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Role of Biological Restraints and Vitamin E in Protecting the Reproductive System of Male Rats from Aflatoxin B1 Toxicity

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ABSTRACT

This study aimed to investigate the effects of mycotoxins, specifically aflatoxin B1, and various bioadsorbents (yeast, zeolite, vitamin E) on the male reproductive system of rats by evaluating their impact on the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. The experiment was conducted with 36 adult male rats, weighing between 60-180 grams. The rats were randomly divided into six groups, each with six replicates. The groups were: T1, the control group with no additives; T2, addition of 0.5 ml of aflatoxin B1; T3, addition of 0.5 ml of aflatoxin B1 + 80 grams of yeast; T4, addition of 0.5 ml of aflatoxin B1 + 1 ml of vitamin E; T5, addition of 0.5 ml of aflatoxin B1 + 40 grams of zeolite; and T6, addition of 0.5 ml of aflatoxin B1 + 80 grams of yeast + 1 ml of vitamin E + 40 grams of zeolite.The results showed a significant increase ($P \leq 0.05$) in the weight of both the testes and the prostate when comparing the different treatments to the control group (T1). However, a noticeable decrease in weight was observed in the group exposed to aflatoxin B1 (T2). Additionally, a significant increase ($P \leq 0.01$) in FSH levels was found in (T2) compared to (T1) and (T6). A significant decrease ($P \leq 0.01$) in LH and testosterone levels was observed in (T2) compared to (T1). Histological analysis revealed tissue damage in the testes, including significant degeneration of the seminiferous tubules, absence of spermatogenic cells, and lack of mature spermatozoa, as well as an increase in the number of deformed spermatozoa due to the toxic effects of aflatoxin B1. Therefore, the study provides significant insights into the impact of aflatoxin B1 and various bio adsorbents on the male reproductive system in rats.

دور المقيدات البيولوجية وفيتامين E في حماية الجهاز التناسلي لذكور الجرذان من سمية الافلاتوكسين B1

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الخلاصة
هدفت الدراسة لمعرفة تأثير السموم الفطرية سم الافلاتوكسين وبعض المقيدات الحيوية (الخميرة ، الزيولايت، فيتامين E) على الجهاز التناسلي لذكور الجرذان من خلال التأثير في مستويات الهرمونات (FSH, LH)وهرمون الشحمون الخصوي T .صممت التجربة باستخدام36 ذكرا من الجرذان البالغة باوزان 60-180 غم ووزعت عشوائيا على أساس الكتلة الحية ، وزعت الجرذان على ستة مجاميع بست معاملات وبواقع ست مكررات كالتالي T1 معاملة السيطرة بدون أي اضافه ، T2 اضافه 0.5 مل سم الافلاتوكسينB1 ، T3اضافه 0.5 مل سم الافلاتوكسين80 + B1 غم الخميرة ، T4 اضافه 0.5 مل سم الافلاتوكسين B1+1مل فيتامينE ، T5 اضافه 0.5 مل سم الافلاتوكسين40 + B1 غم الزيولايت ، T6 اضافه 0.5 مل سم الافلاتوكسين B1+80غم الخميرة +1مل فيتامين40 + E غم الزيولايت. اوضحت نتائج التجربة زيادة معنوية ($P\leq0.05$) في وزن كل من الخصيتين والبروستات عند مقارنة المعاملات مع بعضها بالمقارنة مع السيطرة في حين لوحظ انخفاض وزني ملحوظ في المعاملة التي تعرضت للسموم الفطرية، بينما لوحظ ارتفاع معنوي عالي ($P\leq0.01$) لهرمون FSH في T2 المضاف اليها سم الافلاتوكسين B1 عند مقارنته مع معاملة T1ومعاملةT6 ، لوحظ وجود انخفاض معنوي ($P\leq0.01$) لهرموني LH و Testosteroneفي المعاملة الثانية T2 عند مقارنتها مع معاملة السيطرة، في حين بينت نتائج الدراسة النسيجية وجود تغيرات نسيجية للخصية ومنها انحطاط ملحوظ للأنابيب المنوية مع عدم وجود خلايا مولده للنطف وانعدام تكوين الخلايا المنوية الناضجة وزيادة في عدد الحيوانات المنوية المشوهة بينما لوحظ في المعاملات التي اضيف لها المقيدات مع السموم الفطرية تحسن ملحوظ في التركيب النسيجي للخصيتين ومنها الانابيب المنوية وتكوين الحيوانات المنوية بشكل طبيعي .لذلك، توفر الدراسة رؤى مهمة حول تأثير الأفلاتوكسين ب1 والمواد الماصة الحيوية المختلفة على الجهاز التناسلي الذكري لدى الفئران.
الكلمات الافتتاحية: *Aspergillus flavus* ، الأفلاتوكسين، المعوقات البيولوجية، الجهاز التناسلي

INTRODUCTION

Aspergillus flavus is a widely distributed fungus that grows on plant and animal residues over a wide range of temperatures. It is estimated that 25% of grains are contaminated with fungal toxins as a result of poor harvesting and storage practices. In Iraq, four species of *Aspergillus* genus have been identified, including *A. flavus, A. fumigatus, A. niger, and A. parasiticus*, which are present in yellow corn) Sweany *et al.,* 2011). Aflatoxins are among the most important fungal toxins that cause poisoning, and their effects depend on the type of toxin, its concentration, the duration of animal exposure to the toxin, as well as the species, age, gender, and health status of the affected organism (Juliana et al., 2023). Fungal toxins cause mutations, suppress immunity, and damage the tissues involved in the production of sperm and ova, as well as causing significant deformities in embryonic tissues due to their effects on DNA and RNA, as well as damaging liver tissues (Rai & Varma, 2010). These toxins also affect hormone levels by influencing Sertoli and Leydig cells (Verma & Nair, 2002). Therefore, various strategies have been developed to reduce or eliminate the negative effects of fungal toxins, including toxin binding using zeolite, which has a high adsorption capacity and utilizes cation exchange processes due to the presence of numerous gaps in its crystalline structure, aiding in toxin binding and restriction, preventing aflatoxin absorption and its distribution to other parts of the body (Benjamin *et al.*, 2021). Another study

confirmed the use of Saccharomyces cerevisiae yeast to mitigate the negative effects of fungal toxins by adhering to the cell wall (Alexandros *et al.,* 2021), as well as containing b-glucan and mano-oligosaccharide (MOS) as major components of the yeast, which act as antioxidants (Tsai *et al.,* 2011). Vitamin E, with its high antioxidant activity, is also effective against the negative effects of fungal toxins, as it is abundantly available in nature and exhibits higher biological activity in protecting unsaturated fatty acids by breaking the chain of lipid oxidation reactions, inhibiting hydroxyl radicals, and preventing the formation of free radicals resulting from respiratory processes during exposure to fungal toxins (Ahmed *et al.,* 2015). Additionally, vitamin E acts as a modifier for negative tissue and hormonal changes caused by aflatoxin toxins (Verma & Nair, 2002). Therefore, the current study aimed to investigate the effect of aflatoxin B1 on sex hormones and testicular tissues in male rats and evaluate the effects of yeast, vitamin E, and zeolite as enhancers or binders of these toxins.

MATERIAL AND METHODS

The experiment was conducted at Animal House belonging to the College of Education for Women, University of Anbar, for two months, including a ten-day acclimation period. Aflatoxin B1 was extracted from yeast medium using the Jones (1972) method. Yeast medium was used to grow *Aspergillus flavus* isolates and test their ability to produce Aflatoxin B1 according to the method of Davis et al., 1966. The Vitamin E and the zeolite were obtained commercially and the according to (Ahmed et al., 2015 and Taş et al., 2007) was relied upon to calculate the concentrations used. AflatoxinB1 was also detected in yeast medium by using Thin Layers Chromatography (TLC) plates type TLC Silica gel 60 F254 according to the method of (Cocker et al., 1984).

The experiment was designed with six treatments and six replicates per treatment, with each rat considered as one replicate. The treatments were as follows: T1, control without any additives; T2, addition of 0.5 ml of aflatoxin B_1; T3, addition of 0.5 ml of aflatoxin B_1 + 80 g of yeast; T4, addition of 0.5 ml of aflatoxin B_1 + 1 ml of vitamin E; T5, addition of 0.5 ml of aflatoxin B_1 + 40 g of zeolite; T6, addition of 0.5 ml of aflatoxin B_1 + 80 g of yeast + 1 ml of vitamin E + 40 g of zeolite. Thirty-six male rats of Wistar strain weighing between 60-180 gm. were obtained and randomly distributed based on their body weight. The rats were individually weighed until reaching a uniform body weight for all treatments to ensure no significant differences in weight between the groups at the start of the experiment. The rats were placed in stainless steel cages with plastic clips and clean white wood shavings (1 cm thickness) as bedding material. The rats were kept under controlled conditions of light (12 hours light/12 hours dark) and a temperature of 22 ± 2 C°. The rats were acclimated for ten days to adapt to the experimental conditions. They were fed a standard diet according to Table 1 and provided water using plastic bottles, allowing the rats free access to food and water. The health status of the rats was monitored throughout the experiment.

Table 1. Components of diet used in the experiment

Item	gm/100gm
Starch	**60**
Protein concentrate	**18**
Sunflower oil	**12**
Minerals	**5**
Cellulose	**3**
Vitamins	**2**
Metabolizable energy	**4.460**

Measurement of hormone levels

The enzyme-linked Immunosorbent Assay (ELISA) method was used to estimate the levels of LH, FSH, and T hormones in the serum. The absorbance was read at a wavelength of 450 nm according to the method described by Wistom, 1976.

Preparation of tissue sections

After chloroform anaesthesia, the testes and prostate glands were excised, and the organs were cleaned with 90% NaCl solution and weighed using a sensitive electronic balance to determine the absolute weight. The organs were preserved in 10% formalin solution for 72 hours and then replaced with 70% ethanol until the preparation of histological slides. After the excision of the testis and prostate gland, the organs were weighed using a sensitive balance to calculate the relative weight of the testis relative to the body weight. Tissue sections of the testis were prepared using the Eosin-Hematoxylin staining method according to Titford, (2005).

Statistical analysis

Statistical computations were performed using the SAS software program. Duncan's multiple range test was used to compare means. The statistical model used was: $Yij = \mu + Ti + eij$. Where: Yij: dependent variable, μ: overall mean, Ti: effect of treatment, eij : error term.

RESULTS AND DISCUSSION

Table 2. Showed a significant decrease ($P \leq 0.05$) in testis weight was observed in the second treatment (T2) where aflatoxin B_1 toxins were added compared to the control treatment (T1). Additionally, a significant increase in weight was observed in treatments T3, T4, T5, and T6 compared to the control treatment.

Table 2: Effect of Aflatoxin B_1 and Some biological Determinants on testes and Prostate Weights

Treatments	Means ± Standard Error	
	Testes (gm.)	**Prostate (gm.)**
Control	3.22 ±0.09 ab	1.77 ±0.14 a
AFB1	2.95 ±0.13 b	0.47 ±0.18 b
AFB1+Yeast	3.27 ±0.17 ab	1.27 ±0.08 a
AFB1+Zeolite	3.42 ±0.18 a	1.40 ±0.07 a
AFB1+Vit E	3.95 ±0.12 a	1.15 ±0.06 a
AFB1+Yeast+Zeolite+Vit. E	3.45 ±0.12 a	1.40 ±0.13 a
Level of Sig.	*	**

This means having the different letters in the same column differed significantly, * (P≤0.05), ** (P≤0.01).

Similarly, from the same table, a highly significant decrease (P≤0.01) in prostate weight was observed in the treatment with added aflatoxin B_1 toxins, while a clear increase in weight was observed in treatments with additives (yeast, zeolite, and vitamin E) compared to the control treatment. The decrease in reproductive organ weights may be attributed to the suppressive effect of toxins, particularly sex hormones secreted by the testes. The role of prolactin was also observed in increasing the receptors of luteinizing hormone, which stimulates the synthesis of testosterone and increases the necessary enzymes for androgen synthesis, leading to sperm formation and increased numbers. As for the reason behind the weight increase in treatments with additives, it may be attributed to the role of these additives in inhibiting the negative effects of aflatoxin toxins and their strong antioxidant properties, which support the growth and development of reproductive organs. Additionally, these additives can be absorbed or bound to the intestinal wall and subsequently eliminated from the body, reducing their negative effects on reproductive organs. Furthermore, an increase in the activity of enzymes such as Creatine Phosphokinase and Lactate Dehydrogenase was observed, which ultimately leads to an increase in the availability of nutrients in the digestive tract, improving the weight gain of the animal's organs, including reproductive organs. The additives also help maintain intestinal mucous membranes and support the increase in villus height by enhancing the absorption of digested nutrients, resulting in optimal growth of body organs. Table 2 demonstrates a highly significant increase (P≤0.01) in FSH hormone in the second treatment (T2) where aflatoxin B_1 toxin was added, compared to other experimental treatments. However, no significant difference (P≤0.01) was observed between treatments T3 and T4 or T1 and T6.

The table also demonstrates a significant decrease (P≤0.01) in LH hormone values in the second treatment (T2) as compared to the control treatment and the sixth treatment, while no significant differences (P≤0.01) were observed between treatments T2, T3, T4, and T5. Additionally, a significant decrease in testosterone hormone concentration was observed in the second treatment compared to other experimental treatments, and no significant difference was observed between T4, T5, and T1, nor between T1 and T6. The decrease in FSH hormone levels in serum after adding aflatoxin is attributed to a decrease in the size and thickness of the germinal epithelium of seminiferous tubules, leading to testicular fibrosis and a decrease in sperm production. Since the FSH hormone, is secreted by the anterior pituitary gland, which is

responsible for sperm synthesis, the negative feedback will result in increased secretion of FSH to ensure normal sperm production (Hasanzadeh *et al.*, 2011).

Table 3: Effect of aflatoxin B_1 and some biological determinants on the concentration of Hormones (LH, FSH, Testosterone)

Treatments	Means ± Standard Error		
	FSH (µIu/ml)	LH (µIu/ml)	Testosterone(ng/ml)
Control	0. 740 ±0.01 c	0.741 ±0.10 a	2.42 ±0.09 a
AFB_1	0.678 ±0.04 a	0.301 ±0.04 b	0.851 ±0.02 d
AFB_1+Yeast	0.537 ±0.05 b	0.245 ±0.03 b	1.302 ±0.03 c
AFB_1+Zeolite	0.564 ±0.04 b	0.284 ±0.05 b	1.537 ±0.02 b
AFB_1+Vit E	0.601 ±0.04 ab	0.308 ±0.01 b	1.655 ±0.01 b
AFB_1+Yeast+Zeolite+Vit. E	0. 720 ±0.01 c	0.720 ±0.12 a	2.54 ±0.06 a
Level of Sig.	**	**	**

This means having the different letters in the same column differed significantly, ** ($P \leq 0.01$).

The decrease in testosterone hormone in blood serum may be due to the severe damage caused by mycotoxins to Leydig and Sertoli cells, resulting from the sharp decrease in spermatogonial cells, which can lead to a severe disruption in the function of cells (Carreau, 2001). Furthermore, a decrease in LH hormone in serum may be attributed to the effect of aflatoxin in damaging sperm-generating cells and leading to reduced sperm production. Since there is a direct correlation between sperm production and LH secretion, it is natural that a decrease in sperm production is followed by a decrease in the secretion of hormones from the anterior pituitary gland, which plays a significant role in the production of mature sperm (White *et al.*, 2002; Tajuddin *et al*., 2003)

The return of all hormones to their normal levels may be attributed to the active role of additives (yeast, zeolite, vitamin E) in eliminating the negative effects caused by mycotoxins, especially aflatoxin.

Histological test of testes

Microscopic examination of testes in Fig.1 revealed at a magnification of 10x and 40x, in the control treatment, normal stages of sperm formation, as the histological structure of seminiferous tubules was undamaged and exhibited normal formation, containing a series of undistorted sperm, especially mature ones, in addition to abundance and density of interstitial cells (Leydig and Sertoli cells).

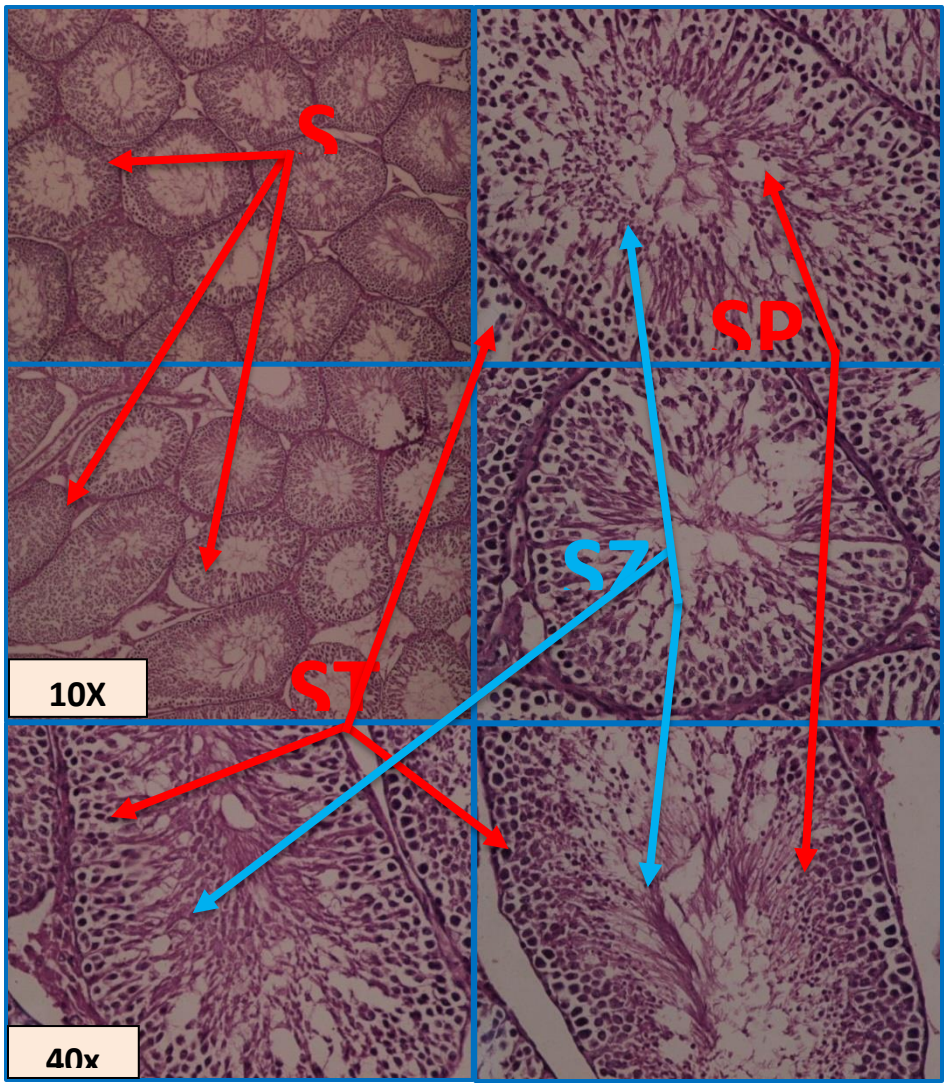

Figure 1: Testis of control rats showing the normal histological structure of active mature functioning seminiferous tubules (S) associated with complete spermatogenic series. The peripheral layer of cells is composed of spermatocytes (SP) followed by a zone of spermatids (ST) and finally spermatozoa (SZ) about to be released into the lumen (H&E).

The study finding revealed that use of aflatoxins B1 toxicity has a negative effect on the histological structure of the male testids, the one that is responsible for the production of sperm and testosterone. They damage to the testicular tissues can disrupt normal reproductive function in the following are some of the effects on the tissues of the male testicals as in Figuer (2) is defect in the tissues that many lead to disruption of the seminiferous tubules responsible for sperm production. That can lead to damage to supporting cell within the tubules and loss of structure safety and this has to do with the hormones imbalance shown in chemical result, including testosterone (Hasanzadeh et . al , 2012).Tissues changes are one of the effects of taking the aflatoxins B1 toxicity side effects of this toxic include abnormalities in the sperm and a difference in the hormones concentration, antioxidant improved drug induced testicular damage in adults male rats that by reducing oxidative stress and testosterone levels (Faicsal et.al, 2008).The results in fig (2) also showed that mycotoxin alone resulted in significant degradation of interstitial tissue

as evidenced by the formation of allvlar vacuoles and tissue bleeding. The degradation of testicular tissue specimen may be due to residual accumulation of fluid and cytoplasm retention in the lumen of some testicular tubules, sertoli cells atrophy directly manifests decreased testosterone levels and leydig cells atrophy mduced by mycotoxin specifically aflatoxins B1(Alia et.al,2019). The bigger rate of germ cell apoptosis and the result in sterility has been attributed to the atrophy of sertoli cell .The biological restraints of vitamin E can restore the typical structure of the seminiferous tubules of the testis and the interstitial spaces (Ahmed et.al,2015).

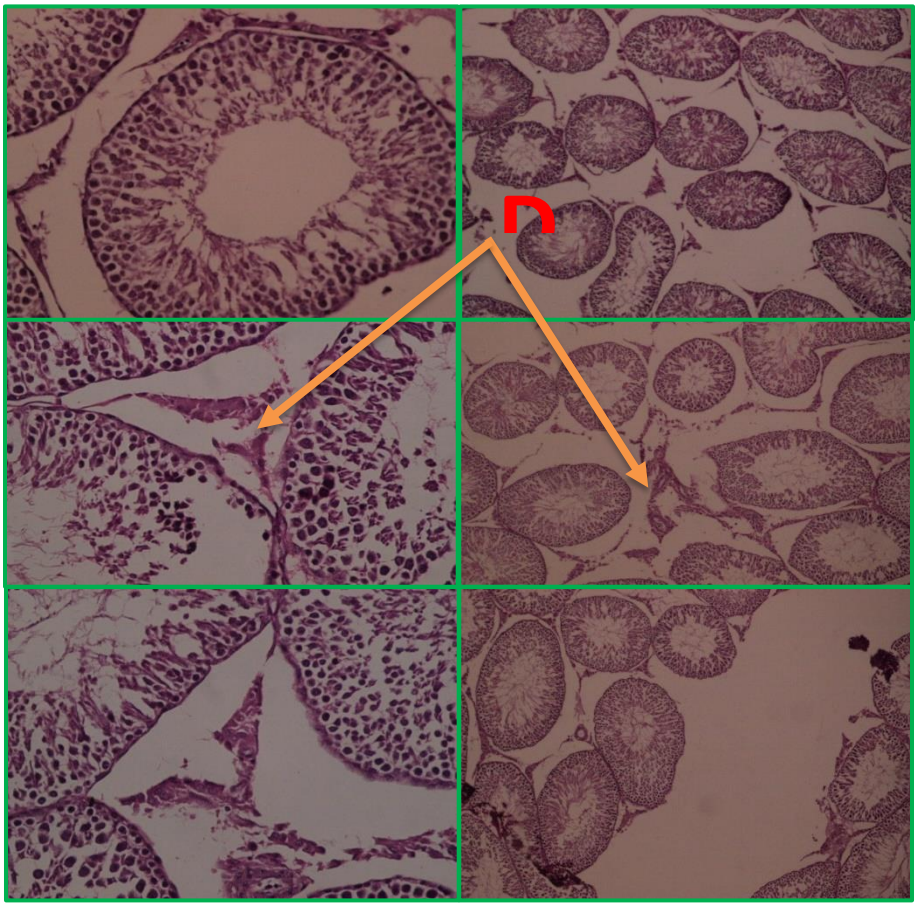

Figure 2: Testis of (Aflatoxin B) treated rats, showing marked degeneration (d) of most seminiferous tubules with the absence of spermatogenic series in the tubular lumen. The arrow indicates mild disintegration of the seminiferous tubules with loss of spermatids and spermatozoa, marked degeneration (arrow) of most spermatocytes, spermatids and congestion in the testis blood vessels (H&E).

The biological restraints of vitamin E can restor the typical structure of the seminiferous tubules of the testis and the interstitial spaces (Ahmed et.al, 2015). Where The group treated with (yeast , zeolite , vitamin E) showed increase in the number of primary and secondary spermatogonia cells and sperm blasts as they appeared in the form of layers showing all stages of sperm and fully mature sperm and appeared density in the within the lumen of the seminiferous tubules compared to the control group (Figure 3,4,5,6).

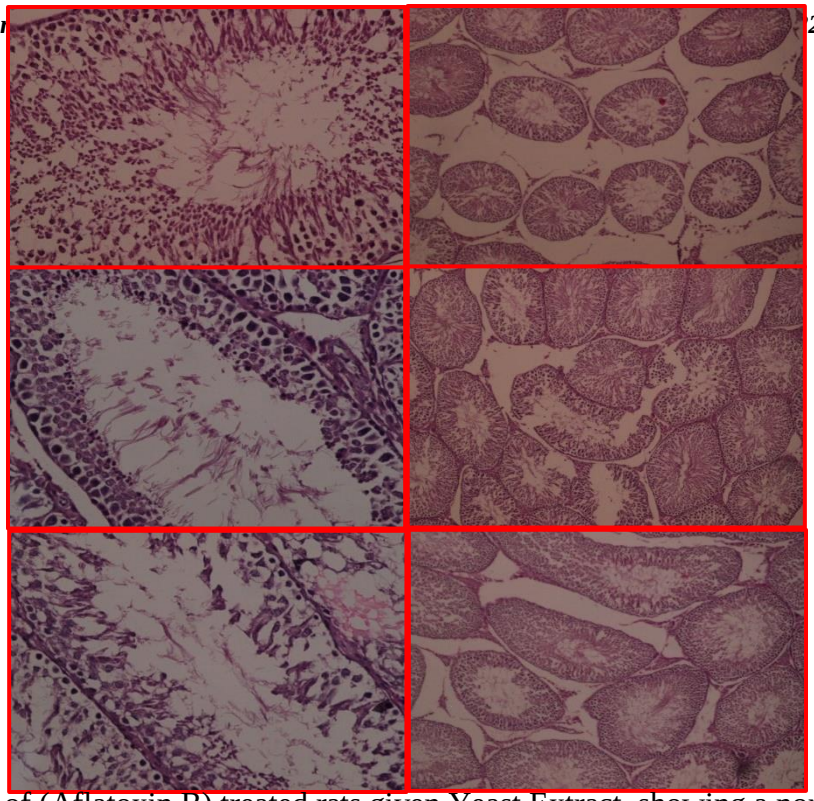

Figure 3: Testis of (Aflatoxin B) treated rats given Yeast Extract, showing a normal histological structure of most seminiferous tubules (H&E).

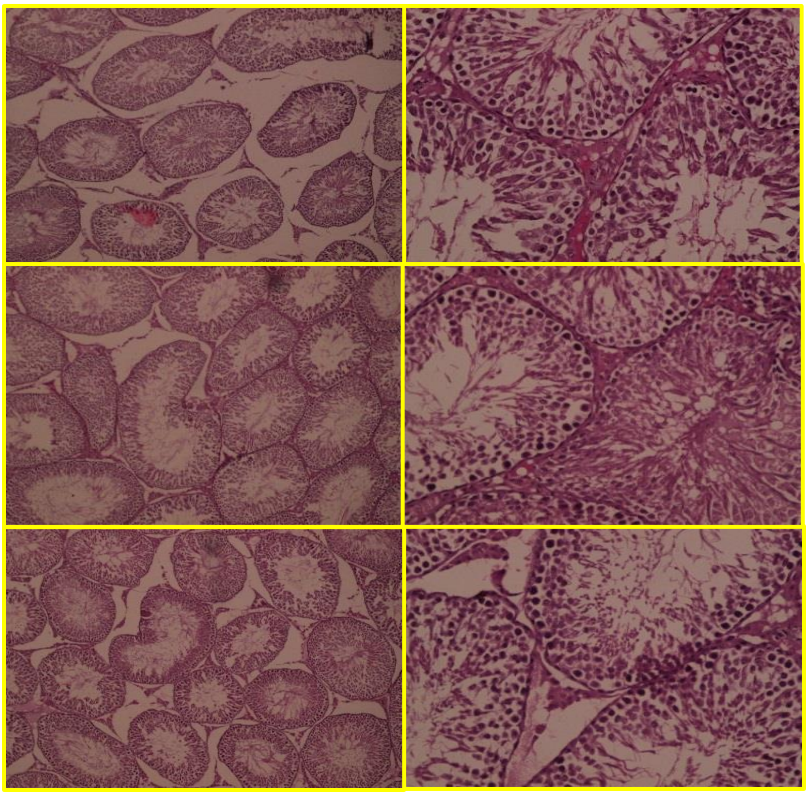

Figure 4: Testis of (Aflatoxin B) treated rats given Ziolet Extract, showing a normal histological structure of most seminiferous tubules (H&E).

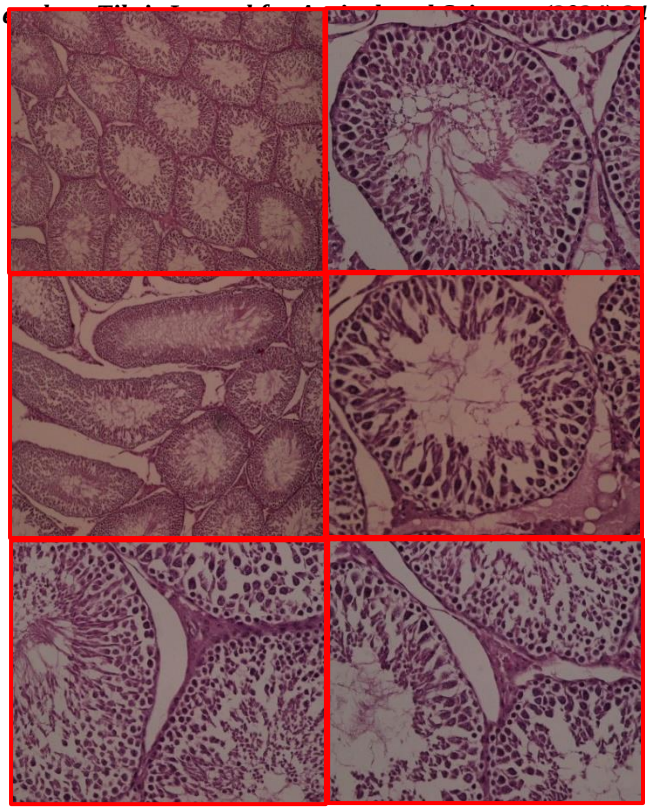

Figure 5: Testis of (Aflatoxin B) treated rats given Vit E, showing a normal histological structure of most seminiferous tubules (H&E).

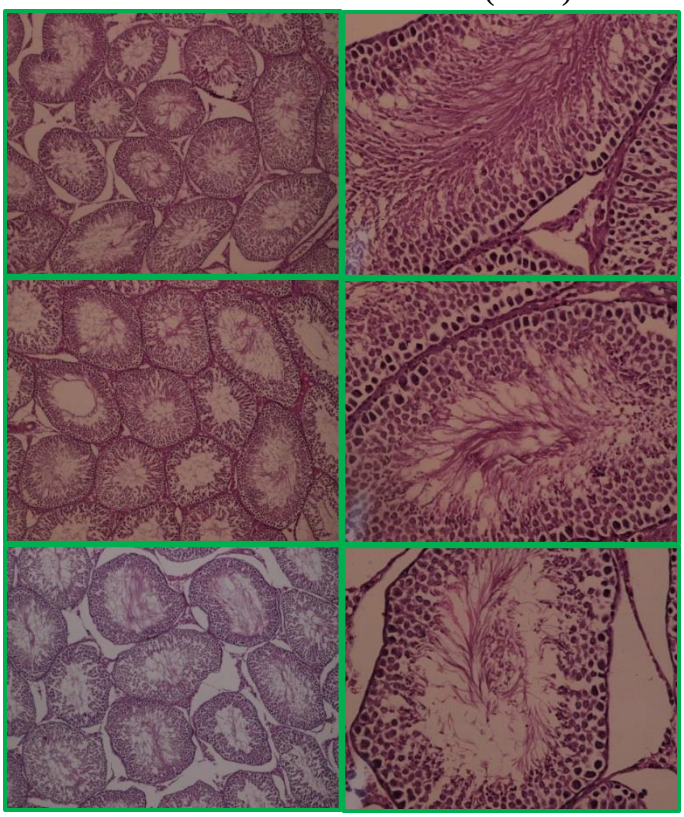

Figure 6: Testis of (Aflatoxin B) treated rats given (Yeast+Ziolet.+Vit E.), showing a normal histological structure of most seminiferous tubules (H&E).

CONCLUSION

These findings indicate that the groups received the adsorbents (yeast, zeolite, and vitamin E) with mycotoxins, a significant improvement in the histological structure of testes, including seminiferous tubules and the formation of spermatozoa, similar to the control group. Thus, the study provides a comprehensive understanding of the detrimental effects of aflatoxin B1 on the male reproductive system and demonstrates the limited potential of bioadsorbents in offering protection. Further investigations are warranted to enhance protective measures and explore more effective solutions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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