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**RESEARCH ARTICLE** **HISTOLOGY**

# Histological Effects of *Ziziphus spina-christi* Leaf Extract on Testes and Epididymides in Male Rabbits

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**ABSTRACT**

The widespread use of medicinal plants in traditional therapy has raised concerns regarding their long-term safety, particularly with respect to reproductive function. *Ziziphus spina-christi* (ZSC) is commonly utilized for its antimicrobial, anti-inflammatory, and antioxidant activities; however, limited data are available concerning its possible impact on male reproductive organs. This study was conducted to investigate the histopathological effects of prolonged oral administration of ZSC leaf extract on the testes and epididymides of adult male rabbits. Animals were divided into two groups: a control group receiving distilled water and a treated group administered ZSC leaf extract at a dose of 200 mg/kg body weight daily for six weeks. At the end of the experimental period, testicular and epididymal tissues were harvested and processed for routine microscopic examination. Tissue changes were evaluated descriptively and graded using a semi-quantitative scoring method. Histological analysis revealed marked structural alterations in the reproductive tissues of treated animals compared with controls. Testicular sections demonstrated degeneration of seminiferous tubules, decreased spermatogenic cell layers, thinning of the germinal epithelium, and vascular congestion. In the epididymis, reduced sperm density, epithelial degeneration, luminal debris, and thickening of connective tissue were observed. These findings indicate that prolonged administration of ZSC leaf extract may adversely influence the histological architecture of male reproductive organs. Further studies are warranted to clarify its reproductive safety and underlying mechanisms.

# التأثيرات النسيجية لمستخلص أوراق نبات السدر *Ziziphus spina-christi* على الخصيتين والبربخ في ذكور الأرانب

أسماء ادريس احمد[1]، سناء عبد اللطيف عياد محمد [2]. عيدة مفتاح عبد الكريم الشيلابي[3]*، فيروز الزبير خالد[4]

**الملخص**

أدى التوسع في استخدام النباتات الطبية في الممارسات العلاجية التقليدية إلى ضرورة تقييم مأمونيتها بصورة علمية منهجية، ولا سيما فيما يتعلق بصحة الجهاز التناسلي. ويُعد نبات السدر (Ziziphus spina-christi) من النباتات الشائعة الاستخدام نظرًا لما يُنسب إليه من خصائص مضادة للميكروبات والالتهاب، إضافةً إلى نشاطه المضاد للأكسدة. ومع ذلك، لا تزال المعلومات المتوافرة حول تأثيره المحتمل في الأعضاء التناسلية الذكرية محدودة. هدفت هذه الدراسة إلى تقصي التغيرات النسيجية المرضية في الخصية والبربخ لدى ذكور الأرانب البالغة بعد إعطائها مستخلص أوراق السدر عن طريق الفم لمدة مطولة. قُسمت الحيوانات إلى مجموعتين: مجموعة ضابطة تلقت ماءً مقطرًا، وأخرى معالجة أعطيت مستخلص أوراق السدر بجرعة 200 ملغم/كغم من وزن الجسم يوميًا لمدة ستة أسابيع متتالية. وعقب انتهاء فترة التجربة، جُمعت عينات الخصية والبربخ وأُعدّت للفحص النسيجي الروتيني، ثم جرى تقييم التغيرات المجهرية وصفيًا وتصنيفها وفق نظام تقدير شبه كمي. أظهر الفحص المجهري حدوث تغيرات بنيوية واضحة في أنسجة الجهاز التناسلي للحيوانات المعالجة مقارنة بالمجموعة الضابطة. وتمثلت التغيرات في الخصية في تنكس الأنابيب المنوية، وانخفاض أعداد الخلايا المولدة للنطاف، وترقق الظهارة الجرثومية، إلى جانب احتقان وعائي ملحوظ. أما في البربخ، فقد لوحظ انخفاض كثافة الحيوانات المنوية داخل التجويف، مع تنكس ظهاري، ووجود حطام خلوي في اللمعة، وزيادة في سماكة النسيج الضام. تشير هذه النتائج إلى أن التعرض المطول لمستخلص أوراق السدر قد يرتبط بحدوث تأثيرات سلبية في البنية النسيجية للأعضاء التناسلية الذكرية، الأمر الذي يستدعي مزيدًا من الدراسات المتعمقة لتقييم سلامته التناسلية وتحديد آليات تأثيره المحتملة.

**الكلمات المفتاحية**

نبات السدر
الجهاز التناسلي الذكري
الخصية
البربخ
الأرانب

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## Introduction

The male reproductive system plays a fundamental role in maintaining fertility, and its efficiency depends largely on the structural and functional integrity of both the testis and epididymis. Within the seminiferous tubules of the testis, spermatogenesis proceeds through a highly coordinated sequence of cellular events involving spermatogonia, Sertoli cells, and Leydig cells. The epididymis, in turn, provides a specialized luminal environment that supports sperm maturation, motility development, and acquisition of fertilizing ability. Disruption of the normal architecture of these organs can interfere with spermatogenic processes and ultimately compromise male fertility [1, 2].

Histopathological assessment remains one of the most reliable approaches for evaluating reproductive toxicity as well as the biological effects of natural and synthetic compounds on male reproductive tissues. Routine microscopic examination allows detailed observation of seminiferous tubule organization, germinal epithelium thickness, tubular diameter, and epididymal epithelial integrity. Such parameters offer sensitive indicators of tissue alterations that may not be evident through biochemical assays or hormonal measurements alone [3, 4, 5].

In recent years, medicinal plants have attracted growing scientific interest as complementary or alternative therapeutic agents. Their accessibility, traditional acceptance, and rich content of bioactive constituents contribute to their widespread use [6, 7]. Phytochemicals such as flavonoids, saponins, alkaloids, and phenolic compounds are known for their antioxidant and anti-inflammatory properties, in addition to their potential influence on endocrine regulation. However, their effects on male reproductive function appear to be dose- and duration-dependent, and may vary according to the experimental model used [8, 9, 10]. Several investigations have shown that plant-derived compounds can modify spermatogenesis and testicular histology. For example, *Tribulus terrestris* has been associated with improved sperm density and preservation of seminiferous tubule structure at moderate doses, whereas higher exposure levels have been linked to mild germinal epithelial alterations [11]. Similarly, extracts of *Withania somnifera* and *Moringa oleifera* have demonstrated protective effects on Leydig cell activity and spermatogenic performance in animal models, although excessive administration has been reported to induce tubular shrinkage and epithelial degeneration [12, 13]. These observations underscore the dual and dose-related nature of phytochemical actions on reproductive tissues.

*Ziziphus spina-christi* (L.), belonging to the family Rhamnaceae, is widely distributed in the Middle East, North Africa, and parts of Asia, where it is traditionally used for managing inflammatory disorders, dermatological conditions, gastrointestinal disturbances, and general fatigue. Phytochemical studies have identified significant amounts of flavonoids, triterpenoid saponins, tannins, and phenolic constituents in its leaves, which are believed to account for its antioxidant and anti-inflammatory activities [14, 15]. Experimental findings also suggest that extracts of *Z. spina-christi* may influence oxidative stress markers and hormonal balance, factors that could potentially affect male reproductive function [16]. Despite these indications, data concerning its direct impact on the histological structure of the testis and epididymis remain scarce [17].

Moreover, comprehensive histopathological investigations of these reproductive tissues following exposure to *Z. spina-christi* leaf extract are limited, particularly in rabbits. Rabbits constitute a suitable experimental model due to their well-defined testicular architecture and characterized spermatogenic cycle. Therefore, the present study was undertaken to perform a detailed histological and semi-quantitative evaluation of testicular and epididymal tissues in male rabbits after prolonged oral administration of *Z. spina-christi* leaf extract, to clarify its potential reproductive safety or toxicity.

## Methodology

### Preparation of ZSC Extract

Fresh leaves of ZSC were collected and thoroughly rinsed under running tap water to remove dust and impurities. The leaves were then air-dried at room temperature in a shaded, well-ventilated area to prevent degradation of heat-sensitive constituents. Once completely dried, the plant material was ground into a fine powder using an electric grinder.

A total of 50 g of the powdered leaves was subjected to extraction by maceration in 500 mL of 80% methanol. The mixture was maintained at 4 ℃ for 72 hours with intermittent shaking to facilitate solvent penetration and extraction of bioactive compounds. Following the extraction period, the suspension was filtered through Whatman No. 1 filter paper to remove plant residues. The filtrate was subsequently concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid crude extract. The final extract was stored at −20 °C until further use in the experimental procedures [14].

### Experimental Design and Animal Grouping

The present study was conducted using clinically healthy adult male rabbits weighing between 1.5 and 2.0 kg. Animals were housed under standard laboratory conditions, including a controlled temperature of 22–25 °C, a 12-hour light/dark cycle, and free access to water and a commercially formulated pellet diet. All experimental procedures were carried out in accordance with institutional guidelines for the care and use of laboratory animals.

The rabbits were randomly allocated into two equal groups (n = 5 per group). The control group received distilled water orally for a period of six weeks. The treated group was administered ZSC leaf extract at a dose of 200 mg/kg body weight once daily by oral gavage for six consecutive weeks [18]. Throughout the experimental period, animals were monitored daily to assess their general condition and detect any signs of distress or adverse effects.

At the end of the treatment period, the animals were anesthetized and euthanized humanely. The testes and epididymides were carefully dissected, cleared of adherent connective tissue, and promptly fixed in 10% neutral buffered formalin for histopathological processing [19]. Tissue sections were prepared according to routine histological techniques and examined under a light microscope. Histopathological changes in both organs were evaluated descriptively and graded using a semi-quantitative scoring system: absent (–), mild (+), moderate (++), and severe (+++), as previously described [5, 20]. For each specimen, a minimum of ten microscopic fields was analyzed to ensure a representative assessment.

## Result and Discussion

### Histopathological Analysis of Testicular Tissues

The seminiferous tubules in the testicular sections from the control group have normal histological architecture, according to microscopic analysis (Figure 1). The tubules contained intact spermatogonia, spermatocytes, spermatids, and mature spermatozoa, with a normal basement membrane and regular interstitial spaces (Figure 2). Moreover,

histopathological evaluation of testicular tissues from rabbits treated with ZSC leaf extract for six weeks demonstrated marked alterations compared to the control group. Moreover, the histological assessment revealed marked structural alterations in the testicular tissue. These changes were characterized by degeneration of the seminiferous tubules, a noticeable reduction of spermatozoa within the tubular lumen, distortion and shrinkage of tubular profiles, expansion of the interstitial spaces, and vascular congestion in the testicular tissue (Figures 3 and 4). More detailed examination demonstrated severe cellular damage, including degeneration and necrosis of spermatogonia, reduction in tubular diameter, attenuation of the germinal epithelium, and complete absence of spermatozoa in some luminal areas (Figure 5). Additionally, cellular debris from damaged spermatogenic cells was evident, accompanied by a lack of spermatozoa in the lumen (Figure 6).

At the gross anatomical level, testes from ZSC-treated rabbits displayed severe tissue damage after six weeks of treatment.

**Histopathological Analysis of Epididymal Tissues**

Microscopically, epididymal tissues from the control animal demonstrated typical histological architecture, including ducts with normal epithelial height lined by pseudostratified columnar epithelium, normal epithelial cells, and high sperm density within the lumen (Figures 7 and 8).

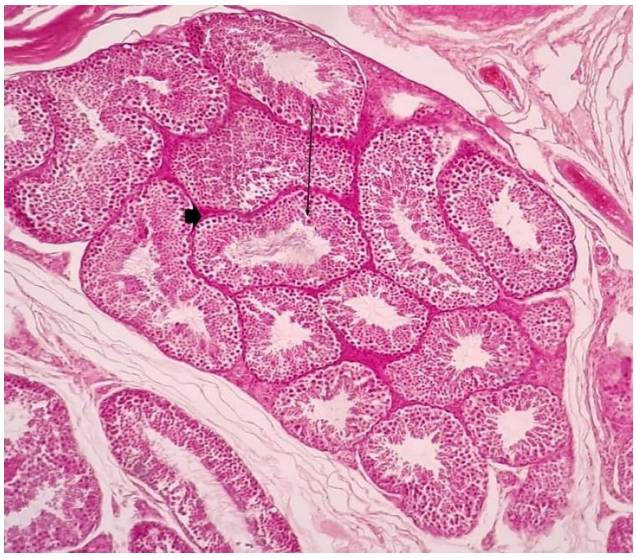

**Figure 1: T**he testis section from control rabbits displays typical seminiferous tubules (arrow) and intact interstitial spaces (thick arrow) (H & E, ×100)

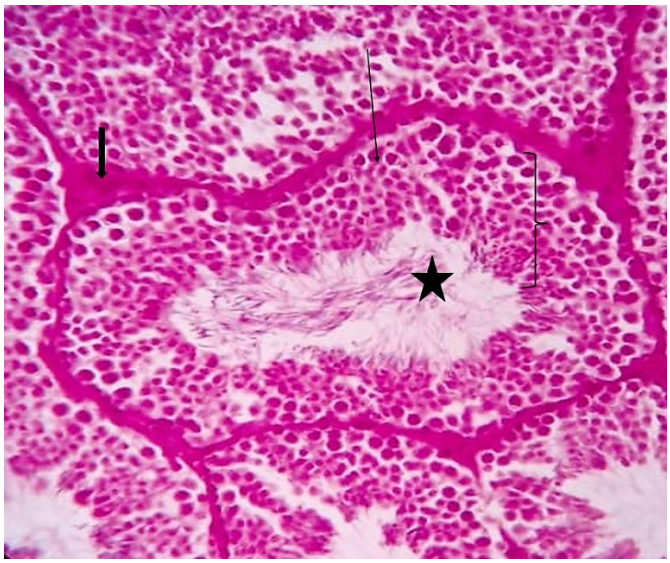

**Figure 2: T**he testis from control rabbits displays intact seminiferous tubules with clearly identifiable spermatogonia (long arrows), primary spermatocytes, spermatids, and spermatozoa (star). Interstitial spaces are normal (thick arrow) (H & E, ×400)

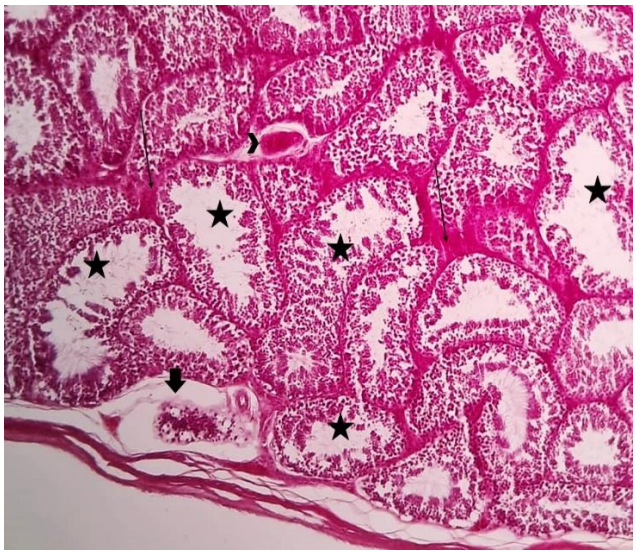

**Figure 3:** The testis section from ZSC rabbits showing degeneration of the seminiferous tubules with reduced spermatozoa in the lumen. The tubules appear markedly shrunken and distorted (stars), the interstitial tissue is expanded (arrow), testicular vessels show congestion (head arrow), and several seminiferous tubules exhibit shrinkage and structural deformation (thick arrow). (H & E, X100)

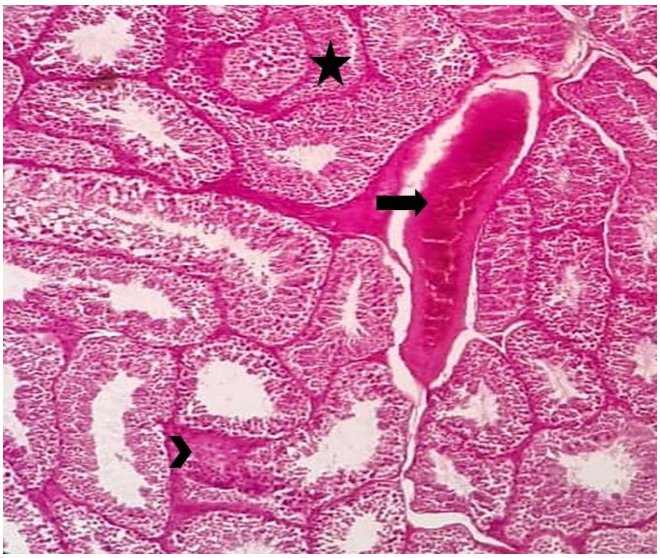

**Figure 4:** The testis section of ZSC rabbits showing marked deterioration of spermatogonial cells lining seminiferous tubules, as well as shrinkage and deformed seminiferous tubules (star), congestion in the testicular vessel (thick arrow), and focal tubular damage (head arrow). (H & E, X100)

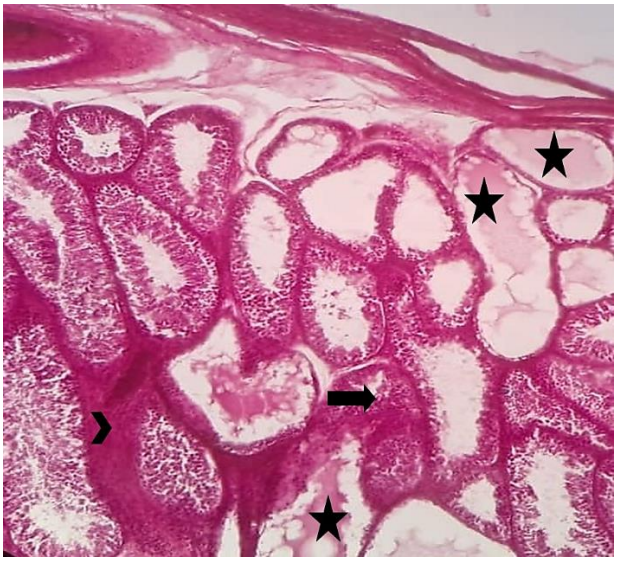

**Figure 5:** The testis section of ZSC rabbits display, marked deterioration and necrosis of spermatogonia cells lining seminiferous tubules (stars), with reduced diameter of seminiferous tubules, and decrease in the thickness of the germinal epithelium with damage of spermatozoa in the lumen, also seen spreading of the interstitial tissue (head arrows), along with shrinkage and deformed seminiferous tubules (thick arrow). (H & E, X100).

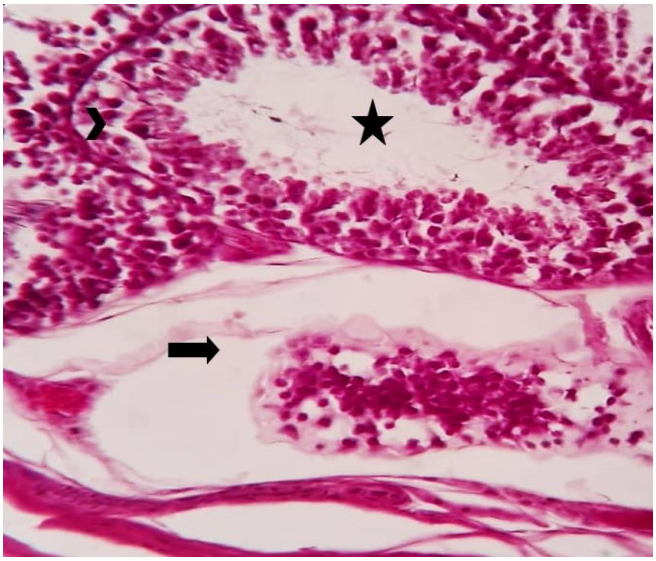

**Figure 6**: The testis section of ZSC rabbits displays deterioration of the seminiferous tubules with debris of damaged spermatogenic cells (head arrow) and absence of spermatozoa in the lumen (stars), and shrinkage and deformed seminiferous tubules (thick arrow). (H & E, X400).

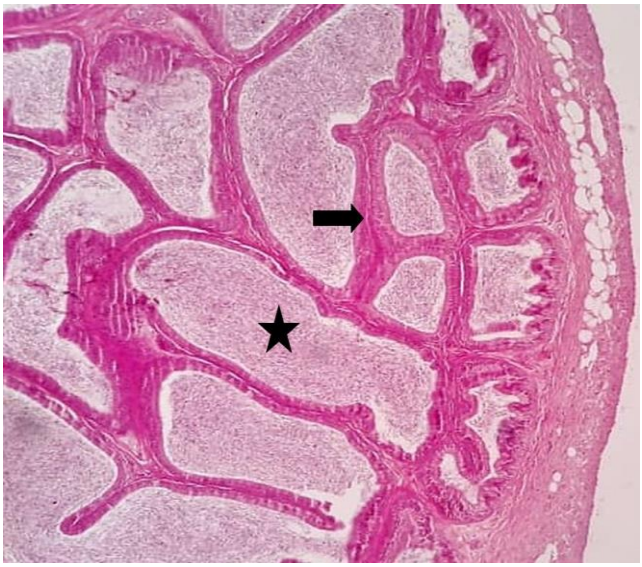

**Figure 7:** The epididymis section of a control rabbit displayed typical histological structure with regular sperm mass (star), regular epithelial cells (arrow) (H & E, X100).

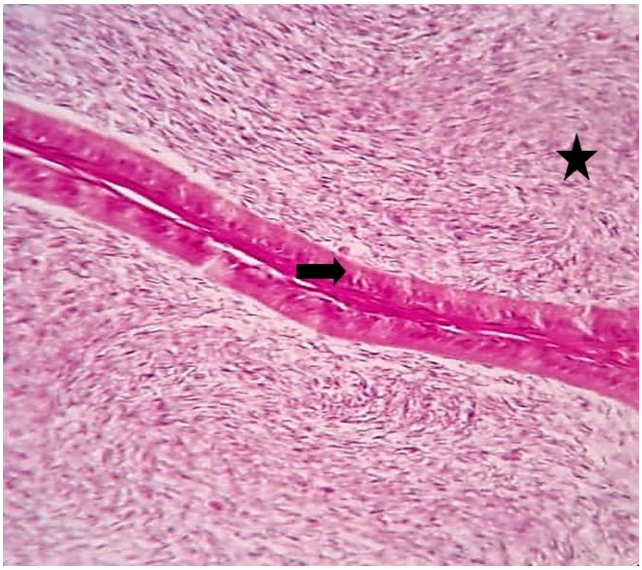

**Figure 8:** The epididymis section of a control rabbit displayed typical histological structure with regular sperm mass (star), and regular epithelial cells (arrow). (H & E, X 400).

In contrast, histological evaluation of the epididymis from rabbits treated with ZSC extract revealed marked pathological alterations. Key observations included partial depletion of sperm within the epididymal ducts, substantial thickening of the surrounding connective tissue, degeneration of the ductal epithelial cells, accumulation of cellular debris in the lumen, distortion of the basement membranes, and overall disruption of the normal ductal architecture (Figures 9 and 10).

Collectively, these findings indicate that ZSC administration for six weeks induces significant histopathological alterations in both testicular and epididymal tissues in male rabbits.

Microscopic examination of testicular tissues from treated rabbits revealed moderate degeneration of seminiferous tubules (++), thinning and disruption of the germinal epithelium (++), mild widening of the tubular lumen (+), vascular congestion (+), tubular shrinkage and distortion (++), and accumulation of cellular debris in the lumen (++). In contrast, control rabbits showed no histopathological alterations (– for all parameters).

Similarly, epididymal tissues from treated rabbits exhibited moderate epithelial degeneration (++), reduced sperm density (++), presence of cellular debris in the lumen (+), and thickening of connective tissue (++). Control animals displayed normal epididymal architecture (–) (Table 1).

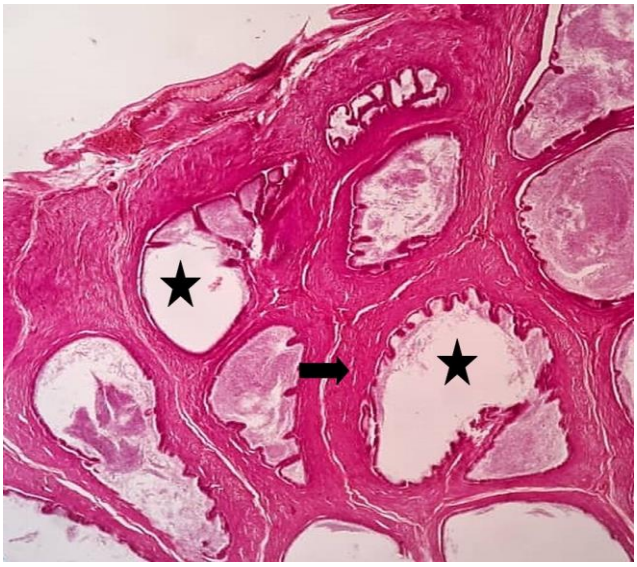

**Figure 9:** The epididymis section of ZSC rabbits displayed some emptying epididymal ducts (stars) with reduced sperm density, and severe thickening of connective tissue (arrow) (H & E, X40).

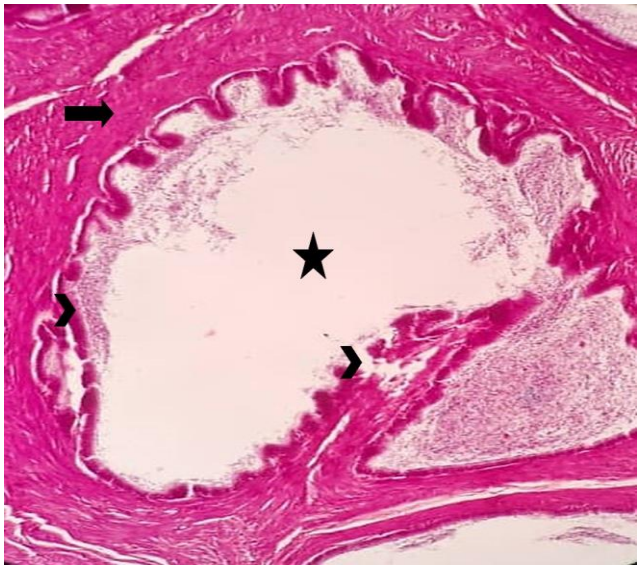

**Figure 10: T**he epididymis section of ZSC rabbits showing degeneration of epididymal ducts (star) containing few sloughed germ cells in the lumen, deterioration of epithelial cells with cellular remains and partial basement membrane (head arrows), and severe thickening of connective tissue (arrow) (H & E, X100).

**Table 1:** Semi-quantitative assessment of histopathological alterations in the testis and epididymis of male rabbits treated with ZSC leaf extract (200 mg/kg, 6 weeks)

| Histopathological alteration | Control | ZSC (200 mg/kg) |
|---|---|---|
| Seminiferous tubule degeneration | – | ++ |
| Germinal epithelium thinning/disruption | – | ++ |
| Luminal widening | – | + |
| Vascular congestion | – | + |
| Tubular shrinkage and distortion | – | ++ |
| Cellular debris in the tubule lumen | – | ++ |
| Epididymal epithelial degeneration | – | ++ |
| Reduced sperm density | – | ++ |
| Epididymal lumen debris | – | + |
| Thickened connective tissue in the epididymis | – | ++ |

**Legend:** – = absent, + = mild, ++ = moderate, +++ = severe.

In the current investigation, testicular tissues from ZSC-treated rabbits exhibited moderate degeneration of seminiferous tubules, thinning and disruption of the germinal epithelium, tubular shrinkage and distortion, vascular congestion, and accumulation of cellular debris within the lumen. Such changes are widely recognized as hallmarks of impaired spermatogenesis and testicular dysfunction. The integrity of the seminiferous epithelium is essential for normal spermatogenic progression, and any structural damage to spermatogonia or supporting Sertoli cells can result in reduced sperm production or complete spermatogenic arrest [21, 22].

The observed thinning of the germinal epithelium and degeneration of spermatogonia in ZSC-treated rabbits may be associated with cellular damage induced by oxidative stress. Previous studies have indicated that excessive exposure to plant-derived bioactive compounds, particularly saponins and phenolic constituents, can disrupt cellular membranes and impair mitochondrial function, leading to apoptosis or necrosis of germ cells [23, 25]. Although ZSC is recognized for its antioxidant properties, prolonged administration or high concentrations may paradoxically exert pro-oxidant effects, particularly in highly sensitive tissues such as the liver and kidney [26]. These findings underscore the need for caution when administering large or extended doses of ZSC.

Vascular congestion observed in the testicular interstitium of treated animals further indicates tissue injury and potential inflammatory responses. Congestion may compromise oxygen and nutrient delivery to the seminiferous tubules, aggravating germ cell degeneration and potentially affecting Leydig cell function, which is critical for testosterone production and maintenance of spermatogenesis [5, 10, 27]. Similar vascular and degenerative alterations have been reported following exposure to other plant extracts rich in flavonoids and saponins, including *Azadirachta indica*, particularly at high doses or prolonged administration [28, 29].

Epididymal tissues from ZSC-treated rabbits also exhibited moderate epithelial degeneration, decreased sperm density, accumulation of luminal debris, and marked thickening of connective tissue. The epididymis plays a crucial role in sperm maturation, storage, and transit, with epithelial integrity being essential for maintaining an optimal luminal microenvironment. Degeneration of the epididymal epithelium may disrupt fluid reabsorption and secretory processes, resulting in reduced sperm concentration and the presence of immature or damaged spermatozoa within the lumen [30, 16, 31]. The reduction in sperm density within the epididymal lumen likely reflects a combination of impaired spermatogenesis in the testis and altered epididymal function [5, 31]. Comparable observations have been reported with other medicinal plant extracts, such as *Withania somnifera* and *Moringa oleifera*, where prolonged exposure led to epithelial degeneration and diminished sperm reserves despite antioxidant benefits at lower doses [12, 13]. These findings support the notion that phytochemicals may exert dual, dose- and duration-dependent effects on male reproductive tissues [9, 13].

The semi-quantitative scoring presented in Table 1 aligns with the microscopic observations, showing moderate (++) severity for most testicular and epididymal alterations, whereas control animals maintained normal histological architecture. This

agreement between descriptive and semi-quantitative assessments reinforces the reliability of the results and highlights the value of histopathological scoring in reproductive toxicology studies [5, 32].

In summary, the histopathological changes observed in both the testes and epididymides suggest that prolonged administration of ZSC leaf extract can adversely affect male reproductive health by disrupting spermatogenesis, impairing epididymal function, and altering tissue architecture. Despite its traditional medicinal use, these findings emphasize the importance of careful and controlled application, particularly regarding dosage and treatment duration. Further investigations integrating hormonal profiling, oxidative stress markers, and fertility assessments are warranted to elucidate the underlying mechanisms of ZSC-induced reproductive effects and to determine safe therapeutic margins.

## Conclusions

Prolonged oral administration of ZSC leaf extract at a dose of 200 mg/kg for six weeks resulted in moderate histopathological changes in the testes and epididymides of male rabbits. Observed alterations included degeneration of the seminiferous tubules, thinning of the germinal epithelium, vascular congestion, and reduced sperm density, collectively indicating compromised spermatogenesis and epididymal function. Semi-quantitative assessment confirmed that control animals maintained normal tissue architecture with no detectable changes. These findings suggest that chronic exposure to ZSC leaf extract may negatively impact male reproductive tissues, highlighting the importance of cautious use and the need for further investigations to establish its reproductive safety.

**Author Contributions**: **Asma** and **Sana:** Conceptualization, Methodology, Data curation, Writing- Original draft preparation. **Eda:** Visualization, Investigation. **Fayrouz Khaled**: Supervision. **Sana, Eda** and **Fayrouz** Software, Validation. **Eda**: Writing- Reviewing and Editing. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest**: "The authors declare no conflict of interest."

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