RESEARCH PAPER

Histomorphological and Immunohistochemical Evaluation of Testicular and Epididymal Alterations in Albino Male Rats Exposed to Food Preservative

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Abstract

In the last years has been notice an increasing use Preservatives on the food and pharmaceutical products, for understanding their potential reproductive toxicity is crucial thus this study to investigate the histological and Immunohistochemistry impacts of sodium benzoate and pimaricin on the testicular tissues and epididymis of albino male rats. Animals were divided into four groups: a control group and three experimental groups treated with sodium benzoate
(600 mg/kg), pimaricin (0.3 mg/kg BW) and mixed group (sodium benzoate , pimaricin). Treatment duration was set for 50 days, Histological examination revealed significant structural changes in the treated groups, including degeneration and necrosis of spermatogenic cells, vascular congestion, inflammatory cell infiltration, and increased collagen fiber deposition—most prominently in the mixed group Immunohistochemical analysis supported these findings, showing altered expression patterns of key markers associated with tissue damage, inflammation, and fibrosis. These results suggest that sodium benzoate and pimaricin, especially in combination, can adverse effect male reproductive tissues and may impair fertility. Further studies are needed to evaluate their long-term toxicological impact.

Keywords: Immunohistochemistry - Histochemistry- Sodium Benzoate- Pimaricin- Testicular Tissues- Epididymis- Albino Male Rats Albino Male Rats.

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Introduction

Food preservatives are used extensively in the food and pharmaceutical industries, raising serious concerns about their safety(1). The antifungal and antibacterial qualities of sodium benzoate and pimaricin make them popular preservatives. A natural antifungal agent called pimaricin is used to prolong the shelf life of a variety of food products, while sodium benzoate is frequently used to prevent the growth of molds and yeasts in acidic foods. (2). While their efficiency in maintaining food quality, new data shows that these compounds may have negative impacts on human health, notably reproductive functions. The male reproductive system is especially sensitive to environmental pollutants and chemical exposures, which can change sperm production, quality, and overall fertility. (3). Research has indicated that exposure to various chemicals can induce histopathological changes in testicular and epididymal tissues, impacting spermatogenesis and hormone levels(4). 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.(2) While its usefulness in food preservation is well-established, the potential influence of pimaricin on male reproductive health, especially testicular tissue, remains completely unexplained .(5)The male reproductive system, including the testes, is known to be exposed to many environmental toxicants and chemical exposures. .(6) Testicular tissue, responsible for spermatogenesis and hormone production, can be

adversely affected by xenobiotics, leading to impaired fertility and hormonal imbalances (7). 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 to evaluate the effects of sodium benzoate and pimaricin dosages on male albino rat testes and epidemics.

Materials & Methods

Experimental design

Forty male albino rat was housed in an animal home for fifty days to get used to the conditions of the laboratory. From the animal house of the college of pharmacy at Karbala University, forty healthy adult male Wistar rats weighing between 800 and 900 g were acquired. They were 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 made feed pellet for 12 hours every day.

The experiment protocol

Ten rats each were divided into four groups. Group one was used as the control group and was fed a regular meal and water.. Group two treated at dose of600mg/kg B.W of sodium benzoatedissolved by water for 60 days' according to method reported by (8) (9) (10).. Group three treated at dose of oral intubation daily 0.3 mg /kg BW of pimaricin for 60 days' .Group four (mix) were given 600mg/kg B.W oral intubation daily of sodium benzoate dissolved by water and given 0.3 mg /kg BW of pimaricin. After completing the experimental period each animal was sacrificed by decapitation.

Tissue histopath

Rats will be killed 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

SDCBP Polyclonal Antibody (MDA) immunohistochemistry was carried out on tissue blocks that were embedded in formalin-fixed paraffin in each instance. In short, 5 mm tissue slices were deparaffinized, rehydrated using xylene, and then subjected to a series of alcohol grades. The antigen retrieval process was then conducted in an autoclave using 5 mm citrate buffer (pH 6.0). 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). (14,15,16)

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

Results

Testicular sections from the control group showed no histopathological alterations and a normal histological structure (Figure (1).In group 2 the testicular section revealed blood vessel thrombus along with interstitial tissue and sub tunica albuginea edema Figure (2).. Moderately thicker tunica albuginea and spermatogenic cell vacuolation and small number of interstitial Leydig cells this was accompanied with oligospermia and detached stereocilia and hydropic degeneration of lining epithelia. Additional to Masson trichrome staining revealed fibrosis thickness of tunica albuginea due to fibrosis Figure (3). the result of epidermis section observe a Mononuclear inflammatory cell recognized in increase interstitial tissue Also, the result revealed oligospermia with hydropic degeneration of lining epithelia. The lining epithelial cells' apical portions in the basal lamina provided PAS with its positive material. Positive reaction recorded in both tunica Albuginea & The connective tissues collagen fibers that encircle the epididymal ducts that stained blue color Figure (4). In group 3 testis section show degenerative of spermatic cells and necrosis with few Leydig cells interstitial, congestion of interstitial blood vessels, atrophy of seminiferous tubules. that gave PAS positive substance and stained purplish color The main testicular finding showed sever increase fibrosis that stained blue color were recorded in tunica Albuginea Figure (5).

The section of epididymis showed intense hyalinization of epididymal lumen, thrombus of blood vessels with fibrin network & large number of degenerative sperm,. Figure (6).

In group 4 histopathological section of the mix group's testis revealed edema in the sub tunica albuginea and

interstitial tissue, along with a thickness of tunica albuginea and inflammatory cell infiltration Figure (7).Atrophy of seminiferous tubules, degenerative and necrosis of the spermatogenic epithelium, cystic dilatation, a deformed and detached portion of the basement membrane, and blood vessel congestion. severe thickening of the foundation membrane that provided Strong reactivity to fibrosis. Figure (8)The epididymis finding is an irregular tubule arrangement surrounded by stroma, with a considerably thicker basement membrane. Collagen fibers and tunica albuginea fibrosis around the epididymis ducts and tiny blood vessels that stained blue are often visible. Figure (9).

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. Figure (10.11).Testicular sections Sodium benzoate group showed positive immunoexpression to (MDA) in spermatogenic in the seminiferous tubules and positive reaction to (MDA) in Leydig cells in the interstitial tissue Figure (12).and the Epididymis revealed presence MDA reaction were recorded in nuclear and cytoplasmic inside the cells that line the epididymal ducts Figure (13).The Pimaricin group showed mild (MDA) immunoexpression was observed in the spermatogenic cells while moderate reaction were recorded in Sertoli cells in the seminiferous tubules& Leydig cells in the interstitial tissue Figure (14). The Epididymis section showed moderate MDA immunoreaction stained nuclear and cytoplasmic in the epithelial cells lining of the epididymal ducts Figure (15). mix group (Sodium + Pimaricin). Representative photomicrographs showing strong nuclear and cytoplasmic positivity staining for MDA in germ cells, Sertoli cells in seminiferous ducts Figure (16). while Epididymis showed strong MDA immunoreaction recorded in both nuclear and cytoplasmic the epithelial cells that line the epididymal ducts Figure (17.18).

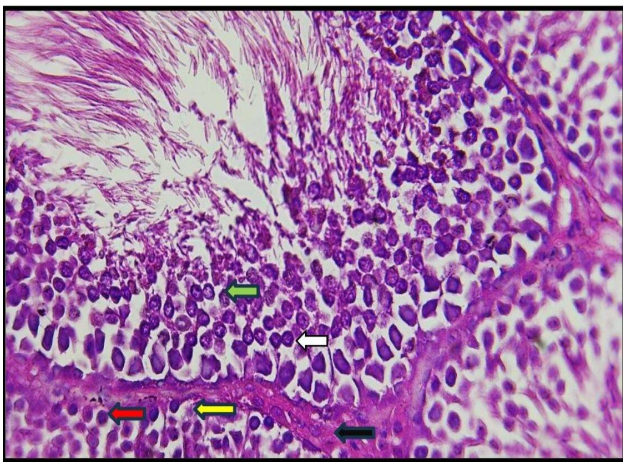

Figure (1): histological section testis of (control group) showed seminiferous tubules lined with series of spermatogenic cells, spermatogonia (yellow arrow), primary spermatocytes (white arrow) and round spermatids (green arrow) with Sertoli cells (red arrow) with attached sperms & interstitial cell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

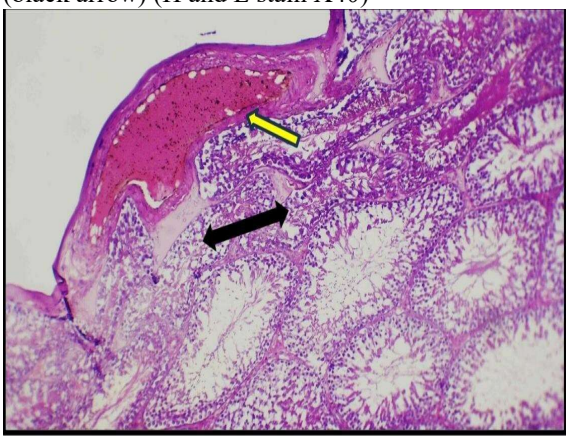

Figure (2): histopathological in the testis section of sodium benzoate group at 50 days post exposed showed thrombus of blood vessels (yellow arrow)with edema in interstitial tissue & sub tunica albuginea(black arrow) (H and E stain X10)

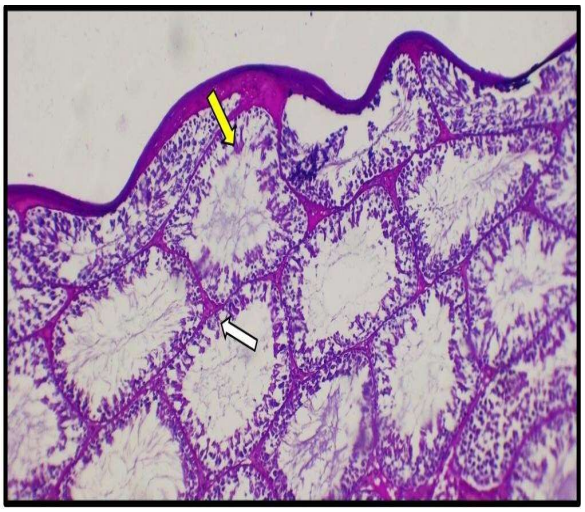

Figure (3): histopathological in the testis section of sodium benzoate group at 50 days post exposed showed lumen full of abundant PAS +ve elongated spermatids (yellow arrow)with moderate increase thickness basement membrane that stained purplish color(white arrow)(PAS stain X10)

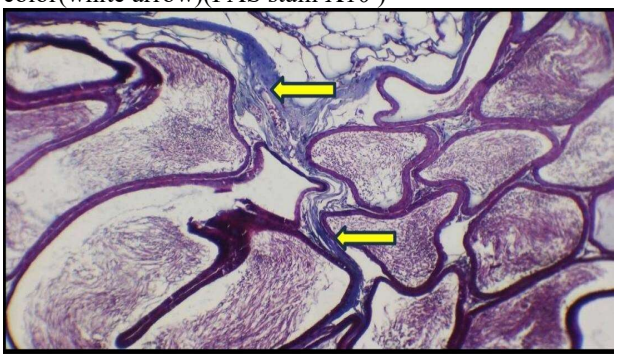

Figure (4): histopathological section in the epididymis of sodium benzoate group at 50 days post exposed showed moderate increase fibrosis in tunica Albuginea & Collagen fibers in the connective tissue around the epididymal ducts that stained blue color (yellow arrow) (Masson trichrome stain X10)

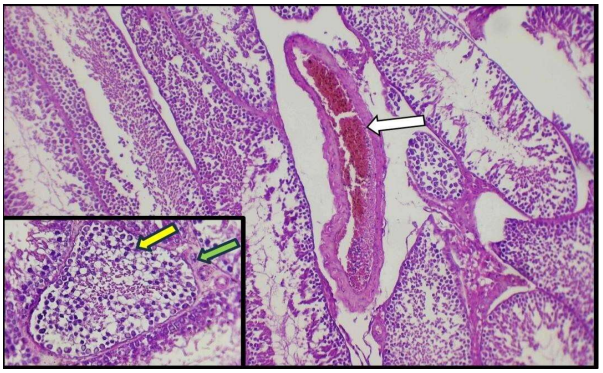

Figure (5): histopathological section of pimaricin group in the testis at 50 days post exposed showed degenerative & necrosis of spermatic cells (yellow arrow) with few Leydig cells interstitial (green arrow) & congestion of interstitial blood vessels (white arrow) (H&E stain X10+40)

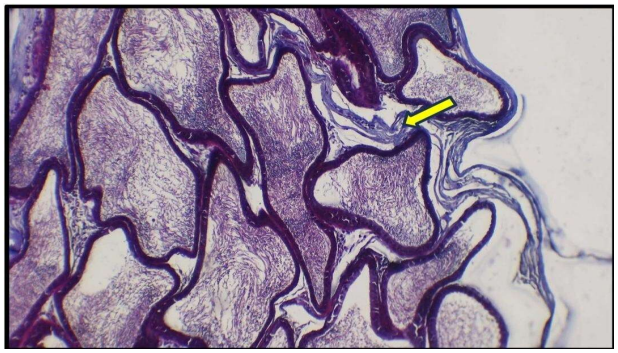

Figure (6):histopathological section of pimaricin group in the epididymis at 50 days post exposed showed sever increase collagen fibers surrounding the epididymal ducts that stained blue color (yellow arrow) (Masson trichrome stain X10)

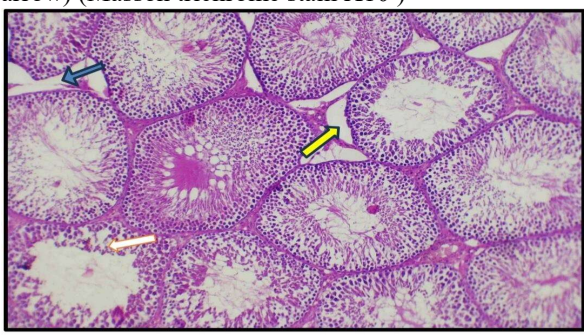

Figure (7): histopathological section of mix group in the testis at 50 days post exposed showed degenerative changes and necrosis of spermatogenic epithelium (white arrow)with atrophy of seminiferous tubules with cystic dilation(yellow arrow) & distorted and detached part of basement membrane (blue arrow)with congestion of B.V(H&E stain X10)

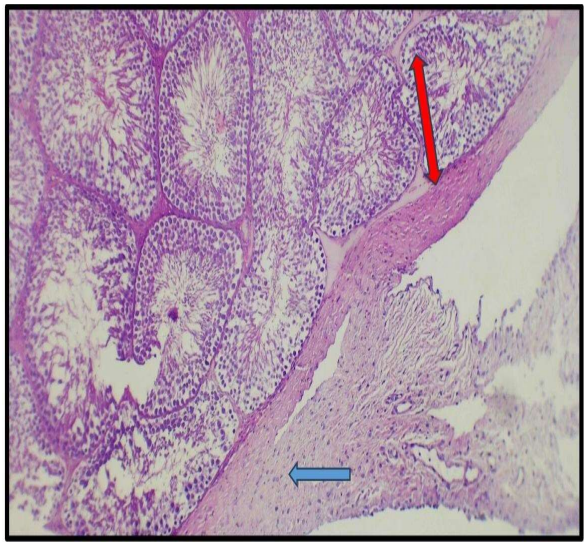

Figure (8): histopathological section in the testis of mix group at 50 days post exposed showed marked increase thickness of tunica albuginea & infiltration of inflammatory cells (blue arrow) with edema in interstitial tissue & sub tunica albuginea (red arrow) (H&E stain X10)

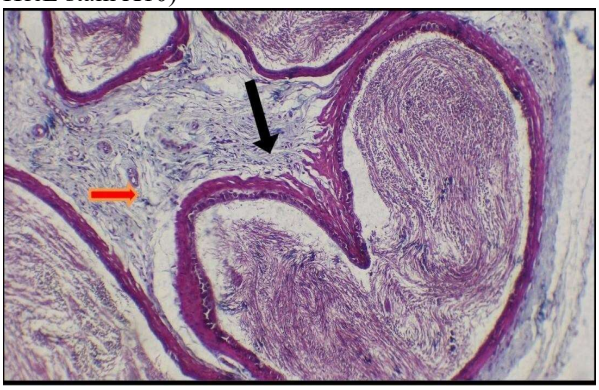

Figure (9):histopathological section of mix group in the epididymis at 50 days post exposed showed marked increase fibrosis in tunica Albuginea& surrounding the epididymal ducts collagen fibers (black arrow) and small blood vessels that stained blue color (red arrow) (Masson trichrome stain X40)

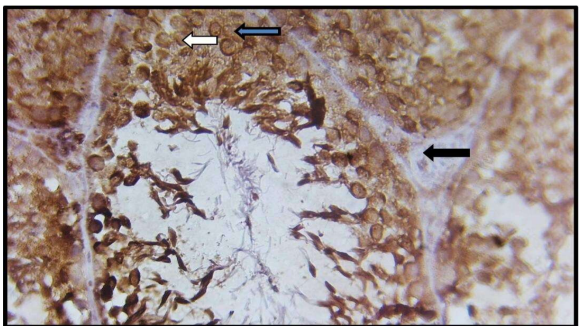

Figure (10):Immunohistochemistry analysis of MDA in formalin fixed tetis sections of normal control rats at 50 days of experiment. Representative photomicrographs demonstrate the presence of MDA immunoexpression in the spermatogenic (white arrow) , Sertoli cells (blue arrow)in the seminiferous tubules& Leydig cells in the interstitial tissue (black arrow) (MDA immunostaining X40)

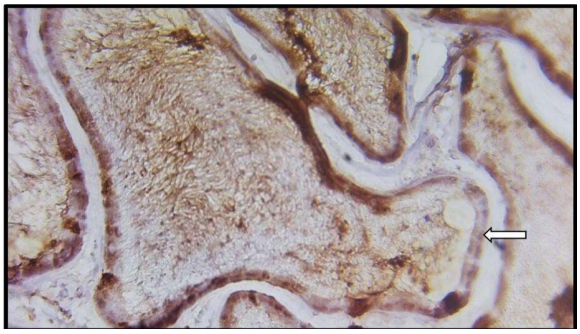

Figure (11):Immunohistochemistry analysis of MDA in formalin fixed cauda epididymis sections of normal control rats at 50 days of experiment. Representative photomicrographs demonstrate the presence of MDA in the nuclear and cytoplasm of the epithelial cells lining the epididymal ducts (white arrow). (MDA immunostaining X40).

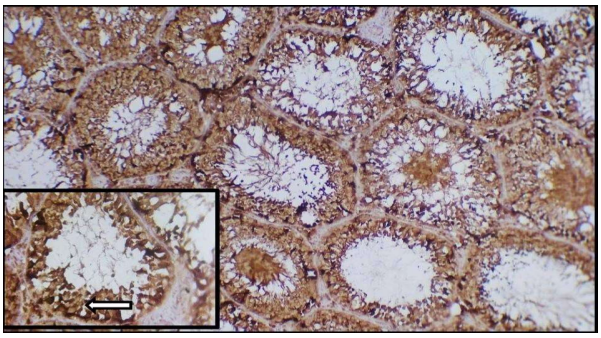

Figure (12): Immunohistochemistry analysis of MDA in formalin fixed testis sections of of sodium group at 50 days of experiment. Representative photomicrographs showing positive (MDA) immunoexpression in the spermatogenic in the seminiferous tubules (white arrow). (MDA immunostaining, X40)

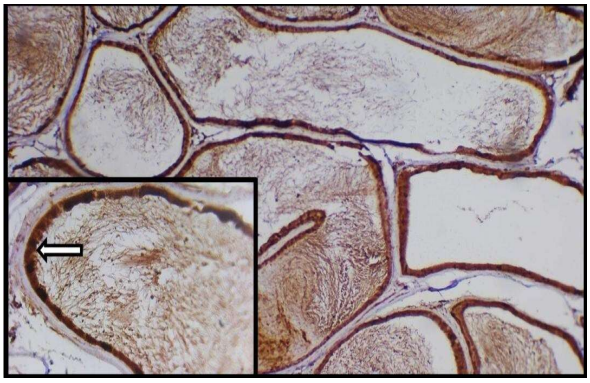

Figure (13): Immunohistochemistry analysis of MDA in formalin fixed cauda epididymis of sodium group at 50 days of experiment. Representative photomicrographs showing strong nuclear and cytoplasmic MDA immunoreaction in the epithelial cells lining the epididymal ducts(white arrow). (MDA immunostaining 10+X40).

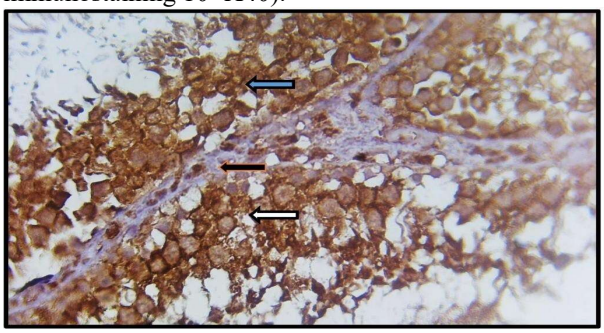

Figure (14): Histopathological section in the testis of pimaricin group at 50 days post exposed showed mild (MDA) immunoexpression in the spermatogenic(white arrow) , moderate rection Sertoli cells (blue arrow)in the seminiferous tubules& Leydig cells in the interstitial tissue (black arrow) (MDA immunostaining X40)

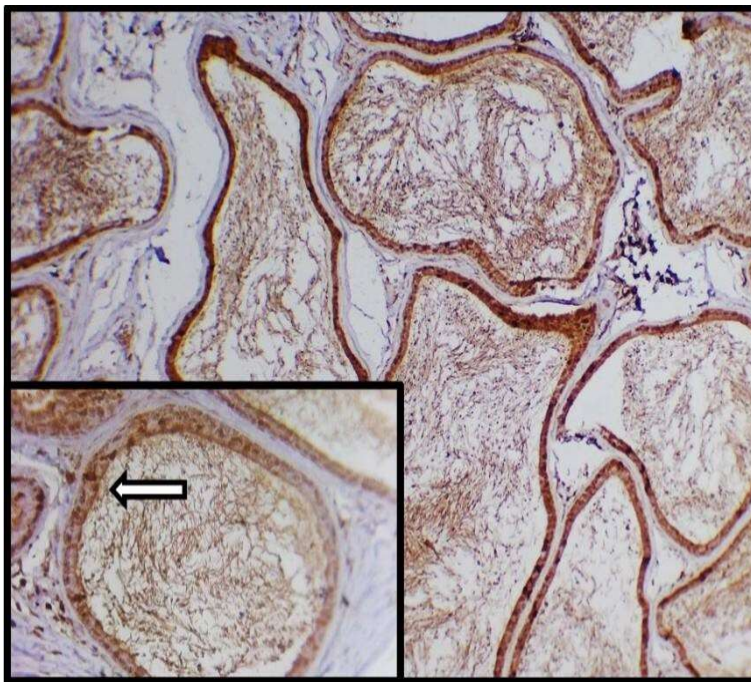

Figure (15): Histopathological section in the cauda epididymis of pimaricin group at 50 days post exposed showed moderate nuclear and cytoplasmic MDA immunoreaction in the epithelial cells lining the epididymal ducts(white arrow) (MDA immunostaining 10+X40)

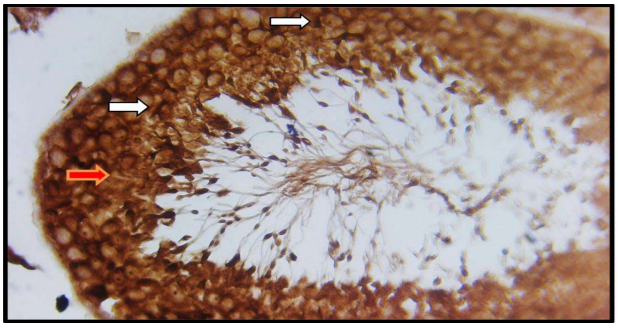

Figure (16): Immunohistochemistry analysis of MDA in formalin fixed testis sections of mix group (Sodium + Pimaricin) at 50 days of experiment. Representative photomicrographs showing strong nuclear and cytoplasmic positivity staining for MDA in germ cells (red arrow) & Sertoli cells (white arrow) in seminiferous ducts. Magnification X40.)

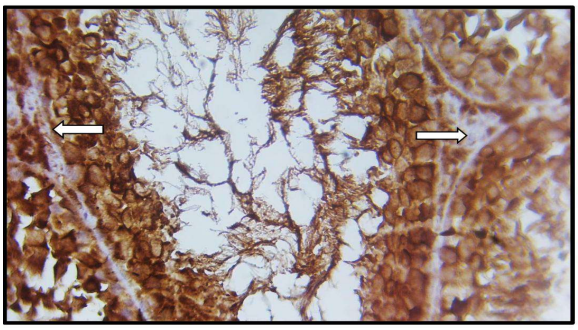

Figure (17): Immunohistochemistry analysis of MDA in formalin fixed cauda epididymis of mix group (Sodium + Pimaricin) at 50 days of experiment. Representative photomicrographs showing strong nuclear and cytoplasmic MDA immunoreaction in the epithelial cells lining the epididymal ducts (white arrow). Magnification 10+X40.

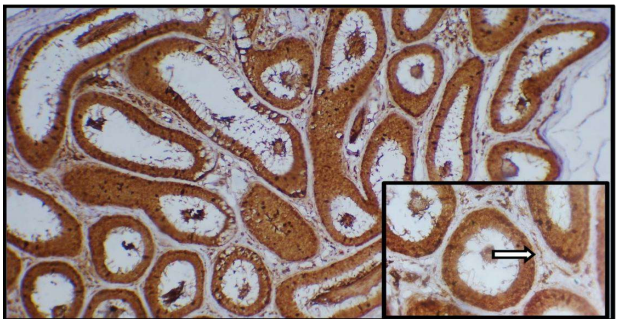

Figure (18): Immunohistochemistry analysis of MDA in formalin fixed cauda epididymis of mix group (Sodium + Pimaricin) at 50 days of experiment. Representative photomicrographs showing strong nuclear and cytoplasmic MDA immunoreaction in the epithelial cells lining the epididymal ducts (white arrow). Magnification 10+X40.

Discussion:

The histological examination of testicular tissues in sodium benzoate revealed thrombus formation with degenerative, necrosis of spermatic cells with few Leydig cells interstitial & congestion of interstitial blood vessels. These results support the findings of some previous researchers (7) have shown that administration of sodium benzoate could altered the histological architecture of testicular which appeared Slight congestion in the interstitial tissue with thrombus with atrophy in some seminal tissues.(17) Our results ,histological section of epididymis sodium benzoate & pimaricin groups revealed increase of interstitial tissue containing mononuclear inflammatory cell This observation was also observed in various previous studies. (8) ,histopathological section in the cauda epididymis of mix group showed irregular arrangement of tubules with increase thickness of basement membrane .The same this result with (18) who reported showing an irregular arrangement of tubules surrounded by stroma with the reduced epithelium. The main features of fibrotic disorders affecting the testis and epididymis are thickening of the basement membrane and excessive extracellular matrix protein deposition. This finding of our study supported by what is stated by (19) Also, the current study found a reduced PAS response in the apical regions of the epidydimal epithelial cells, which may be the result of the loss of the pseudostratified epithelium lining the epididymis. (20) showing a strong PAS reaction in the basal laminae of the epididymal tubes, with intact thin apical PAS reaction in the lining epithelial cells. The epididymis in the mix group exhibited significant collagen fiber deposition in the epididymal tubules, basal lamina and interstitial space. The pimaricin group had severe collagen fiber deposition tubules in the epididymal tubules, basal lamina, interstitial space, and tunica albuginea. The sodium benzoate group had considerable mild collagen fiber deposition in the. Similar results were obtained by (20).

In 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. sodium benzoate group Representative photomicrographs showing nuclear and cytoplasmic in germ cells give positive MDA in the spermatogenic in seminiferous tubules & Leydig cells also , nuclear and cytoplasmic in the epithelial lining the epididymal ducts . The our results same the result of 22) They discovered The cells lining the seminiferous tubule in the first group displayed a strong positive Bcl-2 cytoplasmic immunoreaction, whereas the Bcl-2 immunoreaction was mild in The second group. (20)The epithelial lining of the epididymal ducts had a strong, exclusively nuclear immunoreaction in the form of brown coloring in AR-immunostained sections from the control group. However, the epithelial cells lining the epididymal ducts in The third 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 of the mix group is showing strong nuclear and cytoplasmic positivity staining for MDA and pimaricin group had significant mild and moderate nuclear and cytoplasmic MDA expression.(4). reported TCS-treated group showed an apparently weak nuclear immunoreaction in the epithelial lining

of the epididymal ducts, in addition to some focal cytoplasmic positive immunoreaction.(22) The third group revealed minimal Bcl2 immunoreaction in cells of the seminiferous tubule. The four group showed intense positive Bcl-2 immunoreaction, but mild positive Bcl-2 immunoreaction was seen in cells of the seminiferous tubule of group V(24)

Conclusion

According to the study, pimaricin and sodium benzoate caused tissue damage in the testis and epididymis, including cell degeneration and increased fibrosis. The mix group had the most severe effects. These results suggest a possible negative impact on male fertility and highlight the need for more research and cautious use of these substances

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Conflict of interest
The author claims that there isn't any obvious disagreement at all.

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العنوان

التقييم الهيكلي النسيجي والمناعي للتغيرات التي تطرأ على الخصيتين والبربخ في ذكور الجرذان البيضاء عند تعرضها لمادة حافظة غذائية

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الخلاصة

في السنوات الأخيرة، لوحظ تزايد استخدام المواد الحافظة في الأغذية والمنتجات الصيدلانية، مما يجعل من الضروري فهم سُميتها المحتملة على القدرة الإنجابية. لذلك، هدفت هذه الدراسة إلى البحث في التأثيرات النسجية والمناعية الكيميائية لكل من بنزوات الصوديوم والبيماريسين على أنسجة الخصية والبربخ في ذكور الجرذان البيضاء تم تقسيم الحيوانات إلى أربع مجموعات: مجموعة ضابطة وثلاث مجموعات تجريبية عُولجت إحداها ببنزوات الصوديوم بجرعة 600 ملغم/كغم، والثانية بالبيماريسين بجرعة 0.3 ملغم/كغم من وزن الجسم، والثالثة بخليط من المادتين. استمرت مدة العلاج 50 يومًا. أظهر الفحص النسجي تغيرات هيكلية ملحوظة في المجموعات المعالجة، شملت تنكسًا ونخرًا في الخلايا المولدة للنطف، واحتقانًا وعائيًا، وتسرب خلايا التهابية، وزيادة في ترسيب ألياف الكولاجين، وظهرت هذه التغيرات بشكل أكثر وضوحًا في المجموعة المعالجة بالمزيج (بنزوات الصوديوم والبيماريسين). كما دعمت التحاليل المناعية الكيميائية هذه النتائج، حيث أظهرت أنماط تعبير متغيرة للواسمات المرتبطة بتلف الأنسجة، والالتهاب، والتليف. وتشير هذه النتائج إلى أن بنزوات الصوديوم والبيمارسين، خاصة عند استخدامهما معًا، قد يكون لهما تأثيرات سلبية على أنسجة الجهاز التناسلي الذكري وقد تؤدي إلى ضعف الخصوبة. وتوصي الد

راسة بإجراء أبحاث إضافية لتقييم التأثيرات السمية طويلة الأمد لهذه