed *et al.*, (5) also noted similar pathological alterations in the testes of aflatoxicated rabbits. Akbarsha *et al.*, (8) report similar findings in Swiss mouse and Wistar rat treated with aflatoxin B1. They noted disruption of the seminiferous epithelium that hampered both mitotic and meiotic division of the germ cells consequently impairing spermatogenesis leading to decreased sperm counts. Elham and Mona (9) observed similar testicular changes in male rats fed 7.5/mg/200 grams body weight of AF for three weeks.

Microscopic examination on the epididymis revealed epithelia hyperplasia in all pigs treated with aflatoxin B1. These findings were not dose-dependent since even the 80 ppb treated group showed extensive epithelial hyperplasia and epithelial cell cytoplasmic vacuolation. These findings agrees with that of Ahmed *et al.*, (5) who observed hyperplasia of the epididymal epithelial cells as well as cytoplasmic vacuolation in male rabbits treated with 250, 500 and 1000 ppb aflatoxin for 60 days. These epithelial cells of the epididymis are already terminally differentiated cells and usually, they do not divide unless induced to mitosis. By the fact that all the treated pigs in this study had epithelial hyperplasia, it may be said that aflatoxin B1 is a potent mitogenic agent, in respect to epididymis.

In Conclusion, Aflatoxin caused significant histopathological changes in the liver, testis, and epididymis. The changes in the liver, and testis were dose dependent while those in the epididymis were not.

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