Global Veterinaria 9 (1): 42-52, 2012
ISSN 1992-6197

Pathological Consequences of Aflatoxins in Male Rabbit: Cellular, Genetic and Oxidative Damage

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Abstract: The purpose of the current investigation was to contribute additional insight into links between dietary aflatoxins and the risk of adverse male reproductive. A total number of 28 adult NZ male rabbits were randomly allocated into one of the four treatment groups: the control group received basal diet containing Zero ppb aflatoxins and three aflaoxin treated groups (A, B and C) fed on basal diets containing 250, 500 and 1000 ppb aflatoxins respectively for 60 days. The histopathological examination revealed dose dependant changes varied from mild testicular degeneration to a complete atrophy of seminefrous tubules and loss of all stages of spermatogenesis. Prostate and bulbourethral glands showed marked hyperplasia, cystic dilatation and necrosis. Analysis of apoptosis showed massive necrosis in high doses and increased rate of apoptosis in low doses of aflatoxins. Extracted testicular and spermatic DNA showed marked DNA fragmentation indicating the genotoxic effect of aflatoxins. The activities of antioxidant biomarkers were decreased; however the content of oxidative products were increased due to aflatoxin administration. The Total antioxidant capacity and uric acid levels were decreased and the level of nitric oxide was higher in aflatoxin treated groups. In conclusion, the data presented showed that aflatoxin at graded doses induced severe oxidative damage in the testis and accessories promoting their apoptosis and thus consuming such doses simultaneously may be a greater risk of male infertility.

Key wards: Aflatoxins · Toxipathology · Male · Fertility · Apoptosis · Oxidative Stress · DNA Damage

INTRODUCTION

Aflatoxins are a group of mycotoxins principally produced by *Aspergillus flavus* and *Aspergillus parasiticus* [1]. Although twenty types of aflatoxins have been identified, only aflatoxin B1, B2, G1 and G2 are usually found together in feeds and feedstuffs in various proportions; of which B1 is the most toxic one. In farm animals, direct consequences of consumption of aflatoxins-contaminated feed include: reduced feed intake, feed refusal, poor feed conversion [2], diminished body weight gain, increased disease incidence due to immune-suppression [3] and reduced reproductive capacities [4] which leads to economic losses [5]. Also it has been carcinogenic, mutagenic, teratogenic, tremorgenic-cause tremor or damage the central nervous system, haemorrhagic, as well as causing damage to the liver and kidneys [2, 6].

Epidemiological as well as clinical and experimental studies revealed that short exposure to large doses of aflatoxins produced acute toxicity including fever, oedema, vomiting, abdominal pain, inappetance, lethargy, ataxia, rough hair coat and pale potentially fatal liver failure. During aflatoxicosis in rabbits, histopathological examination revealed vascular congestion, leucocytic infiltration and degenerative change in the affected organs during the initial stage of toxicosis. At its terminal stage, coagulative necrosis, perivascular and periductal fibrocellular reactions along with mononuclear-cellular infiltration and distortion of the hepatic chords were observed in the liver [7].

Sub-symptomatic exposure to aflatoxins is known to produce male reproductive toxic effects with several manifestations, which have been previously reported. The principal target organ in causing male reproductive toxicity is the testis and various aspects of spermatogenesis and androgen biosynthesis are affected [8]. However, the epididymis and Vas deferens also is a target for the action of such reproductive toxicants [9, 10]. The seminiferous tubules showed

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degeneration/denudation of the epithelium and a reduction in the number of mature spermatids in aflatoxin treated rabbits [7]. With a view to find if aflatoxins would produce multinucleate giant cells in the seminiferous epithelium, some authors [11, 12] observed generation of multinucleate giant spermatids or symplastic spermatids as one of the more manifestation of aflatoxicosis.

Aflatoxin is known to be a substrate for the cellular P-450 system resulting in the formation of AF 8, 9-epoxide and this derivative produces DNA adducts causing DNA strand breaks and point mutations. Under this pathological condition, the active process of cellular self-destruction, apoptosis may be occurred [13]. Apoptosis has been defined as ''gene-directed cellular self-destruction'' and is an active process that is tightly regulated by a number of gene products, which promote or block cell death. Apoptotic death can be triggered by a wide variety of stimuli and, importantly, not all cells necessarily undergo apoptosis in response to the same stimulus [14].

Many of the reproductive toxic agents studied exhibit cell-type specificity resulting in increased DNA fragmentation in epididymal or ejaculated sperm. Thus, it appears that there are various mechanisms that result in DNA strand breaks in mature sperm. One of the primary DNA damaging agents receiving a lot of research attention is reactive oxygen species (ROS) [15, 16].

Current focus on male fact or infertility during oxidative stress suggested damage to integrity of DNA in the sperm nucleus resulting in base modification, DNA fragmentation and chromatin cross linking [17, 18]. Strong evidence suggests that high levels of ROS mediate the DNA fragmentation commonly observed in spermatozoa of infertile male [19]. ROS may also initiate a chain of reactions that ultimately lead to apoptosis and the levels of ROS were positively associated with apoptosis in mature spermatozoa. Levels of caspases, which are proteases involved in apoptosis, correlated with levels of ROS [20].

The purpose of the current investigation was to contribute additional insight into links between dietary aflatoxins and the risk of adverse male reproductive events induced by apoptosis and oxidative DNA damage, as measured by new markers of molecular biology.

MATERIALS AND METHODS

Aflatoxins: Aflatoxin production, extraction and quantification were performed in the Laboratory of Marine Toxins, Food Toxicology and Contaminants Department, National Research Centre as described by Conder *et al.* [21] and Stubblefield *et al.* [22].

Animals and Diets: A total number of twenty eight adult New Zealand male rabbits was randomly allocated into one of the four treatment groups (7 males per group): the control group received basal diet containing Zero ppb aflatoxins and three aflaoxin treated groups (A, B and C) were fed on basal diets containing 250, 500 and 1000 ppb aflatoxins/ kg diet, respectively for 60 days. The basal diet was formulated to meet the nutritional requirements of adult male rabbits as recommended by AOAC [23]. Each buck was fed an amount of pellet ration (100 g/day) and supplied with fresh tap water *ad libitum*.

Data Collection Techniques: At the 8th week, semen samples were collected from all experimental animals using artificial vagina and teaser doe. On centrifugation of semen samples, seminal plasma was separated and stored at-80° C till assaying for biomarkers of antioxidant capacity, while sperm cells were used for DNA extraction. At the end of the experimental period, all animals were sacrificed and observations of post mortem examination were recorded. Specimens from testis, epididymis, prostate, bulbourtheral glands were collected and fixed in formol saline 10%, washed, dehydrated, cleared and embedded in paraffin. Paraffin blocks were sectioned at 4-5 micron thickness and stained with Haematoxylin and Eosin [24] for histopathological examination. Moreover, Paraffin blocks of testis, epididymis and prostate were sectioned for morphological analysis of apoptosis. Specimens from testis were kept at-80° C for DNA extraction and analysis of biomarkers of oxidative stress.

Data Analysis Techniques

Morphological Analysis of Apoptosis: Paraffin sections of 4-5µm tissue were cut and fixed on positive charged microscope slides, stained with an acridine orange/ ethidium bromide mixture and viewed under a UV microscope as described by Lam *et al.* [25] and Dhama, *et al.* [26]. The viable cells fluoresce green, while the nuclei in necrotic cells fluoresce orange. Early apoptotic cells are distinguished by the enhanced green fluorescence of their characteristic condensed chromatin, while late apoptotic cells that have lost membrane integrity display enhanced uniform orange fluorescence of their condensed chromatin.

Quantification of DNA Damage: High quality genomic DNA was extracted from preserved testis by phenol/chloroform-based method through precipitation of

protein and other contaminants and further precipitation of high molecular weight genomic DNA by absolute ethanol as described by Sambrook, Fritsch and Maniutis [27]. Spermatic DNA was extracted from sperm cells and separated through agarose gel electrophoresis with some modifications according to Trommelen *et al.* [28].

Determination of Biomarkers of Oxidative Stress: Seminal plasma level of total antioxidant capacity was performed according to Koracevic and Koracevic *et al*. [29]. Seminal plasma levels of nitric oxide and uric acid were determined according to Montgomery and Dymock [30] and Barham and Trinder [31] respectively. Catalase activity was measured in testicular homogenate according to Aebi [32]. Testicular activity of Glutathione Reduced was assayed in testis homogenate using the colorimetric method described by Beutler, Duron and Kelly [33]. The activity of Testicular Superoxide Dismutase was determined according to Nishikimi *et al.* [34]. Testicular homogenate level of Malondialdehyde was performed according to Ohkawa *et al.* [35].

Statistical Analysis: The data were subjected to analysis using PC software, SPSS Version 16 [36]. Data were presented as Mean ± SEM (Standard Error of Mean). Values of $P<0.05$ were considered statistically significant.

RESULTS

Histopathological Variables: Examinations of tissue sections of the testes of treated rabbits are shown in Plate 1. In group A (Fig.1); sections revealed peritubular oedema with atrophy of some seminiferous tubules and

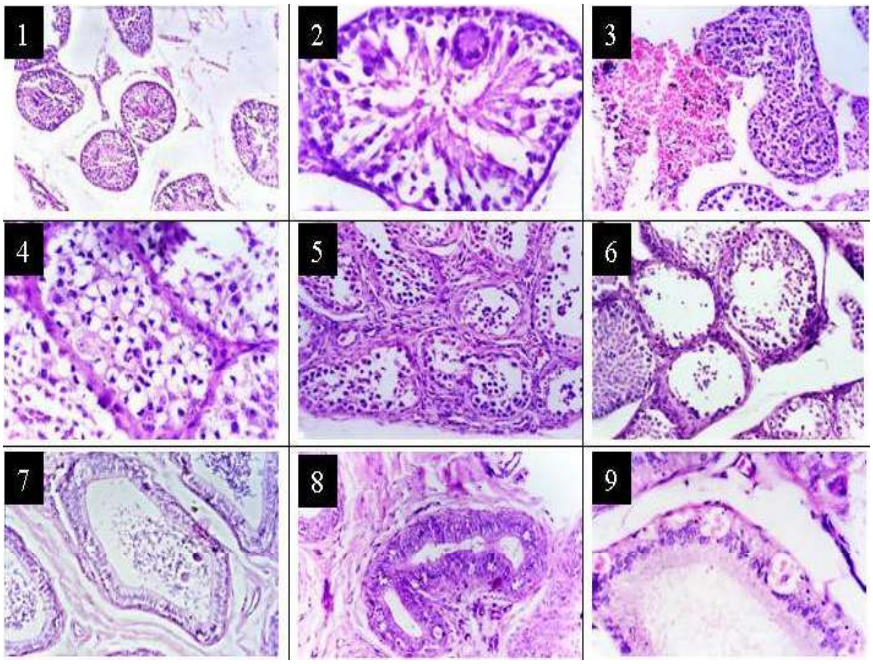

Plate 1:
- Photomicrographs of testicular sections of rabbits treated with aflatoxins (Figures 1-6). Fig.1 shows peritubular oedema and atrophy of seminefrous tubules (H and E X 100). Fig.2 shows degeneration of spermatogonial cells and presence of multinucleated spermatid (symplast). (H and E X 400). Fig.3 shows focal hemorrhage and disturbed process of spermiogenesis (H and E X 200). Fig.4 shows vacuolation of spermatogonial cells and severe nuclear pyknosis (H and E X 400). Fig.5 shows peritubular fibrosis and desquamation of spermatogonial cells (H and E X 200). Fig.6 shows loss of uniform arrangement of seminefrous tubules and complete absence of spermiogenesis. (H and E X 200).
- Photomicrographs of epididymis sections of rabbits treated with aflatoxins (Figures 7-9). Fig.7 shows interstitial oedema with the presence of little number of pale vacuolated epithelial cells (PVEC) (H and E X 200). Fig.8 shows hyperplasia of the luminal epithelium and large number of pale vacuolated epithelial cells which contained homogenous amorphous material (H and E X 400). Fig.9 shows large number of vacuoles which differ in size and diameter (H and E X 400).

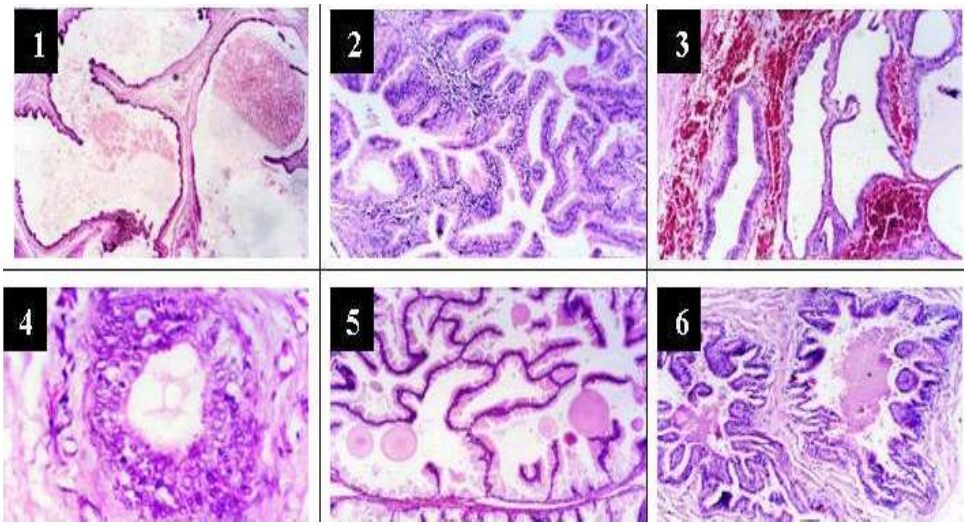

Plate 2:
- Photomicrographs of prostate sections of rabbits treated with aflatoxins (Figures 1-3). Fig.1 shows periacinar edema associated with cystic dilatation of some prostatic acini (H and E X 100). Fig.2 shows marked hyperplasia, with atrophy, degeneration, nuclear pyknosis, desquamation of the epithelium lining and leucocytic cells infiltration (H and E X 100). Fig.3 shows massive hemorrhage and cystic dilatation of prostatic acini. (H and E X 100).
- Photomicrograph of ductus deferns and bulbourethral gland of rabbits treated with aflatoxins, Fig. 4 shows marked hyperplasia, vacuolar degeneration (H and E X 400). Fig. 5 shows marked interstitial oedema, cystic hyperplasia of the epithelium lining acini with newly formed intra luminal acini and presence of intraluminal basophilic corpora amylacia. Fig. 6 shows marked cystic dilatation of some acini whereas other acini showed hyperplasia and corrugations of its lining. (H and E X 100).

Spermatogonial cells showed degeneration and nuclear pyknosis with the presence of multinucleated spermatid giant cells in the lumen of seminiferous tubules (Fig. 2). Moreover, focal hemorrhage and disturbed process of spermiogenesis due to nuclear pyknosis of most spermatogonial cells were also seen (Fig. 3). In group B, aflatoxins caused severe vaculation of the lining spermatogonial cells with severely nuclear pyknosis (Fig. 4), some of sections showed peritubular fibrosis, desqumation of spermatogonial cells and fragmented nuclei were seen in the lumen of seminiferous tubules (Fig. 5). Aflatoxins at a dose of 1000 ppb (group C) caused severe degeneration and necrosis of spermatogonial cells of most of seminiferous tubules with loss of the uniform arrangement and absence of the process of spermiogenesis (Fig. 6).

Examination of epididymal sections revealed pathological changes dependant on toxin dose (Plate 1). Aflatoxicosis caused interstitial edema with the presence of little number of pale vacuolated epithelial cells (PVEC) showed in group A (Fig. 7). However in group B, there were hyperplasia of the luminal epithelium and large number of pale vacuolated epithelial cells which contained homogenous amorphous material (Fig. 8). These vacuoles differ in size and diameter and might gather to form larger vacuoles seen in group C (Fig. 9).

Histopathological sections of prostate gland of rabbits are shown in Plate 2. In group A, showed periacinar edema associated with cystic dilatation of some prostatic acini, whereas other acini showed marked hyperplasia (Fig. 1), with atrophy, degeneration with pyknotic nuclei and desquamation of the epithelium lining. In group B, histopathological examination revealed marked cystic dilatation of some prostatic acini, marked diffuse hemorrhage and leukocytic cells infiltration (Fig. 2). The most conspicuous pathological changes in group C were marked cystic dilatation due to hyperplasia with the presence of intraluminal newly formed acini associated with severe congestion of blood vessels and capillaries and interstitial edema as well as marked hemorrhage (Fig. 3).

Histopathological examination of ductus deferns is shown in Plate 2, whereas ductus deferns showed interstitial edema, degeneration of the epithelial lining and muscle fiber with hyperplasia of the luminal epithelium, clumping of stereocilia, edema in lamina propria and, degeneration of the muscle fiber. Moreover, at high dose of aflatoxins (1000 ppb), there were cystic dilatation of

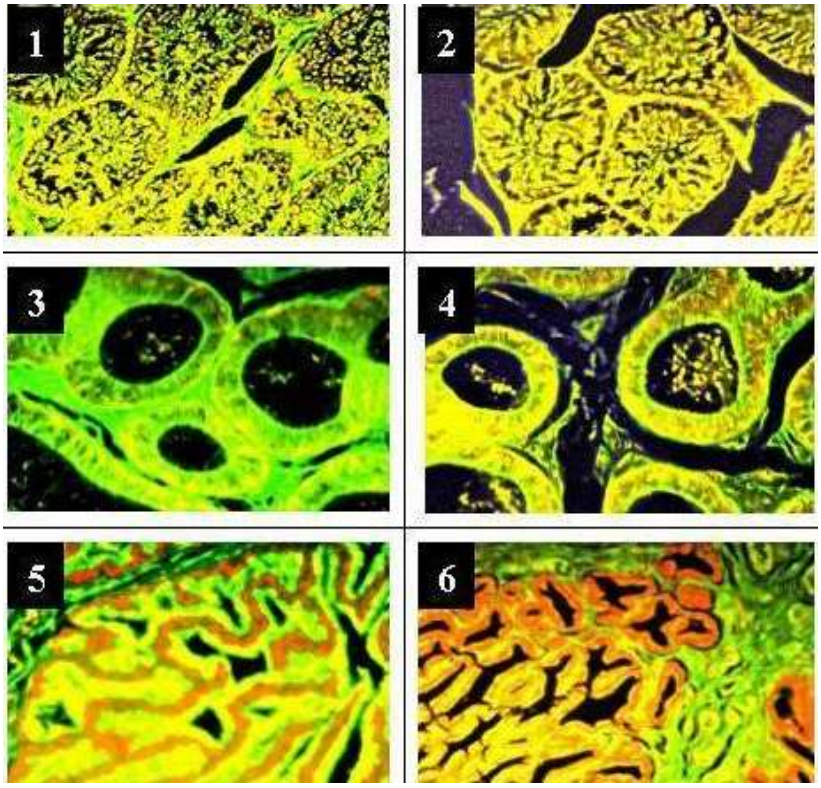

Plate 3:
- Fluorescent photomicrographs of the testis of aflatoxicated rabbit showing marked apoptosis of spermatogonial cells lining the seminefrous tubules (Fig. 1) in group A and B and severe necrosis of spermatogonial cells lining the seminefrous tubules (Fig. 2) with complete absence of the process of spermiogenesis in group C. (A. O and E. B. X 100).
- Fluorescent photomicrographs of the epididymis of aflatoxicated rabbit showing increased levels of apoptosis, with the presence of intraluminal necrotic and apoptotic spermatozoa (Fig. 3) in group A and B and severe necrosis of epithelial cells lining epididymis with increased levels of apoptosis (Fig. 4) in group C. (A. O and E. B. X 100).
- Fluorescent photomicrographs of prostate gland of aflatoxicated rabbit showing hyperplasia and apoptosis of epithelial cells lining acini (Fig.5) in group A and B and marked hyperplasia and apoptosis of epithelial cells lining acini with severe necrosis of hyperplastic cells (Fig. 6) in group C. (A.O. and E.B.X 100).

some ductus associated with atrophy of the lining epithelium whereas other ductus showed marked hyperplasia, vacuolar degeneration and corrugations of its epithelial lining (Fig. 4).

Histopathological examination of bulbourethral gland of rabbits are shown in plate 2, in group A and B revealed marked interstitial edema, cystic hyperplasia of the epithelium lining acini with newly formed intra luminal acini and presence of intraluminal basophilic corpora amylacia (Fig. 5). Aflatoxicosis of rabbits in group C caused marked cystic dilatation of some acini whereas other acini showed marked hyperplasia and corrugations of its lining (Fig. 6). In addition, hyperchromatic nuclei with the presence of large number of basophilic corpora amylacia in their acini were seen.

Morphological Analysis of Apoptosis: Analysis of apoptosis of testicular tissues in rabbits treated with aflatoxins are shown in Plate 3, whereas, groups A and B showed marked apoptosis of spermatogonial cells lining the seminefrous tubules (Fig. 1), in group C, severe necrosis of spermatogonial cells with complete absence of the process of spermiogenesis were seen (Fig. 2). Epididymis of rabbits in group A and B showed dose-dependent induction of apoptosis particularly the cell lining, accompanied with intraluminal necrotic and apoptotic spermatozoa (Fig. 3). Otherwise, there was severe necrosis of epithelial cell lining in group C (Fig. 4). Prostate gland of aflatoxicated rabbits showed dose-dependent hyperplasia and apoptosis of epithelial cells lining

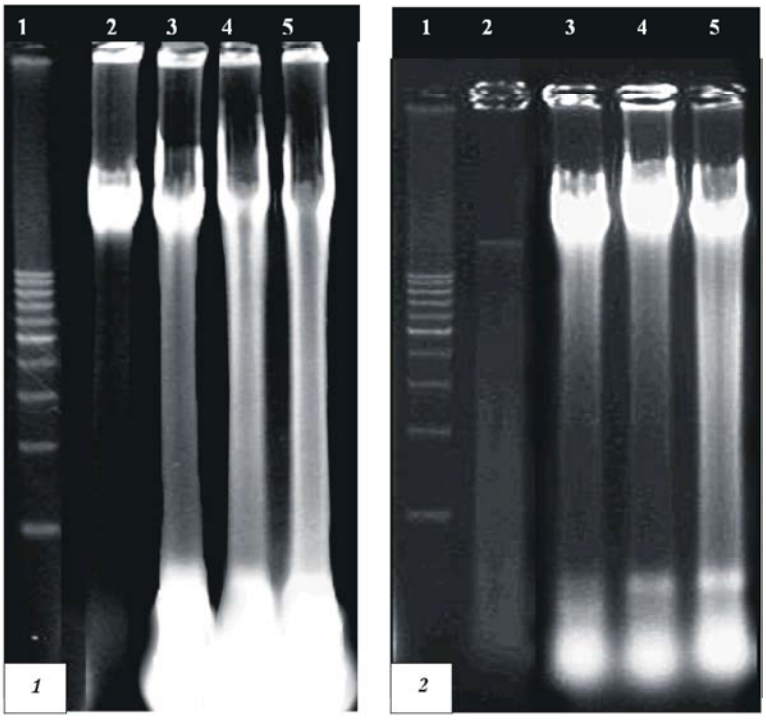

Plate 4: DNA fragmentation on agarose/ethidium bromide gel in testicular (Fig. 1) and Sperm Cells (Fig. 2). Lane (1) 1 Kbp DNA marker; Lane (2) control showed no degree of DNA fragmentation; Lane (3) showed (1+) DNA fragmentation in group A; Lane (4) showed (2+) DNA fragmentation in group B; Lane (5) showed (3+) DNA fragmentation in group C.

Table 1: Activities of catalase, glutathione reduced and superoxide dismutase, malondialdehyde content in testicular homogenate and plasma testosterone level of control and aflatoxin treated male rabbits

	Control	A	B	C
Catalase (U/ml)	3.5± 0.2[a]	3.3±. 01[a]	2.4± 0.4[b]	1.7±0.3[c]
Glutathione reduced (mmol / g)	20.8±1.3[a]	8.5± 0.2[c]	7.6±0.12[c]	6.9± 0.2[c]
Superoxide dismutase (U/ ml)	82.9±0.9[a]	76.1±5.3[bc]	72.7±2.3[bc]	68.0±0.5[c]
Malondialdehyde (nmol / g)	20.9±1.0[d]	42.2± 1.3[b]	44.6± 1.3[b]	53.0±1.5[a]

Table 2: Biomarkers of antioxidant capacity of seminal plasma (total antioxidant capacity, uric acid and nitric oxide) in control and aflatoxin treated male rabbits

	Control	A	B	C
Total antioxidant capacity (mmol/L)	0.6± 0.1[a]	0.23±0.003[b]	0.21±0.007[b]	0.14±0.007[b]
Uric acid (mg/ dl)	3.9± 0.2[a]	2.5± 0.2[b]	2.5± 0.3[b]	2.2± 0.1[b]
Nitric oxide (µmol / L)	6.81±0.48[a]	9.2± 0.19[c]	10.40±. 29[d]	13.90±0.34[d]

Results are expressed as the mean ± SE for seven animals per group.
A, B, C aflatoxins treated groups.
a, b, c, d one way ANOVA was confirmed the treatments within column.
Treatments not sharing common superscripts are significantly different.

acini associated with increase in the rate of necrosis of hyperplastic cells (Fig. 5), while in group C, there was severe necrosis of hyperplastic cells (Fig. 6).

DNA Fragmentation Assay: DNA fragmentation was examined by agarose gel electrophoresis. The results are represented in the testis and sperm cells (Plate 4). A smear on agarose gel had been observed in aflatoxin-treated groups, indicating random DNA fragmentation, a hallmark of necrosis. There are dose dependant DNA damage expressed as (1+), (2+) and (3+) for groups A, B and C, respectively.

Biomarkers of Oxidative Stress: Values of biomarkers of oxidative stress in testicular homogenate of control and aflatoxin treated groups are presented in

table (1). The activities of antioxidant biomarkers (Catalase, glutathione reduced and superoxide dismutase) were significantly decreased due to aflatoxin administration in groups A, B and C comparing to controls. However the content of oxidative product (Malondialdehyde) showed significant ($p < 0.05$) increase in aflatoxin treated groups if compared to controls.

Antioxidant Capacity of Seminal Plasma: The Total antioxidant capacity and uric acid levels showed significant decrease in aflatoxin treated groups compared to control one. In contrast, the levels of nitric oxide are significantly higher in aflatoxin treated groups than control one (Table 2).

DISCUSSION

The clinical signs recorded in aflatoxins (AF) treated groups were erected hair, loss of appetite and marked decrease in body weights, which parallel with the result of Avinash *et al.* [7]. Currently, the post mortem examination of AF treated rabbits receiving 500 and 1000 ppb revealed congestion and enlargement of the liver and blood oozed from their cut surface. The kidneys were pale in colour and slightly enlarged. This was previously described by Koirala *et al*. [37]. Hepatomegaly observed in AF fed rabbits appears to be associated with higher lipid content [7]. In our study, the severity of the pathological alterations observed in the testes of aflatoxicated rabbits was dose dependant. These alterations were described as degeneration and desquamation of spermatogonial cells lining seminiferous tubules, peritubular edema associated with marked atrophy of seminiferous tubules as well as focal hemorrhage and disturbed process of spermiogenesis. The testicular changes are in agreement with the earlier reports [7] and Abdul *et al*. [12]. Studies on adult male rats fed AF for prolonged periods showed regressive changes of different intensity in the germinal epithelium of the seminiferous tubules resulting in a severe dystrophic alteration of the spermatogenic epithelium along with edematous changes in the interstitial tissue [4]. Moreover, prolonged consumption of AF by rats and pigs elicited dystrophy of spermatogenic epithelial cells [38], testicular damage and induce anomalies in meiotic chromosomes and sperm morphology [39]. Most examined sections of AF treated rabbits showed the presence of multinucleated spermatid giant cells in the lumen of seminiferous tubules (Symplasts). The origin of these cells was traced to opening of cytoplasmic bridges. Due to widening of cytoplasmic bridge, the cytoplasm of spermatid(s) in a clone entered a cytoplasm-rich spermatid, followed by the nucleus/nuclei. Subsequently, the bridge(s) collapsed resulting in spherical symplasts. The study, in addition to revealing yet another manifestation of AF induced disruption of spermatogenesis, also provides first direct evidence for opening of cytoplasmic bridges as the mechanism underlying origin of spermatid symplasts [12].

Concerning Epididymis, the severity of the pathological changes were AF dose dependant and summarized as interstitial oedema and atrophy of epididymal tubules, associated with congestion of the blood vessels and capillaries. Hyperplasia of the luminal epithelium of epididymis, together with the presence of pale vacuolated epithelial cells (PVEC) were seen, where some of these vacuoles contain homogenous amorphous material. Previously, [40] concluded that AFB_1 treatment of resulted in pathological changes in the principal cells of the epididymis, whereupon the principal cells form into a fistula and spermatozoa from the lumen gain access into the fistula. The basal cell in the vicinity of the principal cell apparently develops into a PVEC and encloses the disintegrating principal cell¡ including the spermatozoa that have entered it, to prevent an autoimmune response to sperm antigens. Hence, it is proposed that the PVEC develops from the basal cell as a protective device against the autoimmune response to spermatozoa in the context of pathological changes in the principal cells.

AF treatment caused several changes in the Vas Deferens included interstitial edema, congestion of the blood vessels and capillaries and degeneration of epithelial lining with pyknosis of their nuclei. Mild hyperplasia of luminal epithelium was noticed in some examined sections, which are in accordance with the results of Verma and Nair [41]. Degenerative changes and pyknotic nuclei in the vas deferens could be due to oxidative damage and lipid peroxidation which are manifestations of AF induced toxicity [42, 43].

AF treatment exhibit features typical of apoptosis including dense nuclear condensation and cell shrinkage, aflatoxicated rabbits of Group A, B showed marked apoptosis of spermatogonial cells lining the seminefrous tubules, epididymis and prostate gland, whereas in group C, there was severe necrosis in tissues of these organs.

Apoptosis is the process of programmed cell death through a tightly controlled program that plays an important role in many normal processes, ranging from fetal development to adult tissue homeostasis. During apoptosis, the nucleus and cytoplasm condense to

produce membrane-bound apoptotic bodies that are phagocytosed by macrophages or adjacent cells [44]. Several sensitive methods for detecting apoptosis have been developed, based on the different morphological or biochemical features of apoptosis and necrosis [45]. Staining of apoptotic cells with fluorescent dyes such as acridine orange and ethidium bromide is considered the correct method for evaluating the changed nuclear morphology [46, 47]. Apoptotic cells are characterized by a highly condensed nucleus that stains vividly with DNA dyes; in viable cells and early apoptosis excluded the ethidium bromide, but were permeable to acridine orange, which intercalated into the DNA to yield green fluorescent nuclei; in late apoptosis with loss of membrane integrity, both dyes enter the cell and the nucleus is stained orange-red. The balance between germ cell proliferation, differentiation and apoptosis is critical to control spermatogenesis. During establishment of spermatogenesis at the puberal age, early germ cells apoptotic wave occurs, aimed at removing abnormal germ cells and maintaining a proper ratio between maturing germ cells and Sertoli cells [48]. As result of this, up to 75% of the spermatogenic cells are eliminated during the maturation process. While apoptosis is a massive event in the first puberal spermatogenenic wave, in the adult testis it is a rare event and affects mainly spermatogonia [49]. The reason of such change still remains to be clarified; according to some authors only spermatogonia exceeding the supportive capacity of Sertoli cells are eliminated to prevent seminiferous tubule overcrowding. Others suggest that spermatogonia elimination may represent an early selection of abnormal cells before the onset of meiosis [50]. Altering the fine regulation of any of these processes may lead to the onset of testicular diseases. Afiatoxin B_1 is a potent hepatotoxic and hepatocarcinogenic mycotoxin that can damage DNA. One of manifestations of AFB1-induced toxicity is oxidative stress which is an apoptosis inducer [51]. In normal cells, a primary defense system against oxidative damage and apoptosis is provided by antioxidants such as intra-cellular glutathione (GSH) and zinc [52].

Genomic DNA constitutes the total genetic information of an organism. The genomes of almost all organisms are DNA, the only exceptions being some viruses that have RNA genomes. Genomic DNA molecules are generally large and in most organisms are organized into DNA-protein complexes called chromosomes. The size, number of chromosomes and nature of genomic DNA varies between different organisms.

Currently, the DNA of testes and sperms showed a clear fragmentation in all treated groups in a dose dependant manner. This result was previously investigated by Verma [53]. Aflatoxins possess genotoxic potential which is mainly due to adduct formation with DNA, RNA and protein. The adduct formation (DNA Fragmentation) in the tissue cells is so clear and predominant which is parallel to Jia-Sheng and John [54] who concluded that the predominant AFB-DNA adduct was identified as 8, 9-dihydro-8-(N^7-guanyl)-9-hydroxy-AFB_1 (AFB-N^7-Gua), which derives from covalent bond formation between C8 of AFB-8, 9-epoxides and $N^7$of guanine bases in DNA. This may be the most important product from the carcinogenic point of view.

In view of oxidative stress, the present results indicated that aflatoxins induced marked increase in superoxide dismutase and Malondialdehyde while Catalase activity and reduced glutathione were reduced. A significant increase in Malondialdehyde level demonstrated in aflatoxin groups in comparison with the control group were also investigated by Eraslan *et al*. [55] and Eraslan *et al.* [56]. Malondialdehyde is considered to be the most significant indicator of membrane lipid peroxidation, arising from the interaction of reactive oxygen species (ROS) with cell membranes. The induced increase in lipid peroxidation after aflatoxins ingestion may be due to the fact that onset of lipid peroxidation in susceptible sperm leads to the progressive accumulation of lipid hydroperoxides in sperm plasma membranes, which then decomposes to form MDA under stress and toxic conditions [57].

The decline in SOD enzyme activity observed upon administration of aflatoxins was in agreement with [58] who concluded that it may be related to the consumption of highly active components during conversion into H_2O_2 due to the effect of aflatoxins. In our study, catalase was reduced in all treated groups compared to control group. Amongst researches carried out on this subject in different animal species were [59] and Rastogi *et al.* [60] who suggested that aflatoxins decreased catalase in testicles and liver. The significant ($p < 0.05$) reduction in activities of catalase and superoxide dismutase could be responsible for increased lipid peroxidation observed during aflatoxicosis. Significant reductions in superoxide dismutase [61] and catalase [60] have been reported in aflatoxin-fed rat liver. Reduced glutathione is required for the conversion of L dehydroascorbate back to ascorbate. Many investigators [62] have reported significant reduction in glutathione content in aflatoxin-fed rat, as

presently observed. The fall in the level of reduced glutathione decreases the conversion of L dehydroascorbate to ascorbate and this probably explains the lowered level of ascorbic acid in the aflatoxin-treated animals. The overproduction of ROS may play a role in the mechanism of testicular degeneration associated with infertility in case of aflatoxicosis [4].

In conclusion, the results suggested additive interactions on the deleterious effects of aflatoxins on the histological structure of the reproductive system in male rabbits. The data presented showed that aflatoxin at graded doses induced severe oxidative damage in the testis and accessories promoting their apoptosis and thus consuming such doses simultaneously may be a greater risk of male infertility.

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