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Histological and immunohistochemical assessment of testicular and epididymal changes in albino male rats following exposure to the pimaricin

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***Abstract*— Background Pimaricin (natamycin), a polyene antifungal agent commonly used in food preservation and pharmaceuticals, has raised concerns regarding its systemic toxicity, including potential reproductive effects. Objectives: This study aim to investigate the histological, immunohistochemical, and induced by pimaricin exposure in the testis and epididymis of albino male rats. Material and methods: Twenty adult Wistar albino rats were divided into control and treated groups. The treated group received pimaricin orally at a dose of 0.3 mg/kg/day for 50 consecutive days, while the control group received distilled water. At the end of the experiment, serum samples were collected for hormonal analysis of (LH), and (FSH). Testicular and epididymal tissues were processed for histological evaluation using as well as immunohistochemical staining (MDA). Results Histological examination revealed degenerative & necrosis of spermatic cells with few Leydig cells as well as congestion of interstitial blood vessels accompanied with atrophy of seminiferous tubules with severe increase in interstitial spaces & complete absence of tubules were recorded. The section of epididymis showed intense hyalinization of epididymal lumen. Conclusion: According to our findings, immunohistochemical examination showed that the testis and the epithelial lining the epididymal ducts is showing moderate nuclear and cytoplasmic positivity staining for MDA and control group had mild and mild nuclear and cytoplasmic MDA expression .**

Keywords: **Immunohistochemistry, Pimaricin-,Testicular Tissues, Albino Male Rats, epididymis**

INTRODUCTION

THE Food preservatives are used extensively in the food and pharmaceutical industries, raising serious concerns about their safety. The antifungal and antibacterial qualities of pimaricin make them popular preservatives. A naturally occurring antifungal substance, pimaricin (PIM), commonly referred to as natamycin (E235), is frequently employed as a food preservative, particularly in cheese and other dairy products (1). While its usefulness in food preservation is well-established, the potential influence of pimaricin on male reproductive health, especially testicular tissue, remains completely unexplained (2). The initial toxicological analysis of natamycin in rats, mice, and guinea pigs was published in 1957. According to the research, guinea pigs' natamycin LD50 was 450 mg/kg (3) . Male rabbits with male and female rats were the subjects of another study on acute oral toxicity. Following oral treatment, natamycin's LD50 values for male rats, female rats, and male rabbits were 2.73 g/kg, 4.67 g/kg, and 1.42 g/kg, respectively.(4)

Pimaricin, generated by Streptomyces natalensis, is a polyene macrolide antifungal that functions by attaching to ergosterol in fungal cell membranes, causing cell death. (5) Its usage as additive in foos has been permitted by regulatory bodies across the world. However, safety assessments of food additives frequently focus on broad toxicity and carcinogenicity, with less emphasis on specific organ systems such as the male reproductive tract.

The testis and epididymis are essential components of the male reproductive system, responsible for spermatogenesis and sperm maturation, respectively. These organs are highly sensitive to hormonal regulation and toxic insults, which may compromise their histological architecture and physiological function (6). Hormones such as testosterone and follicle-stimulating hormone (FSH) play pivotal roles in maintaining the structural integrity and functional competence of the seminiferous epithelium and accessory reproductive structures (7). Exogenous exposure to pharmacological agents in the presence of hormonal modulation may either exacerbate or

mitigate the observed tissue alterations, depending on the nature and dosage of the compound (8).

The male reproductive system, including the testes, is known to be exposed to many environmental toxicants and chemical exposures. (9) Testicular tissue, responsible for spermatogenesis and hormone production, can be adversely affected by xenobiotics, leading to impaired fertility and hormonal imbalances (10). While studies have investigated the effects of various food additives on testicular function, research specifically addressing the impact of pimaricin on testicular tissue is limited .

The current study's objective is evaluate the changes histomorphological Testicular tissues and Epididymis after exposure to spimaricin. particularly in the context of hormonal influence. Degeneration of Seminiferous Tubules. Disruption of Spermatogenesis. Alterations in Leydig Cells. Interstitial Edema and To analyze the expression of specific biomarkers related to oxidative stress, and inflammation in testicular tissues and epididymis.II. Materials and Methods

A total of one hundred sixty (160) at one-day-old unsexed chicks of the Rose 308 breed were purchased from local hatchery. The birds were fed on basil diet for two-week (14 days), at day 15 of the experiment the bird was randomly divided into 4 groups each group contain forty (40) birds, and each group was subdivided into two groups, each group contain 20 birds, in a sector design. The control group (T) was fed on the basil diet, while the birds in group T1, T2, and T3 were fed 10%, 20%, and 30% of the rice were given by replacing corn in the food ration as shown in table (1), After the end of the experiment, the animals were sacrificed, and intestinal samples were taken and preserved with 10% formalin. Histological sectioning was performed according to (10) and intestine samples were measured using a graduated lens for measuring villi length, villi width, crypt depth, muscular thickness.

MATERIALS & METHODS

EXPERIMENTAL DESIGN

Twenty male (20) albino rats were housed in an animal home for fifty days to get used to the conditions of the laboratory The animals obtained From the animal house of the college of pharmacy at Karbala University, twenty healthy adult male Wistar rats weighing between 800 and 900 g were acquired. two months old. They were housed at the veterinary medical college's animal house at Karbala University. The temperature was kept at 21 to 25 degrees Celsius, the air in the room was continuously changed using a ventilation vacuum, and the animals were given a newly feed pellet for 12 hours every day.

THE EXPERIMENT PROTOCOL

Twenty rats each were divided into tow groups. Group one was used as the control group. Group two Treated at dose of oral intubation daily0.3 mg /kg BW of pimaricin for 60 days. After completing the experimental period each animal was sacrificed .

TISSUE HISTOPATH

Rats will be killed at the end of the exposure period, and their testes will be taken for histological examination. After removing the testes right away, the adhering fat and connective tissues were removed and the testes were cleaned with a cooled saline solution (0.9%). Rat testicles were extracted, fixed with 10% formalin right away, treated with xylol and regular grade alcohol, embedded in paraffin, and then sectioned. Haematoxylin and Eosin (H&E) stain was applied to the sections (11)Periodic acid-Schiff (PAS) (12) Masson trichrome stain (13) Immunohistochemistry (14) (15)

IMMUNOHISTOCHEMISTRY

SDCBP Polyclonal Antibody (MDA) immunohistochemistry was carried out on tissue blocks that were embedded in formalin-fixed paraffin in each instance. thin, 5 mm tissue slices. After blocking the sections, the primary antibody (E-AB-17209) was added and incubated for two hours at 37°C. Add the goat anti-MOUSE linker and rabbit antibody, wash, and then add the secondary antibody using the enzyme (horserdash peroxides) after 30 minutes. Following the manufacturer's instructions, further procedures were carried out using the Immunohistochemistry Kit (Elabasxience, USA). Only negative controls were sections that were treated with phosphate-buffered saline (PBS). (14,15)

DETERMINATION OF REPRODUCTIVE HORMONES

2 LH levels were measured using an ELISA kit, following the method described by (16). Streptavidin -coated wells were used to capture a complex formed by excess biotinylated monoclonal anti-LH antibodies and enzyme-labeled LH antibodies. The sandwich complex, involving the LH antigen, was bound to the plate via streptavidin interaction. Tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) served as substrates for the enzyme reaction, which generated a color change proportional to LH concentration. Absorbance was recorded at 450 nm, with 620 nm as a reference, using a Tecan Infinite F50 microplate reader (Tecan Group, Switzerland).

ETHICAL APPROVE

This investigation was conducted in the anatomical facility of the University of Karbala's College of Veterinary Medicine under reference number UOK.VET. AN. 2024.092

RESULT & DISCUSSION

Testicular sections from the control group showed no histopathological alterations and a normal histological structure (fig:1,2,3) There was degenerative & necrosis of spermatic cells with few Leydig cells interstitial as well as congestion of interstitial blood vessels (fig:4) accompanied with atrophy of seminiferous tubules with severe increase in interstitial spaces & complete absence of tubules were recorded (fig:5) The section of epididymis showed intense hyalinization of epididymal lumen (fig:6) Other observation revealed thrombus of B.V with fibrin network & large number of degenerative sperm (fig:7),The main testicular finding revealed sever increase thickness of basement membrane that gave PAS positive substance and stained purplish color (fig:8), Obviously basement membrane give sever PAS substance reaction and stained more purplish while intact thin apical PAS reaction in the lining epithelial cells (fig:9)The main testicular finding

showed sever increase fibrosis that stained blue color were recorded in tunica Albuginea (fig:10).Recorded increase collagen fiber surrounding the epididymal ducts that stained blue color (fig:11), while another findings revealed strong reaction of tunica Albuginea that stained blue color(fig:12)

IMMUNOHISTOCHEMISTRY STUDY OF EXPERIMENTAL ANIMAL WITH MDA STAIN

Immunohistochemistry analysis of MDA in formalin fixed testis and cauda epididymis sections of normal control rats at 50 days of experiment. Representative photomicrographs demonstrate the presence of MDA immunoexpression in the spermatogenic, Sertoli cells in the seminiferous tubules& Leydig cells in the interstitial tissue. (fig:13,14) The Pimaricin group showed moderate (MDA) immunoexpression was observed in the seminiferous tubules ,spermatogenic cells and Sertoli cells & Leydig cells in the interstitial tissue (fig:15)The Epididymis section showed moderate MDA immunoreaction stained nuclear and cytoplasmic in the epithelial cells lining of the epididymal ducts (fig:16)

The current study showed that the level of the hormone LH in the control group and the group pimaricim in rat dosed with the substance on day zero did not show a significant difference from the control group. However, after 20 days of dosing, it showed a significant low difference from the control group, and after 50 days of the experiment, it showed a low decreased difference from the control group. On the other hand, the study showed that there was a significant decrease in the hormone level on day 50 compared to day 20 of the experiment. (fig:17)

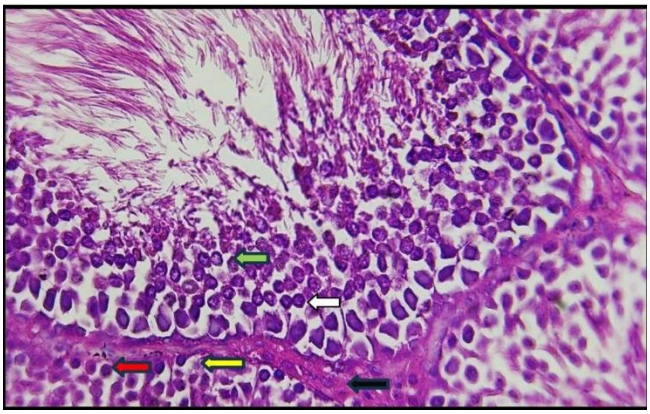

Figure 1.histological section testis of (control group) showed seminiferous tubules lined with series of spermatogenic cells, spermatogonia (yellow arrow), primary spermatocytes (white arrow) and round spermatids (green arrow) with Sertoli cells (red arrow) with attached sperms & interstitial cell of Leydig (black arrow) (H and E stain X40)

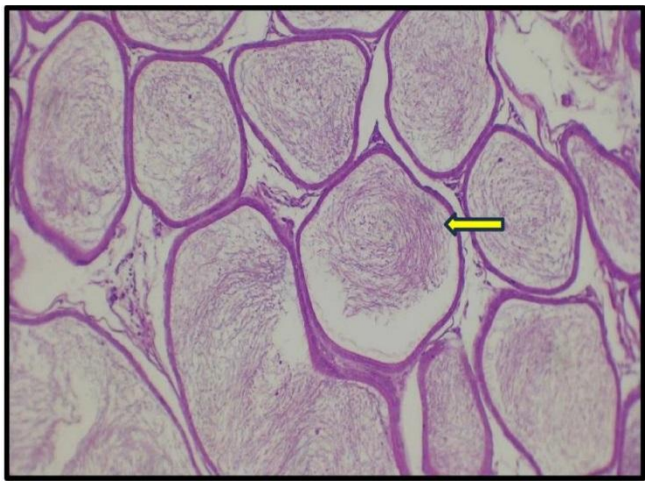

Figure 2. histological section in the epididymis of control group at 50 days post exposed showed Epididymal tubule lined by ciliated pseudostratified columnar epithelium with numerous sperms in the lumen with no pathological lesion(H&E stain X10)

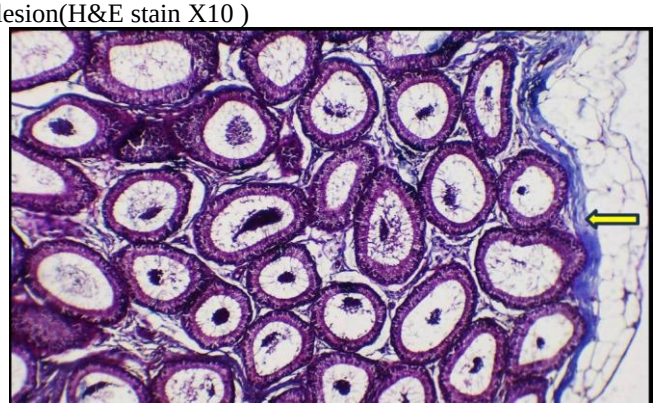

Figure 3.histological section in the epididymis of control group showed masson trichrome +ve for tunica Albuginea that stained blue color (yellow arrow) (masson trichrome stain X10)

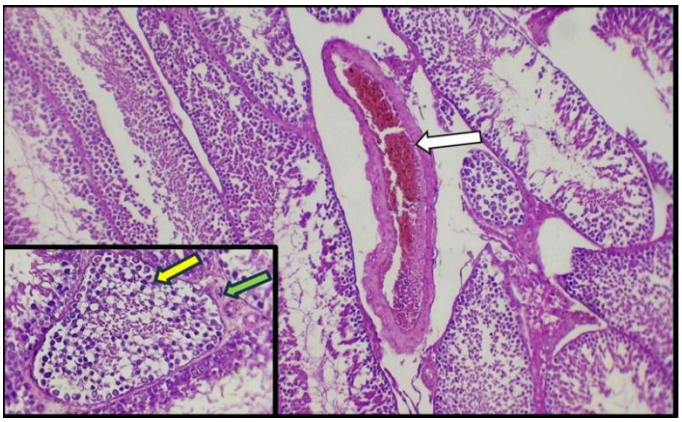

Figure 4.histological section of pimaricin group in the testis at 50 days post exposed showed degenerative & necrosis of spermatic cells (yellow arrow) with few Leydig cells interstitial (green arrow) & congestion of interstitial blood vessels (white arrow) (H&E stain X10+40)

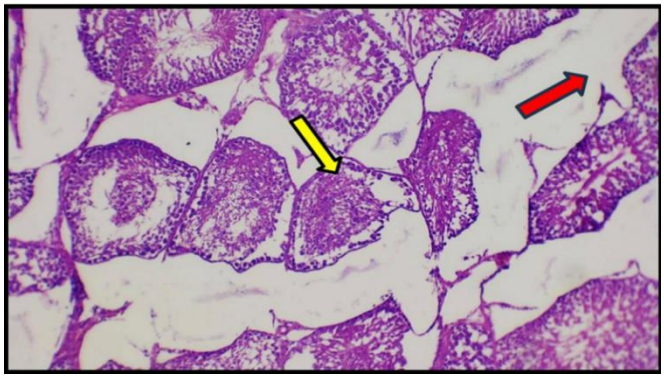

Figure 5. histological section in the testis of pimaricin group at 50 days post exposed showed seminiferous tubules atrophy with severe widening in interstitial spaces (yellow arrow)& complete absence of tubules (red arrow)(H&E stain X10)

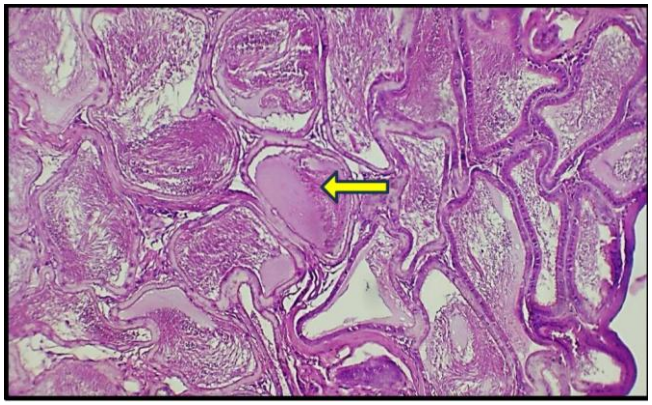

Figure 6. histological section in the of epididymis pimaricin group at 50 days post exposed showed hyalinization of epididymal lumen (yellow arrow) (H&E stain X10)

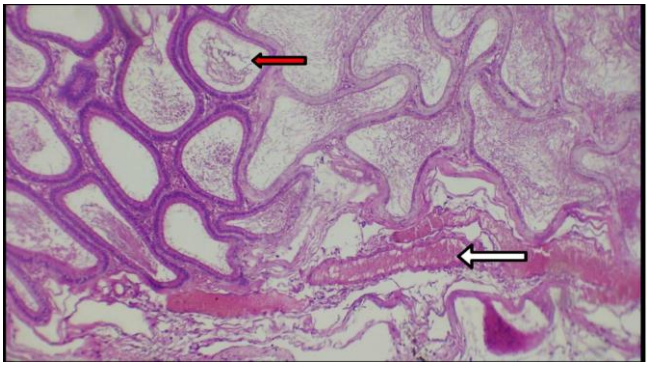

Figure 7. histological section in the of epididymis pimaricin group at 50 days post exposed showed vascular congestion (white arrow) & large number of degenerative sperm(red arrow) (H&E stain X10)

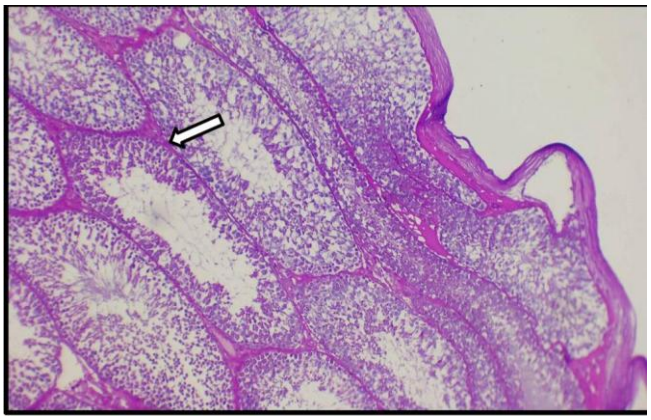

Figure 8. histological section in the testis of pimaricin group at 50 days post exposed showed sever increase thickness of basement membrane (white arrow)(PAS stain X10)

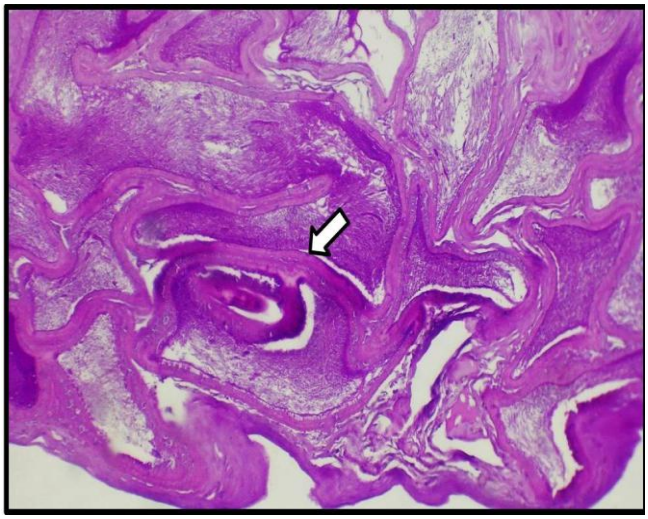

Figure 9. histological section in the epididymis of pimaricin group at 50 days post exposed showed sever increase thickness of basement membrane with intact thin apical PAS reaction in the lining epithelial cells white arrow) (PAS stain X10)

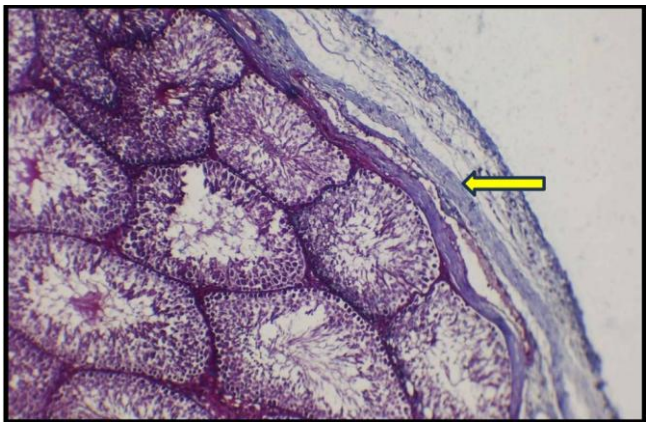

Figure 10. histological section in the testis of pimaricin group at 50 days post exposed showed sever increase thickness of tunica Albuginea due to fibrosis that stained blue color (yellow arrow)(Masson trichrome stain X10)

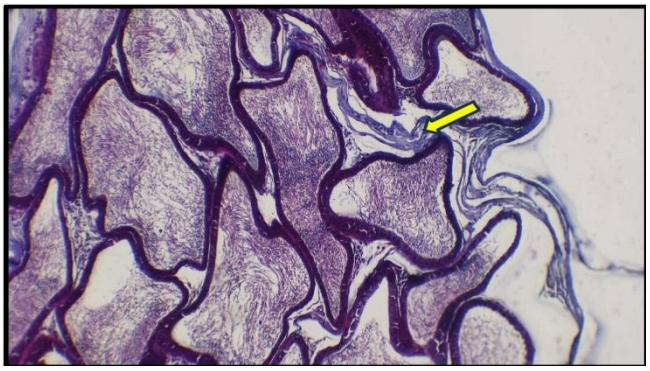

Figure 11. histological section of pimaricin group in the epididymis at 50 days post exposed showed sever increase collagen fibers surrounding the epididymal ducts that stained blue color (yellow arrow) (Masson trichrome stain X10)

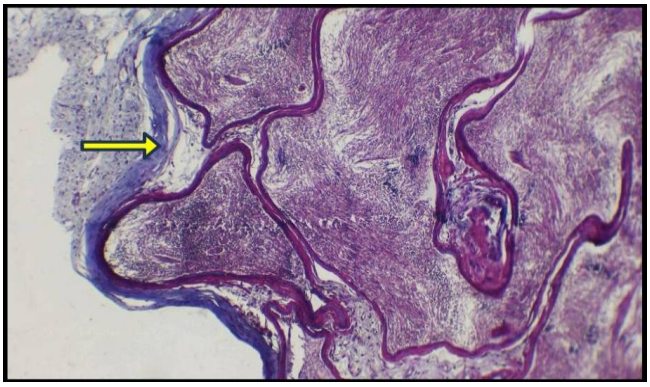

Figure 12. histological section in the epididymis of pimaricin group at 50 days post exposed showed sever increase fibrosis in tunica Albuginea that stained blue color (yellow arrow) (Masson trichrome stain X10)

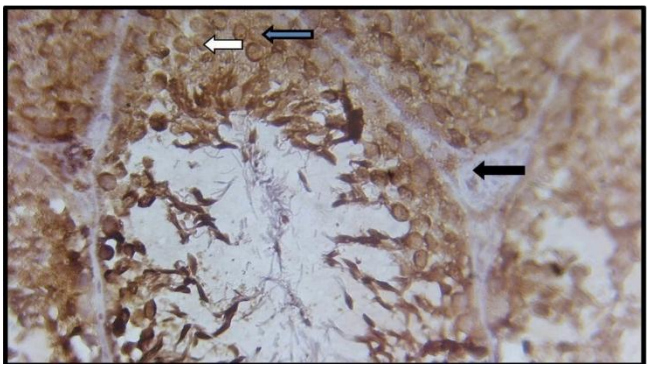

Figure 13. Immunohistochemistry analysis of MDA in formalin fixed testis sections of normal control rats at 50 days of experiment. Representative photomicrographs demonstrate the presence of MDA immunoexpression in the spermatogenic (white arrow) , Sertoli cells (blue arrow)in the seminiferous tubules& Leydig cells in the interstitial tissue (black arrow) (MDA immunostaining X40)

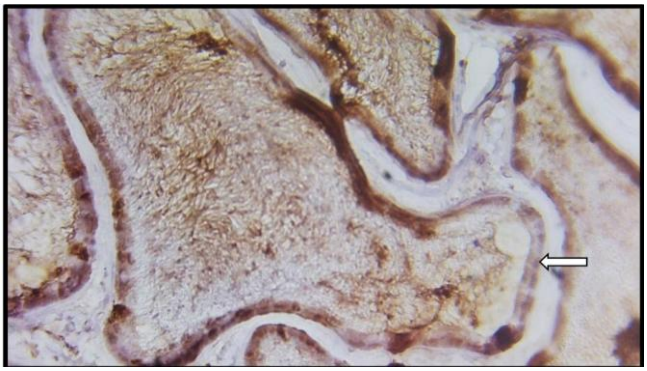

Figure 14. Immunohistochemistry analysis of MDA in formalin fixed cauda epididymis sections of normal control rats at 50 days of experiment. Representative photomicrographs demonstrate the presence of MDA in the nuclear and cytoplasm of the epithelial cells lining the epididymal ducts (white arrow). (MDA immunostaining X40).

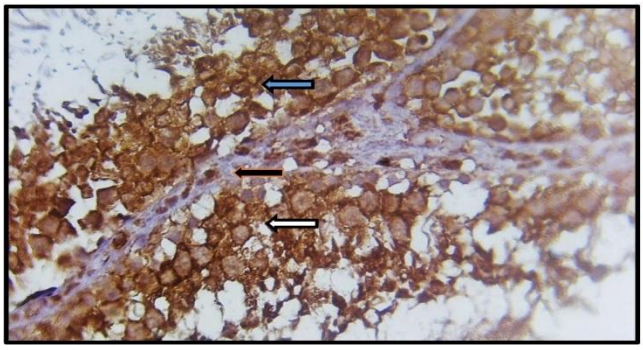

Figure 15. Immunohistochemistry analysis of MDA in formalin fixed testes of pimaricin group at 50 days of experiment. Representative photomicrographs showing moderate (MDA) immunoexpression in the spermatogenic (white arrow) and Sertoli cells (blue arrow) in the seminiferous tubules& Leydig cells in the interstitial tissue (black arrow) (MDA immunostaining X40)

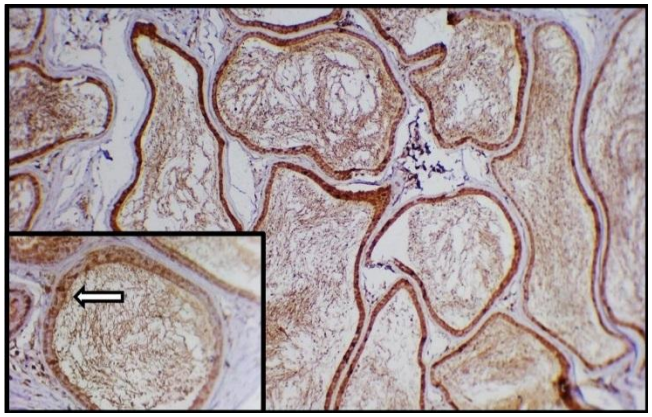

Figure 16. Immunohistochemistry analysis of MDA in formalin fixed cauda epididymis of pimaricin group at 50 days of experiment. Representative photomicrographs showing moderate nuclear and cytoplasmic MDA immunoreaction in the epithelial cells lining the epididymal ducts (white arrow) (MDA immunostaining 10+X40)

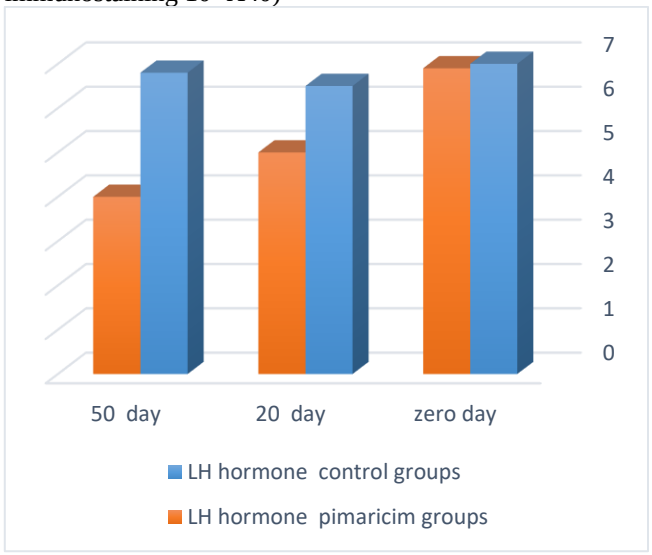

Figure 17. showed that hormonal changes in the LH a significant decrease in experimental animals on the fiftieth day compared to the twentieth day and zero day in control groups respectively. On the other hand, the current study found that there was a significant difference in the pimaricim in the dosage period of 20 days and 50 days.

The present study aim to evaluate the histopathological and immunohistochemical effects of dietary exposure to pimaricin (natamycin) on the testicular and epididymal tissues of albino male rats.

Pimaricin, a polyene macrolide antifungal agent wide used in the food and pharmaceutical industries(17), been generally regarded as safe due to its poor gastrointestinal absorption(18). However, the chronic effects of low-dose systemic exposure, particularly on reproductive organs, remain poorly understood. Our findings demonstrate that prolonged intake of pimaricin-containing food induces significant degenerative changes in the testis and epididymis, suggesting a potential risk to male fertility.

These changes are similar to those reported in other toxicological studies, where exposure to environmental toxins led to similar testicular dysfunction. For example, studies by (19) showed that testicular toxicity, resulting in structural disruption of seminiferous tubules and impaired spermatogenesis, is a common outcome of exposure to various chemicals and environmental pollutants

The main testicular finding showed sever increase fibrosis that stained blue color were recorded in tunica Albuginea and surrounding the epididymal ducts this result agreement with finding of while another findings revealed strong reaction of tunica Albuginea that stained blue color Sertoli cells (20) how examinant Monosodium Glutamate on Epididymis of Adult Male Albino Rats the seminiferous tubules were filled with connective tissue containing groups of Leydig cells, blood vessels and sever increase collagen fibers surrounding the epididymal ducts and tunica albuginea that stained blue color were stained blue by masson trichrome stain. The same result were reported by (21),they showed interstitial cells of Leydig were rounded or polygonal in shape with acidophilic cytoplasm and rounded or oval vesicular nuclei. n the present study, the control group Immunohistochemistry analysis of MDA in the testis and epididymis section of control group at 50 days post exposed showed nuclear and cytoplasmic immunoreaction is present of MDA in germ cells & Sertoli cells in seminiferous ducts.

The epithelial lining of the epididymal ducts had a strong, exclusively nuclear immunoreaction in the mode of of brown coloring in AR-immunostained sections from the control group. However, the epithelial cells lining the epididymal ducts in The pimaricin group showed a high nuclear androgen receptor immunoreaction. According to our findings, immunohistochemical examination showed that the testis and the epithelial lining the epididymal ducts is showing moderate nuclear and cytoplasmic positivity staining for MDA and control group had significant mild nuclear and cytoplasmic MDA expression(22). reported TCS-treated group showed an apparently present nuclear immunoreaction in the epithelial lining of the epididymal ducts, in addition to some focal cytoplasmic positive immunoreaction (23).

CONCLUSION

According to this study, pimaricin caused tissue damage in the testis and epididymis, including cell degeneration and increased fibrosis. These results suggest a possible negative impact on male fertility and highlight the need for more research and cautious use of these substances

REFERENCES

1) Cinar A, Onbaşı E. Mycotoxins: The hidden danger in foods. Mycotoxins food Saf. 2019;1–21.DOI: 10.5772/intechopen.89001
2) Silva MM, Lidon FC. Food preservatives-An overview on applications and side effects. Emirates J Food Agric. 2016;28(6):366. doi: 10.9755/ejfa.2016-04-351

3) STRUYK AP, HOETTE I, DROST G, WAISVISZ JM, VAN EEK T, HOOGERHEIDE JC. Pimaricin, a new antifungal antibiotic. Antibiot Annu. 5:878–85. Book: Antibiot. Ann. 1957-1958, 1958, 878-885 pp.
4) Levinskas GJ, Ribelin WE, Shaffer CB. Acute and chronic toxicity of pimaricin. Toxicol Appl Pharmacol. 1966 Jan;8(1):97–109. https://doi.org/10.1016/0041-008X(66)90105-0
5) Hess RA, De Franca LR. Spermatogenesis and cycle of the seminiferous epithelium. Mol Mech Spermatogenes. 2009;1–15. DOI: 10.1007/978-0-387-09597-4_1
6) Walker WH. Non-classical actions of testosterone and spermatogenesis. Philos Trans R Soc B Biol Sci. 2010;365(1546):1557–69. doi:10.1098/rstb.2009.0258
7) Sharpe RM. Environmental/lifestyle effects on spermatogenesis. Philos Trans R Soc B Biol Sci. 2010;365(1546):1697–712. doi:10.1098/rstb.2009.0206
8) Te Welscher YM, Ten Napel HH, Balagué MM, Souza CM, Riezman H, De Kruijff B, et al. Natamycin blocks fungal growth by binding specifically to ergosterol without permeabilizing the membrane. J Biol Chem. 2008;283(10):6393–401. DOI 10.1074/jbc.M707821200
9) Creasy DM. Pathogenesis of male reproductive toxicity. Toxicol Pathol. 2001;29(1):64–76. DOI: 10.1080/019262301301418865
10) Mathur PP, D'cruz SC. The effect of environmental contaminants on testicular function. Asian J Androl. 2011;13(4):585. doi:10.1038/aja.2011.40; published online 27 June 2011
11) Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier health sciences; 2008. book
12) RD H. A microchemical reaction resulting in the staining of polysaccharide structure in fixed tissue preparations. Arch Biochem. 1948;16:131–41. PMID: 18921848
13) Foot NC. The Masson trichrome staining methods in routine laboratory use. Stain Technol. 1933;8(3):101–10. doi.org/10.3109/10520293309116112
14) Paradis V, Mathurin P, Kollinger M, Imbert-Bismut F, Charlotte F, Piton A, et al. In situ detection of lipid peroxidation in chronic hepatitis C: Correlation with pathological features. J Clin Pathol. 1997;50(5):401–6. https://doi.org/10.1136/jcp.50.5.401
15) Qian XL, Li YQ, Yu B, Gu F, Liu FF, Li WD, et al. Syndecan Binding Protein (SDCBP) Is Overexpressed in Estrogen Receptor Negative Breast Cancers, and Is a Potential Promoter for Tumor Proliferation. PLoS One. 2013;8(3):1–9. doi:10.1371/journal.pone.0060046
16) Tietz NW. Clinical guide to laboratory tests. In: Clinical guide to laboratory tests. 1995. p. 1096.DOI: 10.1136/jcp.50.5.401
17) Meena M, Prajapati P, Ravichandran C, Sehrawat R. Natamycin: a natural preservative for food applications-a review. Food Sci Biotechnol. 2021 Nov;30(12):1481–96. https://doi.org/ 10.1007/s10068-021 -00981-1
18) EastwoocJ J, Baines J. Hydrogenated poly-1-decene (addendum). food Addit Contam. :39. https://doi.org/10.2903/j.efsa.2020.6034
19) Mathur PP, Saradha B, Vaithinathan S. Impact of environmental toxicants on testicular function. Immunol Endocr Metab Agents Med Chem (Formerly Curr Med Chem Endocr Metab Agents). 2008;8(1):79–90. https://doi.org/10.2174/187152208783790705
20) Qasim AS, Numman Waheed I. Protective Effects of Vitamin C and E Against Monosodium Glutamate Induced Histological Changes in The Epididymis of Adult Male Albino Rats. Egypt J Vet Sci. 2023;54(3):525–40. DOI: 10.21608/EJVS.2023.197459.1452
21) El Gharabawy GS, Abd Allah EE-DE, Amr IM, Elmitwalli M. Histological and immunohistochemical study of the effect of cyclophosphamide on testis of male adult albino rats and the possible protective role of vitamin E. Egypt J Hosp Med. 2019;77(6):5930–46. DOI: 10.21608/EJHM.2019.65258
22) Rezaei A, Amirahmadi A, Poozesh V. Journal of Chemical Health Risks. DOI: 10.22034/jchr.2022.1928476.1295
23) Hasanin NA, Sayed NM, Ghoneim FM, Al-Sherief SA. Histological and ultrastructure study of the testes of acrylamide exposed adult male albino rat and evaluation of the possible protective effect of vitamin E intake. J Microsc Ultrastruct. 2018;6(1):23–34. DOI: 10.4103/JMAU.JMAU_7_18
24) El-azab NE, El-mahalaway AM. A Histological and Immunohistochemical Study on Testicular Changes Induced by Sliver Nanoparticles in Adult Rats and the Possible Protective Role of Camel Milk. 2019;1044–58. DOI: 10.21608/ejh.2019.13507.1126