Influence of prepubertal aflatoxicosis on pubertal reproductive activity in male rats

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Abstract

This research aims to document the harmful effects of exposure to aflatoxin-B1 in the prepubertal stage on the reproductive efficiency of male rats after puberty. Sixty male rats (35 days old) were divided equally into control and treatment groups. Control males were orally supplemented with distilled water. Treatment males were orally supplemented with aflatoxin-B1 (0.3mg/kg/day). After 15 days (50 days old; prepubertal stage), 25 days (60 days old; pubertal stage), and 35 days (70 days old; postpubertal stage) of treatment, ten males from each group were weighed, anesthetized, and blood samples were collected for assessment the serum concentrations of reproductive hormones. Pituitary and testicular tissue samples were obtained to analyze the expression levels of pituitary and testicular genes. Testes and epididymis were obtained for histopathological examination. Compared to control, treated males showed a decrease in serum concentration of gonadotrophin releasing hormone, follicle stimulating hormone, luteinizing hormone, and testosterone, and the expression level of pituitary GNRHR1, FSHβ, and LHβ genes and testicular LHR, FSHR, ABP, 3β-HSD, and 17β-HSD genes, in all experimental periods. Histological sections of the testicles of treated males showed atrophy of some seminiferous tubules, empty lumen, and massive vacuolization and exfoliation of the germ cells. The histological results of the epididymis showed obliteration of the lumen, necrosis of the epithelial layer, deformed cavities, a climbing epithelial layer, and epithelium hyperplasia compared to the control group. In conclusion, exposure to aflatoxin-B1 at the prepubertal stage can reduce reproductive efficiency at puberty.

Keywords: Aflatoxin B1, Epididymis, Gonadotrophin, Prepuberty, Testes

Introduction

Among the numerous filamentous fungi that produce mycotoxins as secondary toxic metabolites, Aspergillus, Penicillium, and Fusarium species are significant plant pathogens known to cause infection and create mycotoxins in crops used for human consumption (1). Mycotoxins include Aflatoxin, Fumonisin, Ochratoxin, Zearalenone, T-2 toxins, DON, and DAS (2,3). Aflatoxins are the most notable mycotoxins related to several human and animal health issues out of the more than 400 known mycotoxins (4). Aflatoxins are critical mycotoxins produced primarily by Aspergillus flavus and Aspergillus parasiticus on various foods under suitable environments. Economically, mycotoxicosis affects domestic and international trade and the health of people and animals (5). Furthermore, agricultural products are significantly at risk from mycotoxin contamination (6). Aflatoxicosis is a disease caused by ingesting food contaminated with aflatoxin (4). Aflatoxins are potent mycotoxins containing toxic secondary metabolites created by fungi, such as Aspergillus, Penicillium, and Fusarium (1). The most prevalent AF kinds
are B1, B2, G1, and G2, AFB1 being the most dangerous for animals (7) and Nile tilapia (8). Raw peanuts contain aflatoxin (9), and it was discovered that the liver produces aflatoxin-B1 due to aflatoxicosis (10). Aflatoxin B1 causes liver, kidney, and genital organ damage in mature males, and histopathological changes in the testicles and epididymis (11). Several male reproductive toxicity experiments in rodent models show a decrease in testosterone production, even if the compound's overall effects on male reproductive parameters depend on the type of chemical (12). According to the compound, testicular interstitium atrophy, decreased serum testosterone concentrations, decreased sperm count, altered motility/morphology, and increased testicular and epididymal weights are the main findings (13). Aflatoxin B1 may have other impacts on the endocrine and reproductive systems in both research animals and adult male rats (14). Aflatoxin-B1 can also negatively impact adult rats' male reproductive organs, and this deleterious effect is time-dependent (15,16). Aflatoxins are known to directly influence Leydig cells, which may impair their capacity to carry out crucial functions related to necessary enzymes and hormone production. AFB1 has been demonstrated to specifically block the expression of certain dehydrogenase enzymes and decrease testosterone concentrations dose-dependently. It is critical to comprehend how mycotoxins like AFB1 might affect both hormonal and enzymatic mechanisms because the process of steroidogenesis depends on both systems (17).

The functional activities of the adult male reproductive system are impacted by aflatoxins. So, the current study hypothesized that prepubertal exposure to aflatoxin may also impair these activities during puberty by affecting the development of male reproductive organs and male reproductive competence in adulthood.

Materials and methods

Chemicals
Sigma Company, USA bought aflatoxin-B1 (from Aspergillus flavus 98% purity, Formula C17H12O6, Formula Weight 312.3 AMU). All the extra substances utilized in the study were highly analytical quality and obtained from neighboring commercial establishments.

Animal ethics and care
This research was conducted under the guidelines provided by the National Research Council for the Care and Use of Laboratory Animals. The Ethical Council in the College of Veterinary Medicine, University of Al-Qadisiyah permitted the experimentation.

Experimental animals
Premature male rats (aged 35 days and weighted 75-88 g) were used in the present study. The animals were kept at 22 to 25 °C, 75-76% relative humidity, and 12:12 hours of dark and light cycle. The animals had free access to water and laboratory food at all times. After acclimatization, the animals were weighed before treatment (at day 35 of age) after each experiment period.

Experimental design
Sixty premature Wistar rats (35 days old) were allocated to control and treatment groups (30 males each). Control male rats were administered daily with distilled water per os. Treated male rats were administered daily with Aflatoxin-B1 per os (0.3mg/kg/day) (18,19). After 15, 25, and 35 days of treatment (prepubertal, pubertal, and postpubertal subgroups, respectively), ten males from each group were anesthetized, sacrificed, and blood samples were collected for assessment of the serum concentrations of GnRH, FSH, LH, and testosterone, by using ELISA kits, according to the instructions provided by the manufacturer (Cusabio, China). Tissue samples from the testis and epididymis were dissected and fixed in a formalin buffer solution, and microscopic slides were prepared at 5μm of thickness and stained with hematoxylin and eosin stains for histopathological examination (20). Additional tissue samples from the pituitary and testicles were also collected to analyze the gene expression levels of pituitary GnrH, FSHβ and LHβ genes, and testicular FSHR, LHR, ANB, 3β-HSD, and 17β-HSD genes.

Molecular analysis of pituitary and testicular genes
According to the instructions from Bioneer company (Korea), the total RNA from pituitary and testicular tissues using an Accuzol® kit was extracted, and cDNA was synthesized. Quantitative real-time PCR (qRT-PCR) was used to quantitate the target specific genes, including pituitary GnrHR, FSHβ and LHβ genes, and testicular FSHR, LHR, ANB, 3β-HSD, and 17β-HSD genes, and normalize the results using the GAPDH gene as a housekeeping gene. The procedure followed the method previously described in a publication by Burow et al. (21). The qRT-PCR data were analyzed using the fold-change method for relative expression of the target genes and GAPDH, following the protocol outlined in a publication by Livak and Schmittgen (22).

Statistical analysis
The statistical analysis was conducted using GraphPad Prism version 5. The mean ± standard deviation (M±SD) was used to present the data. One-way ANOVA with Newman-Keuls posthoc analysis determined the significant differences between the periods within each group. The student’s t-test was also used to determine the significant differences between the groups at each period. The level of (P<0.05) was considered significant (23).
Results

Reproductive hormones

As shown in figure 1, male rats treated with AFB1 showed a significant decrease (P<0.05) in serum concentrations of GnRH, FSH, LH, and T in the three periods (50, 60, and 70 days) in comparison with control. When comparing the three periods for the control group, the concentration increased with age, whereas the concentrations of the treated group showed little increase at 70 days compared to 60 days old.

Figure 1: Serum concentration of GnRH, FSH, LH, and testosterone in AFB1 treated male rats. The values are depicted as Mean ± SD. The different uppercase letters indicate significant differences (P<0.05) between groups during each period. Lowercase letters indicate significant differences (P<0.05) between the three time periods for each group.

Pituitary GnRHR, FSHβ, and LHβ Genes

In all experimental periods, male rats treated with AFB1 showed a significant decrease (P<0.05) in the expression level (fold changes) of pituitary GnRHR, FSHβ, and LHβ genes compared with the corresponding periods of control male rats (Figure 2). Compared to the 50 days, the expression level of pituitary GnRHR, FSHβ, and LHβ genes of control males at 60 and 70 day periods showed a noticeable percentage of the increase, while in the treatment group, the increase was slight.

Testicular FSHR, LHR, ANB, 3β-HSD and 17β-HSD

In all experimental periods, the expression levels (fold changes) of testicular FSHR, LHR, ANB, 3β-HSD, and 17β-HSD genes of treated male rats showed a significant decrease (P<0.05) compared with the corresponding periods of control male rats (Figure 3). When comparing to 50 days period, the expression level of testicular FSHR, LHR, ANB, 3β-HSD, and 17β-HSD genes of control males at 60 and 70 days periods showed a highly significant increase, while in the treatment group there is a slight increase.

Figure 2: The expression levels of pituitary GnRHR, FSHβ, and LHβ genes in AFB1 treated male rats. The values are depicted as Mean ± SD. The different uppercase letters indicate significant differences (P<0.05) between groups during each period. Lowercase letters indicate significant differences (P<0.05) between the three time periods for each group.

Figure 3: The expression levels of testicular FSHR, LHR, ANB, 3β-HSD, and 17β-HSD genes in AFB1 treated male rats. The values are depicted as Mean ± SD. The different uppercase letters indicate significant differences (P<0.05) between groups during each period. Lowercase letters indicate significant differences (P<0.05) between the three time periods for each group.
Testes

The histological findings of AF treated male rat’s testes of 50 days old (prepubertal period) showed atrophy of some seminiferous tubules, empty lumen, irregular shape of seminiferous tubules, a massive discharge of germ cell cytoplasm, and marked loss of cells, compared to the control group of the same period, that showed normalization of the seminiferous tubules, the presence of spermatogonia, spermatocytes, and Sertoli cells (Figure 4).

At 60 days (pubertal period), the histological findings of the AF-treated male rat’s testes showed thickening of the wall of the seminiferous tubules, exfoliation of germ cells in the lumen of seminiferous tubules, irregular shape of seminiferous tubules, distributed vacuolation of germ cell, marked loss of cells compared with the control male rat’s testes, which showed the normal shape of seminiferous tubules, presence of spermatogonia, spermatocytes, and spermatids (Figure 5). At 70 days (postpubertal period), the histological findings of the AF-treated male rat’s testes showed thickening of the wall of the seminiferous tubules, atrophy of some seminiferous tubules, irregular shape of seminiferous tubules, huge vacuolation of germ cells, empty lumen, marked loss of cells compared with control male rat’s testes (Figure 6).

Epididymis

The histological findings of the epididymis of AF treated male rats at 50 days old (prepubertal period) showed obliteration of the lumen, mild fibrosis, clumped spermatzoa, and necrosis of the epithelial layer compared with the control group (Figure 7).

Figure 4: Histological section from AF-treated (A) and control (B) male rat testes at the prepubertal period (50 days period) showed atrophy of some seminiferous tubules (black arrow), empty lumen (red arrow), irregular shape of seminiferous tubules (blue arrow), vacuolation of germ cell cytoplasm (green arrows), marked loss of cells, whereas control male rat’s testes showed normal seminiferous tubules, presence of spermatogonia cells (SG), spermatocytes (SPC), spermatids (SPT), and Sertoli cells (SC). H&E, 100X and 400X.

Figure 5: Histological section from AF-treated (A) and control (B) male rat testes at the pubertal period (60 days period) showed thickening of the wall of the seminiferous tubules (black arrows), exfoliation of germ cells in the lumen of seminiferous tubules, irregular shape of seminiferous tubules, huge vacuolation of germ cell (red arrows), marked loss of cells compare with the group control (B) that normal showed shape seminiferous tubules, presence of spermatogonia cells (SG), spermatocytes (SPC), and spermatids (SPT). H&E, 100X and 400X.

Figure 6: Histological section from AF-treated (A) and control (B) male rat testes at the postpubertal period (70 days period) showed thickening of the wall of the seminiferous tubules (black arrows), atrophy of some seminiferous tubules, irregular shape of seminiferous tubules, huge vacuolation of germ cell (red arrows), huge vacuolation of germ cell (red arrows), empty lumen (EL), marked loss of cells compare with the group control (B) that normal showed shape seminiferous tubules, presence of spermatogonia cells, spermatocytes, and spermatids. H&E, 100X and 400X.
Figure 7: Histological section from AF-treated (A) and control (B) male rat epididymis at the prepubertal period (50 days period) showed obliteration of the lumen (black arrows), mild fibrosis (red arrows), clumped spermatozoa (blue arrow), and necrosis of the epithelial layer (green arrow) compared with the control group (B) that showed normal shape tubules, normal muscular layer (red arrow), basement membrane (black arrow), columnar epithelial cells (green arrow), and thin connective tissue (pink arrow). H&E, 40X, 100X and 400X.

At 60 days (pubertal period), the histological findings of the AF-treated male rat’s epididymis showed a distorted lumen, mild fibrosis, hemorrhage in the lumen, and sloughed epithelial layer, compared with the control (Figure 8).

Figure 8: Histological section from AF-treated (A) and control (B) male rat epididymis at the pubertal period (60 days period) showed distorted lumen (red arrow), mild fibrosis (blue arrows), hemorrhage in the lumen (black arrow), and sloughed epithelial layer (green arrow) compared with the control group (B) that showed normal epididymis structures. H&E, 100X and 400X.

At 70 days (postpubertal period), the histological findings of the AF-treated male rat’s epididymis showed a distorted lumen, mild fibrosis, and epithelial hypertrophy compared with the control group (Figure 9).

Figure 9: Histological section from AF-treated (A) and control (B) male rat epididymis at the postpubertal period (70 days period) showed distorted lumen (black arrow), and mild fibrosis (red arrow), and hypertrophy of the epithelial compared with the control group (B) that showed normal epididymis structures. H&E, 100X and 400X.

Discussion

In the current study, exposure to AFB1 at the prepubertal stage reduced the serum concentrations of GnRH, FSH, LH, and testosterone in both the prepubertal and postpubertal stages. This result indicates that exposure to aflatoxin in the prepubertal stage leads to structural and functional changes reflected in male’s reproductive efficiency. AFB1 was found to harm the reproductive activity, as the hypothalamic-pituitary-gonadal (HPG) axis is essential for gametogenesis, maintenance of gonadal hormone synthesis, and reproductive organ functions (24). Previous research has also suggested that AFB1 can be toxic to Leydig cells and affects testicular steroidogenesis (25). The study conducted by Owumi et al. (17) found that FSH and testosterone concentrations in rats treated with AFB1 were significantly reduced, which may affect the semen quality, as the composition of seminal fluid was significantly affected in A. flavus exposed male rats (26). These results are consistent with the findings of our study.

Moreover, the current decline in pituitary GnRHR, FSHβ, and LHβ gene expression corroborates the previous findings and indicates that the effect may be central via the hypothalamus and/or pituitary gland. This result was consistent with the decrease in serum FSH and LH concentrations, as their decrease came from a decline in the
influence of GnRH. After administering AFB1 to premature male rats, the LH concentrations decreased significantly accompanied by decreased testosterone concentrations, indicating disruption of the HPGA axis. Previous studies have suggested that AFB1 can suppress LH in adult rats (15,17,27,28).

On the other hand, the decrease in the expression concentrations of testicular FSHR, LHR, ANB, 3β-HSD, and 17β-HSD genes may confirm that the effect of AFB1 is also on the concentration of testses, whether in the puberty stage, as indicated by Supriya et al. (29) and Mohammed et al. (11), or in the prepubertal stage, as indicated by the results of the current study. Moreover, the current pathological changes observed in the testicular and epididymal tissues of both immature and mature male rats as a result of AFB1 treatment are additional evidence that aflatoxicosis is effective in the prepubertal stage and continues its effect until after puberty. These findings were in agreement with those reported by Mukumu and Macharia (30), Owumi et al. (17), and Mohammed et al. (11), who found similar pathological changes in the adult testicular tissues.

Conclusion

The study suggests that prepubertal exposure to Aflatoxin B1 is exceptionally toxic to the male reproductive system of rats, as it can adversely affect the rat’s male reproductive organs resulting in structural and functional alteration of the HPG axis.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication and or funding of this manuscript.

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تأثير الأفلاتوكسيكوز قبل البلوغ على فعالية ذكور الجرذان التكاثرية بعد البلوغ

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الخلاصة

تهدف الدراسة الحالية إلى توثيق تأثيرات الأفلاتوكسيكوز-ب على الغدة النخامية والخصى لدى الذكور في مرحلة البلوغ. تم تقسيم 100 ذكرًا على مجموعتين: سيطرة ومعالجة. تم تجرب ذكور السيطرة بالبقاء الطبيعية وتجريع ذكور المعالجة بالأفلاتوكسيكوز-ب (1.3 ملغم/كغ/يوم). بعد مرور 15 يومًا (عمر 50 يومًا وتمثل مرحلة البلوغ) و 35 يومًا (عمر 70 يومًا وتمثل مرحلة بعد البلوغ)، تم وزن عشرة ذكور من كل مجموعة وتحديد هرمونات الفضلات ومستوى تعبير الجينات المراد قياسه من خلال دراسة القناعات والهرمونات النخامية والخصية في مصل الدم. تم أخذ عينات من الأنسجة النخامية والخصية لقياس مستويات هرمونات نحلية ومستوى تعبير الجينات المراد قياسه من خلال دراسة القناعات والهرمونات النخامية والخصية. أظهرت الدراسة أنه تعرض الذكور بالأفلاتوكسيكوز قبل البلوغ يقلل من الكفاءة التكاثرية بعد البلوغ.

مصادر


