Iraqi Journal of Veterinary Sciences

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Influence of prepubertal aflatoxicosis on pubertal reproductive activity in male rats

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Article information

Article history:
Received 08 July, 2023
Accepted 31 August, 2023
Published online 31 January, 2024

Keywords:
Aflatoxin B1
Epididymis
Gonadotrophin
Prepuberty
Testes

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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.

DOI: 10.33899/ijvs.2023.141632.3125,

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 after 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 to 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 *GnRHR*, *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.

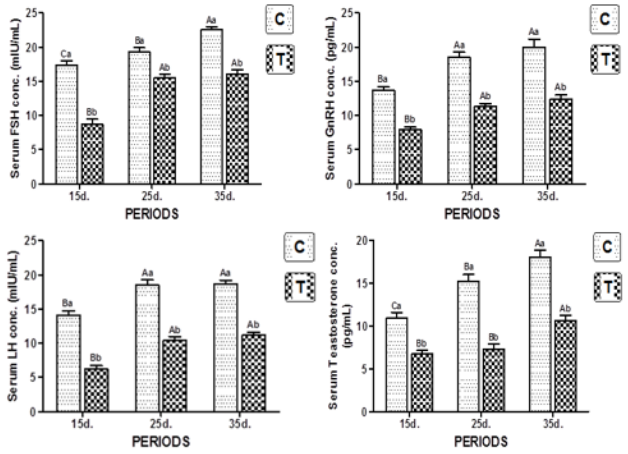

Figure 1: Serum concentration of GnRH, FSH, LH, and testosterone in AFB1 treated male rats. The values are depicted as Mean ± SD. The different uppercase letters indicate significant differences (P<0.05) between groups during each period. Lowercase letters indicate significant differences (P<0.05) between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

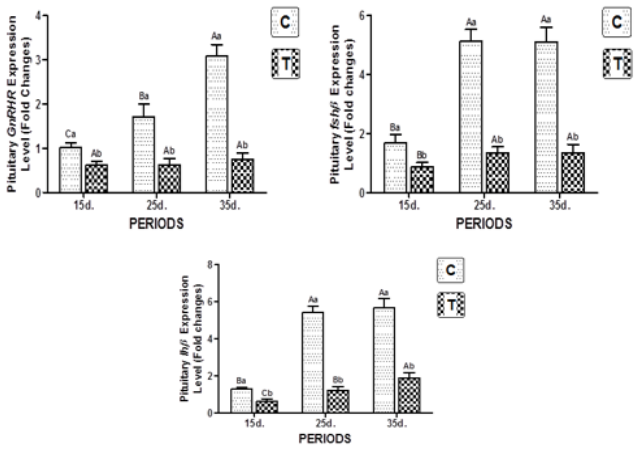

Figure 2: The expression levels of pituitary GnRHR, FSHβ, and LHβ genes in AFB1 treated male rats. The values are depicted as Mean ± SD. The different uppercase letters indicate significant differences (P<0.05) between groups during each period. Lowercase letters indicate significant differences (P<0.05)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

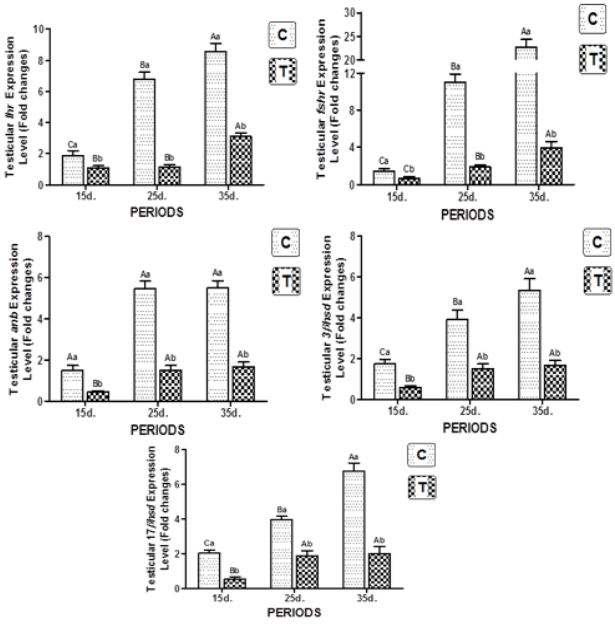

Figure 3: The expression levels of testicular *FSHR*, *LHR*, *ANB*, *3β-HSD*, and *17β-HSD* genes in AFB1 treated male rats. The values are depicted as Mean ± SD. The different uppercase letters indicate significant differences (P<0.05) between groups during each period. Lowercase letters indicate significant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

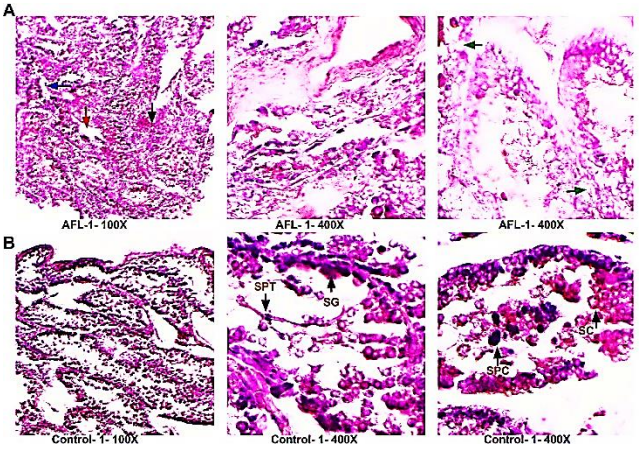

Figure 4: Histological section from AF-treated (A) and control (B) male rat testes at the prepubertal period (50 days period) showed atrophy of some seminiferous tubules (black arrow), empty lumen (red arrow), irregular shape of seminiferous tubules (blue arrow), vacuolation of germ cell cytoplasm (green 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.

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 spermatozoa, and necrosis of the epithelial layer compared with the control group (Figure 7).

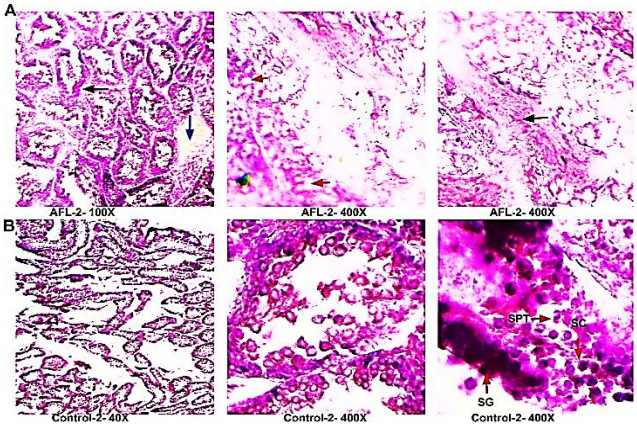

Figure 5: Histological section from AF-treated (A) and control (B) male rat testes at the pubertal period (60 days period) showed thickening of the wall of the seminiferous tubules (black arrows), exfoliation of germ cells in the lumen of seminiferous tubules, irregular shape of seminiferous tubules, 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.

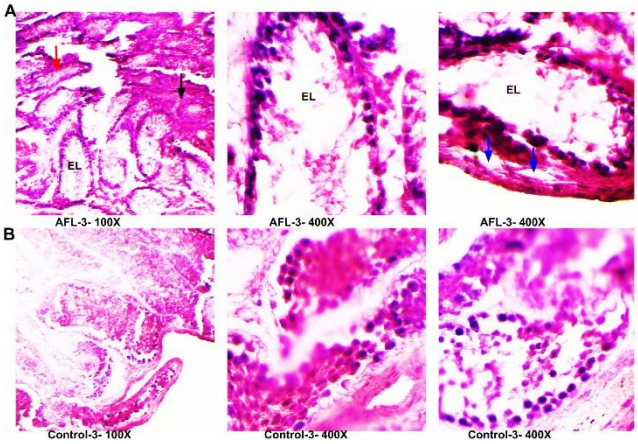

Figure 6: Histological section from AF-treated (A) and control (B) male rat testes at the postpubertal period (70 days period) showed thickening of the wall of the seminiferous tubules (black arrows), atrophy of some seminiferous tubules, irregular shape of seminiferous tubules (red arrows), huge vacuolation 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.

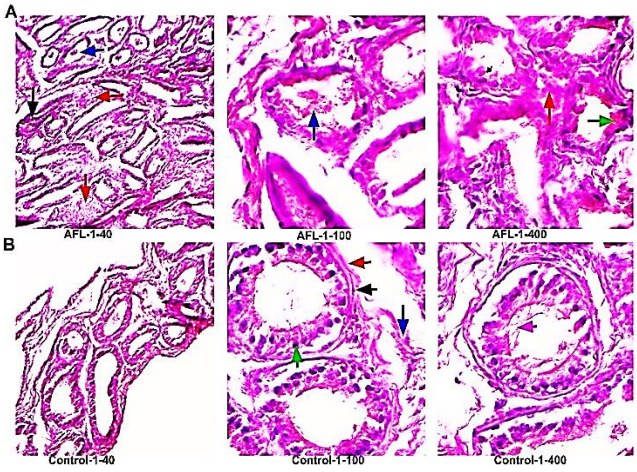

Figure 7: Histological section from AF-treated (A) and control (B) male rat epididymis at the prepubertal period (50 days period) showed obliteration of the lumen (black arrows), mild fibrosis (red arrows), clumped spermatozoa (blue arrow), and necrosis of the epithelial layer (green arrow) compare with 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).

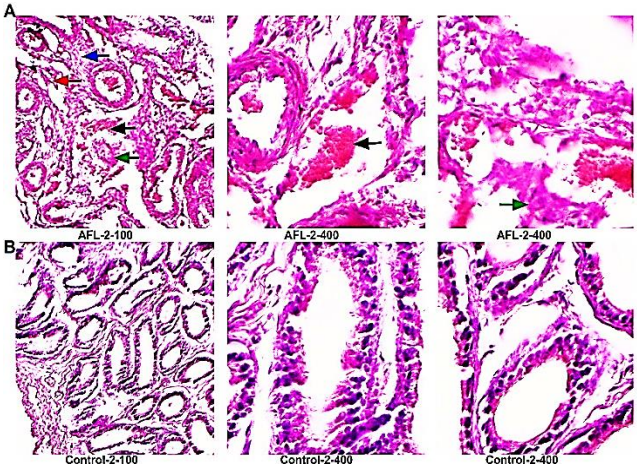

Figure 8: Histological section from AF-treated (A) and control (B) male rat epididymis at the pubertal period (60 days period) showed distorted lumen (red arrow), mild fibrosis (blue arrows), hemorrhage in the lumen (black arrow), and sloughed epithelial layer (green arrow) compare with the control group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

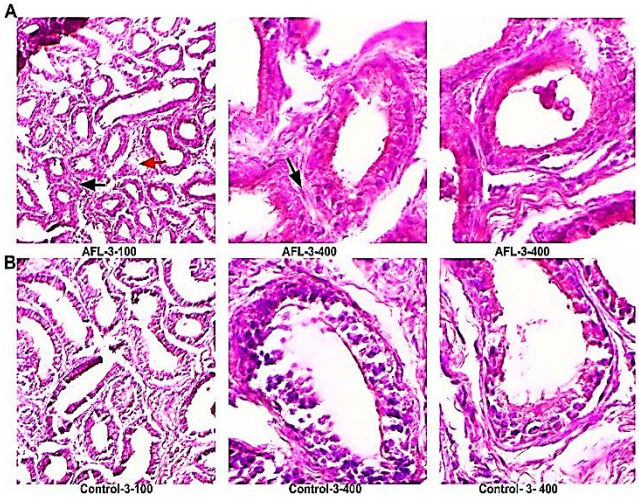

Figure 9: Histological section from AF-treated (A) and control (B) male rat epididymis at the postpubertal period (70 days period) showed distorted lumen (black arrow), and mild fibrosis (red arrow), and hypertrophy of the epithelial compared with the control group (B) that showed normal epididymis structures.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

Acknowledgment

The authors would like to acknowledge the Department of Physiology, Pharmacology, and Biochemistry, College of Veterinary Medicine, University of Al-Qadisiyah for their provision to perform the current work.

Conflict of interest

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

References

1. Nazhand A, Durazzo A, Lucarini M, Souto EB, Santini A. Characteristics, occurrence, detection and detoxification of aflatoxins in foods and feeds. Foods. 2020;9(5):644. DOI: 10.3390/foods9050644
2. Imran M, Cao S, Wan SF, Chen Z, Saleemi MK, Wang N, Naseem MN, Munawar J. Mycotoxins - a global one health concern: A review. Agrobiol Rec. 2020;2:1-16. DOI: 10.47278/journal.abr/2020.008
3. Gohar UF, Mukhtar H, Mushtaq A, Farooq A, Saleem F, Hussain MA, Ghani MU. Fungi: A potential source of biopharmaceuticals. Agrobiol. Rec. 2020;2:49-62. DOI: 10.47278/journal.abr/2020.011
4. Gurikar C, Shivaprasad DP, Sabillón L, Nanje Gowda, NA, Siliveru K. Impact of mycotoxins and their metabolites associated with food grains. Grain Oil Sci Technol. 2023;6(1):1-9. DOI: 10.1016/j.gaost.2022.10.001
5. Moretti A, Pascale M, Logrieco AF. Mycotoxin risks under a climate change scenario in Europe. Trends Food Sci Technol. 2019;84:38-40. DOI: 10.1016/j.tifs.2018.03.008
6. Moussa AI, Sobeih AK, Al-Hawary II, Elkassas WM, Barakat R. Efficacy of kaolin and bentonite clay to reduce aflatoxin M1 content in contaminated milk and effects on milk quality. Pak Vet J. 2020;40:181-186. DOI: 10.29261/pakvetj/2020.001
7. Alnaemi HS. Estimation of aflatoxin M1 levels in some dairy products manufactured from raw milk experimentally inoculated with toxin. Iraqi J Vet Med. 2019;43(1):50–8. DOI: 10.30539/iraqijvm.v43i1.471
8. Marijani E, Charo-Karisa H, Gnonlonfin GJ, Kigadye E, Okoth S. Effects of aflatoxin B1 on reproductive performance of farmed Nile tilapia. Int J Vet Sci Med. 2019;7(1):35–42. DOI: 10.1080/23144599.2019.1678315
9. Khalil R, Yassin M, Yaseen SJ. Detection some aflatoxins in some locally marketed raw peanuts. Res J Pharm Technol. 2021;14(3):1431-1437. DOI: 10.5958/0974-360X.2021.00255.9
10. Sukmanadi M. Effendi MH. The protective effect of Capsaicin (*Capsicum annum L*) against the induction of aflatoxin B1 in hepatocytes: A study of liver histopathology in mice (Musmusculus). Res J Pharm Technol. 2021;14(2):813-816. DOI: 10.5958/0974-360X.2021.00143.8
11. Mohammed BJ, Al-Saaidi JA, Al-Zwean DY. The protective role of camel milk against reprotoxicity, hepatotoxicity, and nephrotoxicity in aflatoxic-induced male rats. Res J Pharm Technol. 2023;16(3):1072-8. DOI: 10.52711/0974-360X.2023.00179
12. Kuzukiran O, Simsek I, Filazi A, Yurdakok-Dikmen B. Perfluorooctane sulfonate and perfluorooctanoic acid. In: Gupta RC, editor. Reproductive and developmental toxicology. 3rd ed. USA: Academic Press; 2022. 815-831 p. DOI: 10.1016/B978-0-323-89773-0.00041-2
13. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Leblanc JC, Nebbia CS, Nielsen E, Ntzani E, Petersen A, Sand S, Vleminckx C, Wallace H, Barregård L, Ceccatelli S, Cravedi JP, Halldorsson TI, Haug LS, Johansson N, Knutsen HK, Rose M, Roudot AC, Van Loveren H, Vollmer G, Mackay K, Riolo F, Schwerdtle T. Scientific opinion on the risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J. 2020;18(9):6223. DOI: 10.2903/j.efsa.2020.6223
14. Hasanzadeh S, Hosseini E, Rezazadeh L. Effects of aflatoxin B1 on profiles of gonadotropic (FSH and LH), steroid (testosterone and 17β-estradiol) and prolactin hormones in adult male rat. Iran J Vet Res. 2011;12:332–336. DOI: 10.22099/IJVR.2011.85
15. Mohammed BJ. Role of camel milk in ameliorating reprotoxic and hepato/ renotoxic-induced by aflatoxin B1 in male rats [Ph.D. dissertation]. Baghdad: College of Veterinary Medicine, University of Baghdad; 2022.
16. Mohammed BJ, Al-Saaidi JA, Al-Zwean DY. Reprotoxicological ameliorating effect of camel milk against aflatoxicosis in male rats. J Pharm Negat Results. 2022;13(4):456-462. DOI: 10.47750/pnr.2022.13.04.058
17. Owumi SE, Adedara IA, Akomolafe AP, Farombi EO, Oyelere AK. Gallic acid enhances reproductive function by modulating oxido-inflammatory and apoptosis mediators in rats exposed to aflatoxin-B1. Exp Biol Med. 2020;245(12):1016–28. DOI: 10.1177/1535370220936206
18. Dhanasekaran D, Shanmugapriya S, Thajuddin N, Panneerselvam A. Aflatoxins and aflatoxicosis in human and animals. In: Guevara-Gonzalez RG, editor. Aflatoxins-Biochemistry and Molecular Biology. USA: InTech; 2011. 221-254 p. [available at]
19. Kudayer AM, Alsandaqchi AT, Saleh FM, Alwan NA. Toxic effect of aflatoxin B1 on heart, lung, and testis of male albino rats: histopathology study. IOP Conf Ser Mater Sci Eng. 2019;571(1):012055. DOI: 10.1088/1757-899X/571/1/012055
20. Bilinska B, Hejmej A, Kotula-Balak M. Preparation of testicular samples for histology and immunohistochemistry. In: Alves MG,

تأثير الأفلاتوكسيكوسز قبل البلوع على فعالية ذكور الجرذان التكاثرية بعد البلوغ

حسين حامد الحجامي و جبار عباس أحمد الساعدي

فرع الفسلجة والأدوية والكيمياء الحياتية، كلية الطب البيطري، جامعة القادسية، القادسية، العراق

الخلاصة

تهدف الدراسة الحالية الى توثيق تأثيرات الأفلاتوكسين-ب١ الضارة في مرحلة ما قبل البلوغ على الكفاءة التكاثرية لذكور الجرذان لعد البلوغ. تم تقسيم ٦٠ جرذا ذكرا بعمر ٣٥ يوما بالتساوي على مجموعتي سيطرة ومعاملة. تم تجريع ذكور السيطرة بالماء المقطر وتجريع ذكور المعاملة بالأفلاتوكسين-ب١ (٠,٣ ملغم/كغم/يوم). بعد مرور ١٥ يوم (عمر ٥٠ يوما وتمثل مرحلة قبل البلوغ) و ٢٥ يوم (عمر ٦٠ يوما وتمثل مرحلة البلوغ) و ٣٥ يوم (عمر ٧٠ يوما وتمثل مرحلة بعد البلوغ)، تم وزن عشرة ذكور من كل مجموعة وتخديرها وجمع عينات الدم منها لغرض قياس مستوى الهرمونات التكاثرية في مصل الدم. تم أخذ عينات من أنسجة النخامية والخصى لغرض حساب مستوى تعبير الجينات المراد دراستها. كما تم أخذ عينات من الخصى والبرابخ لغرض الدراسة النسجية-المرضية. بالمقارنة مع السيطرة، أظهرت ذكور المعاملة انخفاضا في تركيز الهرمون المحرر لمحرضات القند والهرمون محفز الجريب والهرمون المصفر في مصل الدم ومستوى تعبير جينات *GNRHR1* و *FSHβ* و *LHβ* في الغدة النخامية و جينات *LHR* و *FSHR* و*ABP* و *3β-HSD* و *17β-HSD* في الخصى، وفي جميع مراحل الدراسة. أظهرت المقاطع النسجية لخصى ذكور المعاملة ضمورا في بعض النبيبات المنوية وخلو تجاويفها مع وجود فجوات وتقشر للخلايا الجرثومية. وأظهرت مقاطع البرابخ النسجية عدم وجود تجاويف وتنكس الطبقة الظهارية وتشوه بعض التجاويف وتثخن البطانة بالمقارنة مع مقاطع السيطرة. يستنتج أن تعرض الذكور للأفلاتوكسين في مرحلة ما قبل البلوغ يقلل الكفاءة التكاثرية بعد البلوغ.

Oliveira PF, editors. Sertoli cells: Methods Protocols. USA: Humana Press; 2018. 17-36 p. DOI: 10.1007/978-1-4939-7698-0_3

21. Burow S, Fontaine R, von Krogh K, Mayer I, Nourizadeh-Lillabadi R, Hollander-Cohen L. Medaka follicle-stimulating hormone (FSH) and luteinizing hormone (LH): Developmental profiles of pituitary protein and gene expression levels. Gen Comp Endocrinol. 2019;272(2):93–108. [available at]
22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8. [available at]
23. Schefler WC. Statistics for the biological sciences. UK: Addison-Wesley Publishing Company; 1979. DOI: 10.1002/bimj.19700120316
24. Squires EY. Applied animal endocrinology. 2nd ed. UK: Cambridge University Press; 2010. 39 p.
25. Chen X, Li C, Chen Y, Ni C, Chen X, Zhang L, Xu X, Chen M, Ma X, Zhan H, Xu A. Aflatoxin B1 impairs Leydig cells through inhibiting AMPK/mTOR-mediated autophagy flux pathway. Chemosphere. 2019;233(10):261–72. [available at]
26. Kadhim SO, Faleh IB. Pathological study of genitourinary invasion of Aspergillus flavus in male rats. Iraqi J Vet Sci. 2021;35(I-III):87-94. DOI: 10.33899/ijvs.2021.131580.1974
27. Onuzulu CD, Rotimi OA, Rotimi SO. Epigenetic modifications associated with in utero exposure to endocrine disrupting chemicals BPA, DDT and Pb. Rev Environ Health. 2019;34(4):209-325. [available at]
28. Amin YA, Mohamed RH, Zakaria AM, Wehrend A, Hussein HA. Effects of aflatoxins on some reproductive hormones and composition of buffalo's milk. Comp Clin Pathol. 2019;28(4):1191–6. [available at]
29. Supriya C, Girish BP, Sreenivasula Reddy P. Aflatoxin B1- induced reproductive toxicity in male rats: Possible mechanism of action. Int J Toxicol. 2014;33(3):155–161. DOI: 10.1177/1091581814530764
30. Mukumu CK, Macharia BN, Lusweti D, and Mwaniki DM. Effects of aflatoxin B1 (AFB1) on follicle stimulating hormone, luteinizing hormone, testosterone, and estradiol hormone levels in reproductively mature male pigs. African J. Environ. Sci. and Tech; 2017; 11(7): 370-374. DOI: 10.5897/AJEST2016.2220.