Histopathological Study of Shilajit Inhibits the Toxic Effect of Bisphenol in the Testis and Epididymis

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Received:1/5/2026 **Accepted:** 31/5/2026 **Published:** 15/6/2026

***Abstract*—** Bisphenol -A is a broadly used substance in our environment .The current study was established to established to investigate whether Shilajit inhibits the toxic effect of bisphenol in the testis and epididymis. Three groups of thirty rabbits were created: G1 received water and pellets as a control, G2 received bisphenol A (1 ml/kg), and G3 received bisphenol A (1 ml/kg) plus shilajit (0.2 g/kg). Histopathological findings showed characteristic testicular changes, including severe thickening of the tunica albuginea due to fibrosis and degeneration of primary spermatogonia, atrophy of seminiferous tubules with vacuolation ,severe widening of interstitial spaces and a distorted , detached basement membrane .There was thickening of the basement membrane with inflammatory cells in the interstitial tissue in G2.Also, the histopathological findings of the epididymis showed hydropic degeneration of the lining epithelia and severe collagen deposition and widening of the interstitial tissue containing mononuclear inflammatory cells as well as Oligospermia hyperplasia of the lining epithelia with thick stroma due to hypertrophy of smooth muscle fibers of G2.The third group showed closely packed seminiferous tubules with a regular appearance , sperm in the lumen ,a narrow interstitium, intercellular vacuolations and mild changes in the epididymal duct with scanty stereocilia ,numerous spermatozoa in the lumen with few vacuolated cells and narrowing interstitial tissue. Conclusion :The results of our study showed that BPA administration induced abnormalities and histopathological alteration in the testis and epididymis . Co-treatment with shilajit provided a protective antioxidant role against such adverse effects.

Keywords **—** Bisphenol A, shilajit, testis, epididymis, histopathological alterations

INTRODUCTION

One bisphenol is known as bisphenol A or 2,2-bis (hydroxyphenyl). Monomers and polycarbonate are used to make plastic. Epoxy, one of the substances that disrupts hormones, was recently outlawed. The cause of this is estrogenic efficacy, or an endocrine-disrupting drug.Numerous health effects of bisphenol were initially discovered by accident. In 1993 AD, it was found that polycarbonate flasks leach bisphenol. In autoclaved breast tissue, cancer cells multiply throughout the breast(1). Human beings and lab animals both absorb bisphenol. Very little of it is consumed by mouth; the majority is converted into its original, undamaged state. It is not more than 90% metabolized. binds to plasma-based proteins (1).

The bisphenol that enters the body is metabolized as it passes through the liver (2). Adult rhesus monkeys were considered a reliable model for the study. The researchers have discovered that the metabolism of bisphenol in humans, mice, and monkeys is highly comparable.(3). BPA is a poor peripheral estrogen based on its relative alpha and beta binding strengths. (4). According to recent research, bisphenol can occasionally be confused for estrogen and can even trigger particular biological processes at low quantities. (5). Shilajit is a naturally occurring mineral that is a gift from natural resources. (6).

Despite being mentioned in traditional literature from antiquity, Shilajit is not commonly known in the West today. There is little to no information from clinical trials, and the processes underlying its therapeutic efficacy are poorly understood.Important elements of the various WHO strategies to promote safe, effective, and affordable traditional medicine (TM) in the current millennium include the documentation of TM and remedies, as well as the development of a strong evidence base on the safety, efficacy and quality of TM product and practices (7). Research also requires prior literature reviews and documentation of TM remedies in the form of a basic data bank. This paper's comprehensive review highlights Shilajit's significance, definition, source, synonyms, varieties, traditional uses, origin, physical characteristics, chemical constituents, bioactivity, toxicity, and contraindications while accounting for the previously mentioned facts. It is created when many plants undergo a protracted humification process, according to (8, 9, 10). (11, 12, 13) have detailed chemical analyses that show humus makes up 60–70% of shilajit. Ayurvedic medicine, a branch of Indian traditional medicine, uses shilajit extensively

to treat a variety of illnesses and chronic conditions because of its therapeutic qualities (13, 14).

MATERIALS AND MTHODS

Animals

Before the experiment began, every animal was inspected to make sure there were no wounds or abnormalities. Prophylactic doses of an intestinal, hepatic, and internal antihelmintic medication were administered to the animals .In order to avoid coccidiosis, rabbits received subcutaneous injections of 3% albendazole (Higro, Dox-al Italia S.p.A,Italy) and 0.1 ml/rabbit of ivermectin (Ivermic, Al-Sakab Company for Agricultural and Animal Services and General Trade ,Uruguay) and for four days, they received injections of Amprolium (Interchemie werken "De Adelaar" B.V., Netherlands) at a dosage of 0.6 milliliters per liter of drinking water to prevent internal and external worms. Pellets were used as animal feed. Tap water was provided ad libitum. All study protocols were approved (UOK.VET.PA. 2024.112) by the Ethics Committee of the Faculty of Veterinary Medicine (University of Kerbala 's Faculty of Veterinary Medicine).

Experimental design

A total of 30 rabbits were split up into 3 groups. As a control group, G1 received treatment with water and pellets and G2 received treatment with bisphenol A (1 ml/kg) .G3 received bisphenol A (1 ml/kg) plus shilajit (0.2 g/kg).From March 1, 2025, to May 2, 2025, the experiment was carried out at the University of Kerbala's Faculty of Veterinary Medicine. where the experiment's rabbits were housed in special cages.

Preparation of Shilajit:6 g of Shilajit is put in distilled water (30 ml), then put on a magnetic stirrer.

Preparation of bisphenol : Distelled water 75 ml added to 25ml (Dimethyl sulfoxide (DMSO) then taken 30 ml mix with 3 g bisphenol A then put on Magnetic stirrer (4).

Histopathology:

Testicular and epididymal fragments of normal internal organs were gathered and preserved in 10% formalin. The standard paraffin embedding technique was used to process the fixed tissue samples. The samples were, in short, cut into pieces that were 2-3 mm thick, rinsed under water for a few hours, dehydrated in increasing alcohol grades, cleared in xylene, and then embedded in paraffin. Using standard techniques and protocols, tissue sections with a thickness of 4-5 um were stained with hematoxylin and eosin. (15)

RESULT AND DISCUSSION

Histopathological finding:

Control group:

Testis: The section of testis showed spermatogonia ,primary spermatocytes and round spermatids with Sertoli cells (Figure 1).

Epididymis: The histological section in the epididymis revealed epididymal tubule lined by ciliated pseudostratified columnar epithelium with numerus sperms in the lumen (Figure 2). Also, another section record columnar cells with basal nuclei and stereocilia & basal cells resting on the basal lamina and clear cells with vacuolated cytoplasm with thin layer of smooth muscle fiber surrounds each epidydimal duct and numerus spermatozoa in the epididymal lumen (Figure 3).

Bisphenol group:

Testis: the main histopathological finding revealed severe thickening of the tunica albuginea due to fibrosis with degeneration of primary spermatogonia (Figure 4). Other sections revealed atrophy of seminiferous tubules and vacuolation with severe widening of interstitial spaces and distorted and detached part of the basement membrane (Figure 5). In addition ,thickening of basement membrane with inflammatory cells in the interstitial tissue (Figure 6).

Epididymis: epididymal lesion showed hydropic degeneration of lining epithelia and severe collagen deposition, widening of interstitial tissue containing mononuclear inflammatory cells (Figure 7). Other lesions showed Oligospermia hyperplasia of lining epithelia with thick stroma due to hypertrophy of smooth muscle fiber (Figure 8).

Bisphenol +Shilajit group:

Testis: the main histopathological finding showed closely packed seminiferous tubules with regular appearance & sperm in the lumen with narrow interstitium and intercellular vacuolations (Figure 9).

Epididymis : epididymal lesion showed mild changes in the epididymal duct with scanty stereocilia and numerus in spermatozoa in the lumen with few vacuolated cells and narrowing interstitial tissue (Figure 10) .

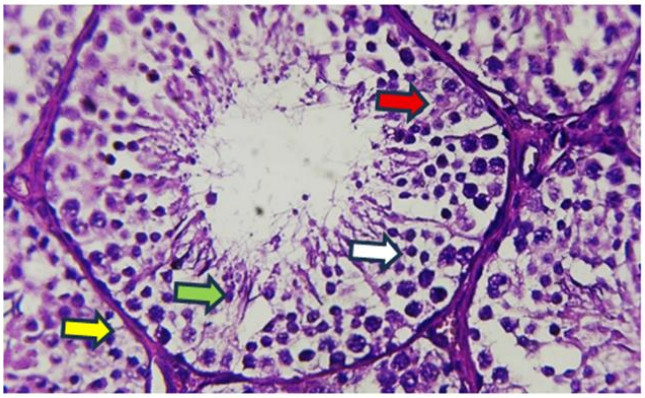

Figure1. histological section in the testis of rabbit of control group at 21 days post challenge spermatogonia (yellow arrow) with primary spermatocytes (white arrow)and round spermatid green arrow with Sertoli cells (red arrow) (H&E stain X40).

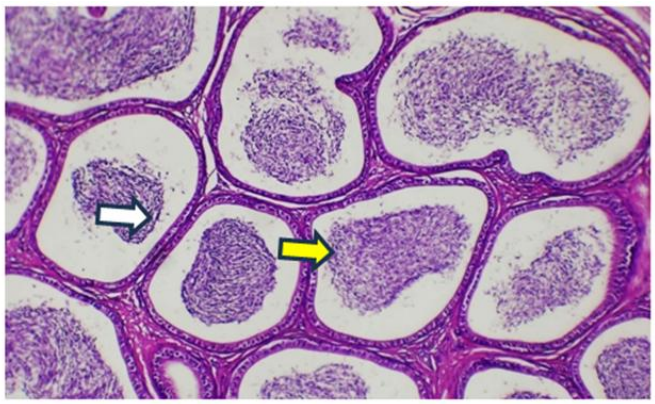

Figure 2. histological section in the epididymis of rabbit of control group at 21 days post challenge shows epididymal tubule lined by ciliated pseudostratified columnar epithelium (white arrow) with numerous sperm in the lumen (yellow arrow) (H& E stain X10)

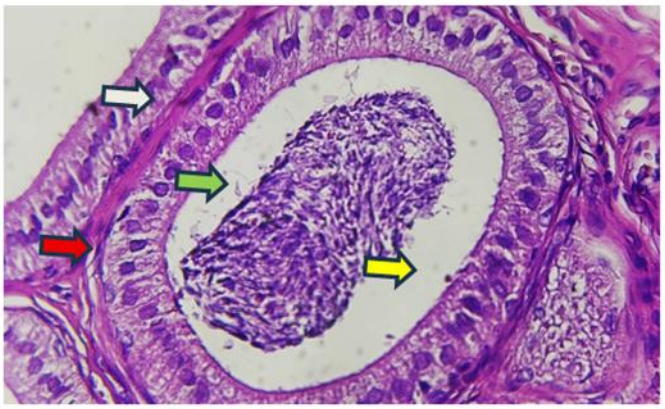

Figure 3. histological section in the epididymis of rabbit of control group at 21 days post challenge shows columnar cells with basal nuclei and stereocilia (yellow arrow) & basal cells resting on the basal lamina and clear cells with vacuolated cytoplasm (red arrow) with thin layer of smooth muscle fiber surrounds each epidydimal duct (white arrow) and numerus spermatozoa in the epididymal lumen (green arrow) (H&E satin X40)

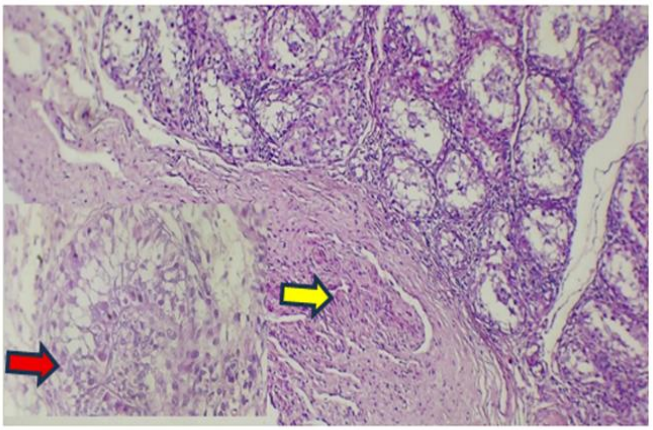

Figure 4. histopathological section in the testis of rabbit of bisphenol group at 21 days post challenge shows severe thickening of tunica albuginea due to fibrosis (yellow arrow) with degenerative of primary spermatogonia (red arrow)(H&E stain 10,40)

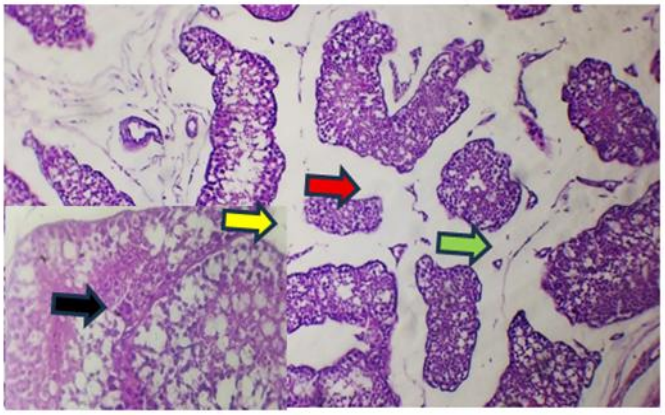

Figure 5. histopathological section in the testis of rabbit of bisphenol group at 21 days post challenge shows atrophy of seminiferous tubules (yellow arrow) with vacuolation (black arrow) with severe widening in interstitial spaces (red arrow)& distorted and detached part of basement membrane (green arrow) (H&E stain X10,40)

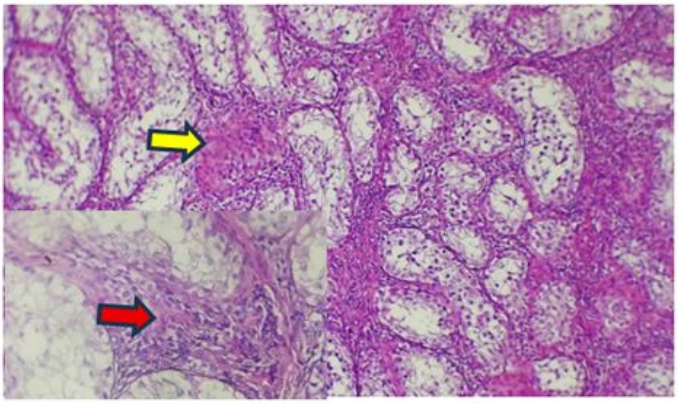

Figure 6. histopathological section in the testis of rabbit of bisphenol group at 21 days post challenge shows thickening of basement membrane (yellow arrow) with inflammatory cells in the interstitial tissue (red arrow) (H&E stain X10,40)

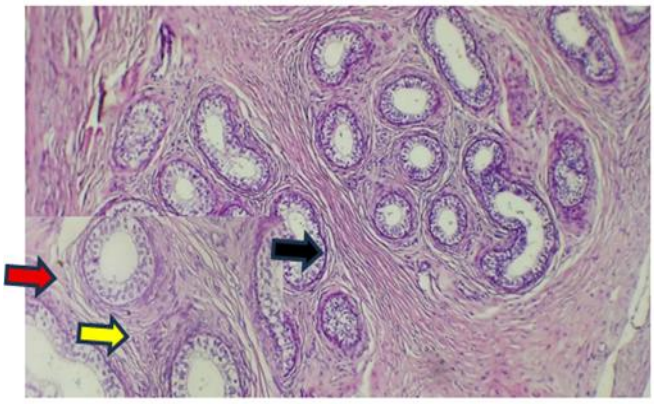

Figure 7. histopathological section in the epididymis of rabbit of bisphenol group at 21 days post challenge shows hydropic degeneration of lining epithelia (red arrow)and sever collagen deposition (black arrow)&widening of interstitial tissue containing mononuclear inflammatory cell (yellow arrow)

(H&E stain X10,40) .

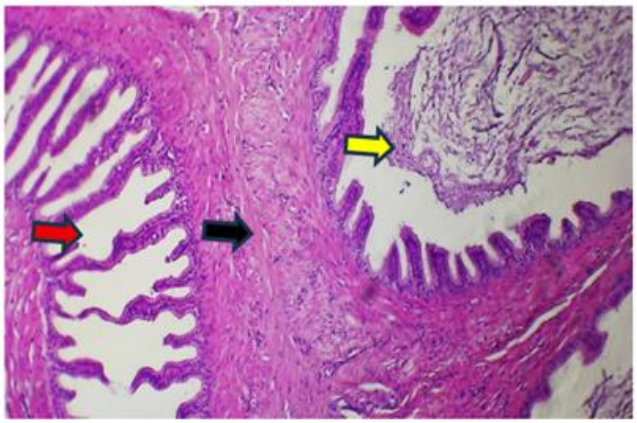

Figure 8. histopathological section in the epididymis of rabbit of bisphenol group at 21 days post challenge shows Oligospermia (yellow arrow) hyperplasia of lining epithelia (red arrow) with thick stroma due to hypertrophy of smooth muscle fiber (black arrow) (H&E stain X10)

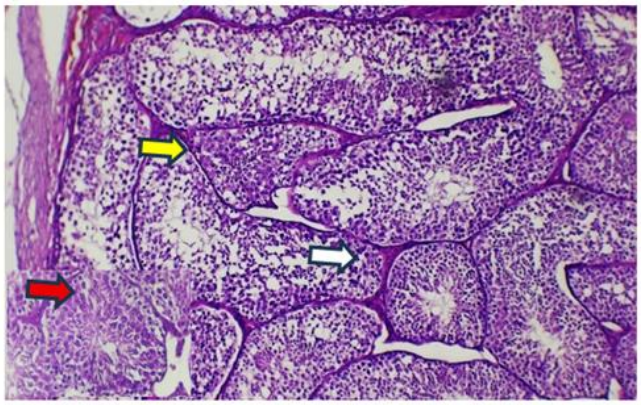

Figure 9. histopathological section in the testis of rabbit of bisphenol + shilajit group at 21 days post challenge shows closely packed seminiferous tubules (yellow arrow) &with regular appearance & sperms in lumen with narrow interstitium (white arrow) & intercellular vacuolations (red arrow) (H&E stain X10,40) .

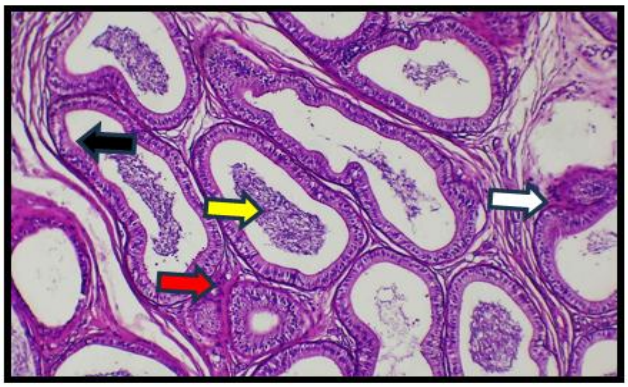

Figure 10. histopathological section in the epididymis of bisphenol +shilajit group at 21 days post challenge shows minimal changes in the epididymal duct with scanty stereocilia (black arrow) & numerous in spermatozoa in lumen (yellow arrow) with few vacuolated cells (white arrow) & narrowing interstitial tissue(red arrow) (H&E stain X10+40)

In the present study, the control group of the testis revealed spermatogonia , primary spermatocytes and round spermatids with Sertoli cells .Our results same the result of(16). They discovered that the stratified epithelial tissue lining the lumens was constructed from sperm line cells, starting with spermatogonial cells, which were small, spherical cells with round nuclei. Large, spherical nuclei arranged in single or multiple layers were considered to be primary spermatocytes. The nuclei of spermatids were pale, spherical, and tiny. The mature sperm were then found within the lumens of the tubule. The triangular nuclei of sertoli cells were discovered affixed to the testicular tubules' basement membranes.The control group of epididymis revealed epididymal tubule lined by ciliated pseudostratified columnar epithelium with numerus sperms in the lumen and columnar cells with basal nuclei and stereocilia & basal cells resting on the basal lamina and clear cells with vacuolated cytoplasm with thin layer of smooth muscle fiber surrounds each epidydimal duct and numerus spermatozoa in the epididymal lumen.

The same result with (17) they discovered The adult male albino rats in control groups I and II had cauda epididymis made up of circular tubules with a lot of sperm in their lumina, separated by interstitial tissues that contained connective tissue cells and smooth muscle fibers. The epididymal tubules were lined with pseudostratified epithelium , which contained three different typer of cells :principle cells , basal cells and clear cells. In contrast to basal cells ,which positioned the bases of the principal cells and had oval nuclei that rested on the basement membrane, the principal cells were columnar to cubical cells with long microvilli called stereocilia that extended into the tubule lumina The cytoplasm of the basal cells was occupied by oval indented nuclei. The clear cells were characterized by pale cytoplasm with central nucleus . On the other hand, section of testis of bisphenol group that revealed atrophy of seminiferous tubules and vacuolation with severe widening of interstitial spaces and distorted and detached part of the basement membrane. Also ,thickening of basement membrane with inflammatory cells in the interstitial tissue.(16)observed a widening of the interstitium tissue, as well as sloughing of the germinal epithelium, resulting in a loss of the usual configuration of seminiferous tubules. Blood arteries in the interstitial spaces were clogged and dilated ,and they had been invaded by mononuclear cells infiltration. The lumens of seminiferous tubules included debris of small spermatid and degraded Sertoli cells with pyknotic nuclei. The germinal epithelium deteriorated, dispersed and detached from the basement membrane. The mitochondria on the spermatids were disordered and unevenly distributed. Some of them have smaller nuclei .

The intracellular spaces show progressive degenerative alterations compromising cell membrane integrity due to oxidative stress caused by BPA. Free radical oxygen species induce oxidative phosphorylation events in the cells membrane, resulting in disturbance of the integrity of the intercellular junctional complex (18). Furthermore, it was found that neonatal exposure to BPA changes the ectoplasmic specialization of the Sertoli cells and spermatids when the

animal enters puberty. (19,20).It was also proposed that oxidative stress specifically impairs cadherin/catenin complex and cell-cell adhesion (21). Both control groups' cauda of the epididymis showed severe collagen deposition, hydropic degeneration of the lining epithelia, and expansion of the interstitial tissue harboring mononuclear inflammatory cells. Oligospermia thick stroma and hyperplasia of the lining epithelia as a result of smooth muscle fiber hypertrophy(22)The latest work revealed the disruption of seminiferous tubules of BP-treated rabbits with germinal epithelium sloughing; these results were agreement with (23).The findings were attributed to the increasing smooth muscle activity due to inflammation, which influences the epithelial tissue responses and then causes disproportionate stretch of the epithelial lining through the production of chemotactic chemicals.(24).

The findings of the present study explained the BPA treated rabbits' cellular hypertrophy and disorganization of the epididymal tubules, with the epithelial layer's shape changing from columnar to flat, the findings were constant (18) result ,who liked the exposure to the BPA forging material to the defense mechanisms. In the same way, (25) was used to describe the epididymal main cells' phagocytosis function against the waste of any forgin material. It was assumed that the hallo cells were lymphocytes that served as the male reproductive system's immune component.(26).

The injured immature sperms were hunted down by clear cells, which also absorbed sperm cytoplasm droplets. (27).The hisopathological examination of testis in the third group revealed closely packed seminiferous tubules &with regular appearance & sperms in lumen with narrow interstitium & intercellular vacuolations. The same results (28) They discovered by increasing the number of germ cells, low-dose Shilajit treatment enhanced tubular histoarchitecture in CPA-exposed mice. When high-dose Shilajit was administered to CPA-exposed mice, the histoarchitecture of the seminiferous tubules was nearly identical to that of the controls.The third group's cauda of epididymis displayed narrowing interstitial tissue, few stereocilia, and a large number of spermatozoa in the lumen with few vacuolated cells. Similar findings were found by (28).

CONCLUSION

The results of our study showed that BPA administration induced abnormalities and histopathological alterations in the testis and epididymis .Co-treatment with shilajit provided a protective antioxidant role against such adverse effects.

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