

Research Article



Effects of Aqueous Extract from *Asparagus officinalis* on the Histomorphological Structure of the Testis in Local Male Rabbits (*Oryctolagus cuniculus*)

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Abstract | The goal of this study was to investigate the effect of *Asparagus officinalis* extract on the male rabbit reproductive system (*Oryctolagus cuniculus*). Thirty adult male rabbits were equally divided into three groups and administered 0, 400, and 800 mg/kg of *A. officinalis* extract, respectively. The testes of rabbits in the control group showed typical histological features, including a tunica albuginea capsule, septa, seminiferous tubules, and interstitial tissue containing Leydig cells, blood vessels, and nerves. However, marked degeneration of the germinal epithelium was observed in the treated groups. In contrast, after 60 days of treatment with asparagus extract (400–800 mg/kg), the rabbit testes displayed well-structured, enlarged seminiferous tubules filled with abundant spermatozoa in the lumen, enhanced Sertoli cells, and better-organized parenchymal tissue with branching trabeculae. Treatment of asparagus extract greatly enhanced ($P \leq 0.05$) body weight, epididymal and testicular morphometry (diameter and length) in comparison with controls. Histological parameters of the treated groups demonstrated hypertrophy of the tunica albuginea (up to $210.26 \pm 2.08 \mu\text{m}$) compared to the control group ($113.33 \pm 2.42 \mu\text{m}$), along with increased prominence of trabeculae, increased thickness of the germinal epithelium, and an increased number of Sertoli cells ($P \leq 0.05$). The high dose show clear appearance of interstitial adipose tissue. These findings collectively demonstrate that *Asparagus officinalis* extract has a remarkable positive modulatory effect on the structure and function of the testis, enhancing spermatogenic efficiency and male reproductive capacity in rabbits.

Keywords | *Asparagus officinalis*, Spermatogenesis, Sertoli cells, Rabbit, Testis

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INTRODUCTION

Asparagus officinalis is an herbaceous plant species that is perennial in nature and typically attains a height of about 100–150 cm. The plant is characterized by its photosynthetic cladodes, small scale-leaves, hollow tuberous roots, and red, toxic berries. It is common in subtropical and tropical areas. Buds are bell-shaped with

color variations from greenish white through yellowish tints, while fruits produce small, toxic red berries (Hamza, 2005; Smith, 2018). It is highly nutritious with regard to proteins, lipids, vitamins, mineral components, and antioxidant chemicals, with content also higher than much of that in most vegetables (Guo *et al.*, 2020). It was used in ancient Chinese medicine with medicinal potential in the suppression of inflammation, malignancies, infections,

and respiratory problems such as cough. The medicinal application of the roots is also significant in the treatment of gout, rheumatism, arthritis, and hypertension (Kumar *et al.*, 2023). It also possesses neuroprotective and anti-aging activities in the form of extracts of the roots that improve cognitive functions such as memory (Al-Saffar and Al-Samawy, 2016; Sui *et al.*, 2017).

Domestic rabbits (*Oryctolagus cuniculus*), descendants of the European wild rabbit, are globally distributed and have been integral to genetic and breeding research since the early 20th century. Males of this species typically attain sexual maturity around 32 weeks of age. As small herbivores, rabbits exhibit selective feeding habits, prioritizing young, nutrient-rich plant material a behavior classifying them as concentrate pickers over coarse, high-fiber vegetation (Cunha and Cheeke, 2012; Onuoha, 2020).

The male reproductive system is charged with the production, maturation, storage, and discharge of spermatozoa. In the rabbit (*Oryctolagus cuniculus*), the testes are round and flat, and they sit in the scrotum between the hind limbs, with the left testis being slightly lower and more posterior than the right. It has a caudal pole that is curved caudolaterally, a penis that is located between the testes, and it is in observable as far as the bursa testicularis is concerned (Dyce *et al.*, 2010; Jawad, 2016; Türkmenoglu and Abacıoglu, 2021).

Histologically, the seminiferous tubules contain well-ordered layers of germ cells in progressive stages of spermatogenesis. Spermatogonia, the most primitive germ-line, exist in intimate association with the basal membrane and proliferate by mitosis to form primary spermatocytes.

These larger cells undergo their first meiotic division to give origin to secondary spermatocytes, while these latter, in turn, rapidly pass through their second meiotic division to give origin to spermatids. Mature spermatids of the advanced stages contain small, oval to elongated dark nuclei with long tails projecting into the tubular lumen. Spermatids, as they exit the lumen, change into mature spermatozoa (Zamora *et al.*, 2014; Setyawati *et al.*, 2018; Amao and Oladele, 2016; Onuoha, 2020). This study was carried out in order to assess the effects of aqueous *Asparagus officinalis* extracts on testicular histomorphology in local male rabbits and to compare structural change with that of the control.

MATERIALS AND METHODS

PREPARATION OF *ASPARAGUS OFFICINALIS* EXTRACT

Fresh *Asparagus officinalis* rhizomes were acquired from a local Baghdad Province, Iraq, herbal market. The sliced rhizomes were dried in the shade for seven days and then mechanically comminuted into a fine powder with an industrial electric blender (Figure 1). To prepare the aqueous extract, 15 g of dried *Asparagus officinalis* rhizome powder was steadily added to 1500 mL of distilled water and stirred with a magnetic stirrer at room temperature for six hours with no external heat supplied. It was left to stand at room temperature for 24 hours, and then another stirring was conducted, and finally filtered with the help of Whatman cellulose filter papers. The filtrate was then condensed in a water bath with a temperature range of 40–50 °C to obtain a semisolid mass, and thereafter stored in a clean glass bottle at 4 °C for subsequent use (Sharma *et al.*, 2012; Hammodi and Hamza, 2022).



Figure 1: The steps of preparing *A. officinalis* powder: (A) dried *A. officinalis*; (B) powdered *A. officinalis*; (C) *A. officinalis* extract.

EXPERIMENTAL ANIMALS

Thirty adult male rabbits, in the range of about 1368–1398 g in weight and aged between 6 months and 1 year, were collected from a local market. The experimental protocol was sanctioned by the Animal Ethics Committee of Baghdad University, Iraq. Before experimentation, the rabbits were also acclimatized for two weeks to laboratory conditions. Animals were reared in cages with a maximum of two rabbits per cage and were reared in ordinary laboratory conditions with a maximum of 25 ± 2 °C temperature and a 12-hour light/dark photoperiod. During the course of the study, the animals were provided with free access to standard pellet feed and drinking water, and sterile and hygienic conditions were stringently followed.

The rabbits were randomly divided into three groups ($n = 10$ per group). The control group (Group 1) received oral administration of distilled water (1 mL/kg body weight) for 60 days. Group 2 received *A. officinalis* extract at a dose of 400 mg/kg body weight orally for 60 days, while Group 3 received *A. officinalis* extract at a dose of 800 mg/kg body weight orally for the same duration.

The solutions were ready by dissolution of the dried extract in distilled water to make concentrations of 400 mg and 800 mg/1 mL, and they were kept at 4 °C until being used (Zhu *et al.*, 2010).

SAMPLE PREPARATION AND HISTOLOGICAL PROTOCOL

Following injection of the euthanasia solution, the testes were removed with care from the anterior to the posterior margin and rinsed once with normal saline solution to rid them of attached fat tissue (Hasan and Hamza (2021)). The testes were weighed and morphometric measurements were taken. Then they were transferred into a large volume of fixative solution, i.e., Bouin's solution, in such a manner that the volume of the solution, at least, was ten times that of the tissue portion for reducing postmortem histological changes. The portions were cut into minute fragments with the help of a fine scalpel. The cut portions were transferred into containers with Bouin's solution (Latendresse *et al.*, 2002). To prepare tissue slides, the portions were treated with running water for the removal of excessive fixative in order to prevent interference with the subsequent processing procedure, as well as staining with Hematoxylin and eosin (H&E), and staining with Masson's Trichrome stain (Nazar and Al-Aaraji, 2024; Obead and Hamza, 2025).

STATISTICAL ANALYSIS

The data of various experimental groups were subjected to statistical analysis in order to determine the magnitudes of the treatment effects, along with significant differences among concentrations of extracts. Several comparisons

of groups were carried out by using one-way analysis of variance (ANOVA), for which a P -value ≤ 0.05 was taken as an index of statistical significance (Steel and Torrie, 1981).

RESULTS

The morphological assessment revealed that the male genital system of the rabbit is comprised of paired testes contained in specific scrotal sacs, as well as the epididymis, vas deferens, accessory sex glands, urethra, and penis. Two testes were located in separate compartments of the scrotum, a fibromuscular sac that is separated by a median septum and located bilaterally on the ventral midline between the preputial orifice and the anus. Two testes were covered externally by the tunica vaginalis, a membrane that lines the scrotal sac, and internally by the tunica albuginea, a thick, fibrous capsule that prevents excessive pressure from forming. The epididymis was located posterolateral to the testes and consisted of three anatomical regions. The caput (head) appeared as a flattened, expanded tube firmly attached to the cranial end of the testis, formed by conical lobules originating from the efferent ductules. The corpus (body) presented as a narrow cord running along the posterolateral testicular border, distinguished by its loose attachment via a distinct mesenteric fold the mesoepididymis which allowed it to be easily elevated. The cauda (tail) was a curved, knuckle-like structure at the caudal pole, resembling a pendulous nodule that distinctly transitioned into the thick-walled vas deferens, a defining feature confirming its identity. These anatomical divisions were essential for sperm maturation, concentration, and storage. Additionally, observations revealed age-related differences in testicular size and morphology, indicating progressive development of spermatogenic activity (Figure 2).

At experimental termination, mean weights of control and *A. officinalis* extract-treated rabbit reproductive organs at 400 mg/kg and 800 mg/kg were tabulated in Table 1. Both treated groups showed a significant ($P \leq 0.05$) gain in body weight relative to controls. Considerable increases in the paired testicular and epididymal weights were also observed in Asparagus-treated rabbit males. Furthermore, testicular length and diameter were substantially increased in treated groups relative to controls (Table 1), supporting the possibility that *Asparagus officinalis* extract enhances gonadal accretion and architectural maturation in male rabbits.

Histologically, the testes of the control rabbits in the present study displayed the expected tissue architecture. This included a dense fibrous capsule (the tunica albuginea) covered by mesothelium. Extending radially from the inner

surface of the tunica albuginea were septal projections that divided the testicular parenchyma into numerous lobules, which were roughly quadrilateral or triangular in shape. Each lobule contained multiple convoluted seminiferous tubules the primary sites of spermatogenesis embedded within a thin interstitial stroma of loose connective tissue. This stroma not only demarcated adjacent lobules but also provided the structural support for Leydig cells, blood vessels, and nerves (Figure 3).

In addition, Histological inspection revealed a dose-dependent improvement in testicular architecture following 60-day administration of asparagus extract (400mg/kg and 800 mg/kg). Treated groups exhibited marked spermatogenic hyperplasia, characterized by dilated

seminiferous tubules with a highly organized, stratified epithelium comprising spermatogonia, spermatocytes, and abundant luminal spermatozoa anchored to Sertoli cells, as shown in Figure 4. The Sertoli cells exhibited complex cytomorphology with cytoplasmic projections, suggesting enhanced supportive and barrier functions. The parenchymal organization was more advanced, with branching of the trabeculae which carried the requisite vascular and neural supply and a greater density of tubular parts. In contrast, the control group exhibited degeneration of the germinal epithelium, characterized by extensive vacuolization, the presence of apoptotic cells, and a marked decrease in mature spermatozoa within the seminiferous tubule lumina, as shown in Figure 5.

Table 1: Effect of Asparagus extract in different concentrations on the morphometric parameter of testes.

Parameters	Groups		
	Control	400 mg/kg	800mg/kg
Animal weight before administration (g)	1398.50 ± 15.2 ^a	1370.17 ± 12.5 ^b	1368.55 ± 14.8 ^c
Animal weight after administration (g)	1718.70 ± 18.3 ^c	1896.27 ± 16.7 ^b	1994.78 ±20.1 ^a
Increase in weight (g)	320.20 ± 10.5 ^c	526.10 ±12.3 ^b	626.23 ±14.6 ^a
Weight of right and left testes (g)	3.52 ±0.18 ^c	4.61 ± 0.15 ^b	4.88 ± 0.16 ^a
Testicle length (mm)	29.04 ± 0.85 ^c	30.21 ± 0.92 ^b	33.09 ± 0.88 ^a
Testicle diameter (mm)	7.92 ± 0.25 ^c	8.63 ± 0.22 ^b	9.28 ± 0.24 ^a
Testicle weight to body weight ratio (%)	0.20 ± 0.03 ^b	0.24 ± 0.03 ^a	0.23 ± 0.03 ^a
Weight of right and left epididymis (g)	0.471 ± 0.03 ^c	0.488 ± 0.02 ^b	0.497 ± 0.03 ^a

Values are presented as mean± SEM for 10 rabbits (n=10). Different superscript letters in a row refer to significant difference between the treatments (P≤ 0.05).

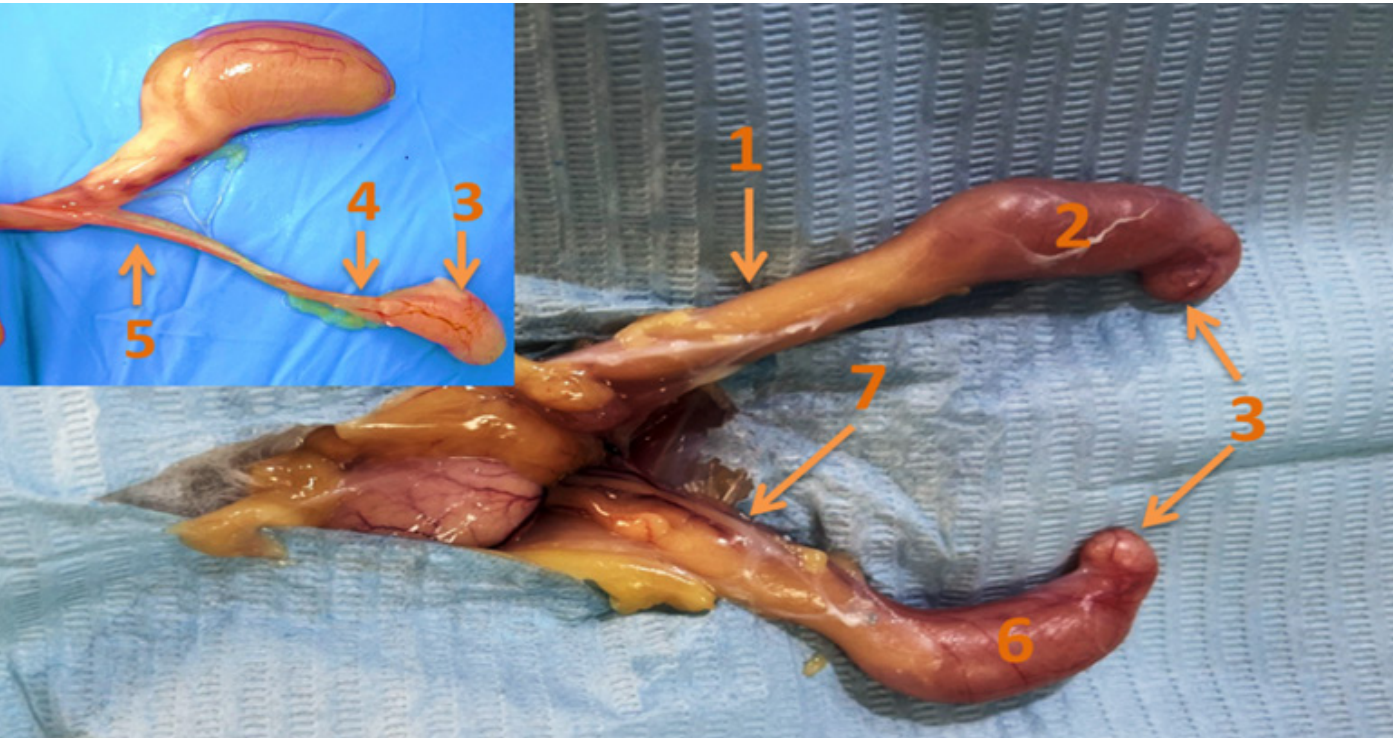


Figure 2: A photograph of rabbit testes showed: cremaster muscle (1), left testes (2), caput epididymis (3), corpus epididymis (4), cauda epididymis (5), right testes (6), vas deferens (7).

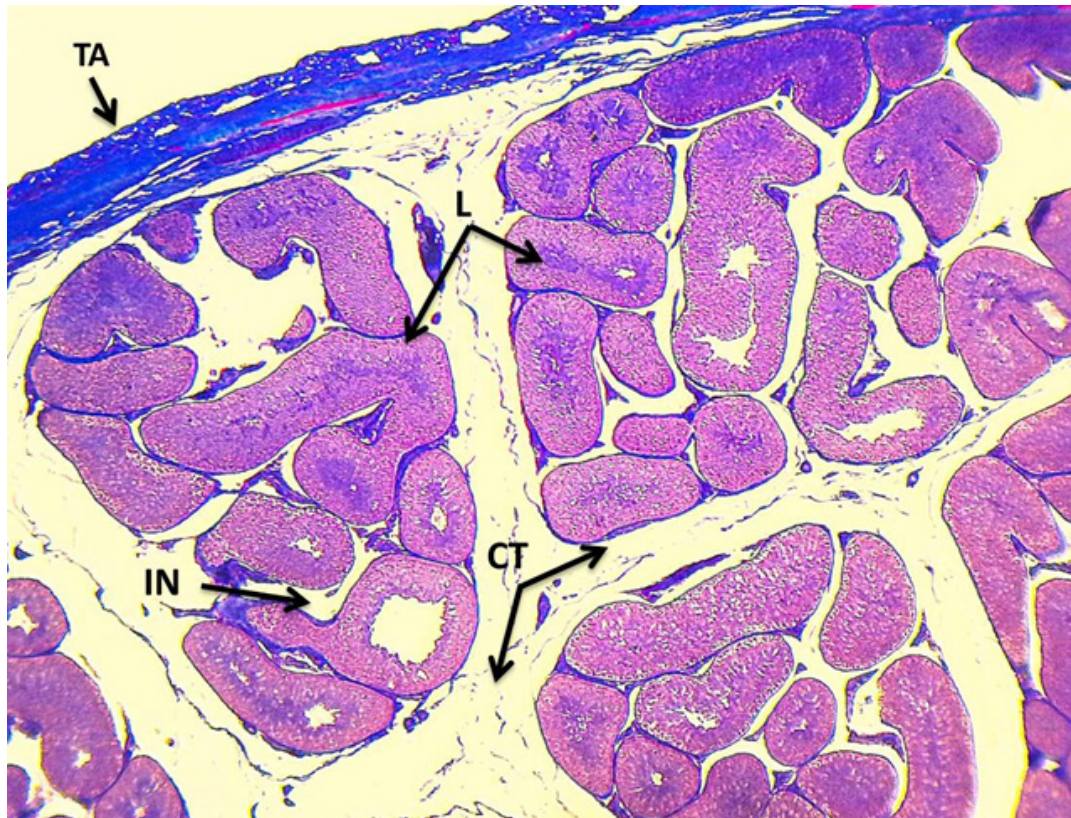


Figure 3: Histological architecture of testes showed many partitions projecting from the connective tissue capsule (CT), to separate the parenchyma into highly number lobules (L). Each lobule comprises different numbers of ST; the interstitial tissue (IN) lobules appeared either quadrilateral or triangular in shape (Control group); Masson's trichrome stain 100x.

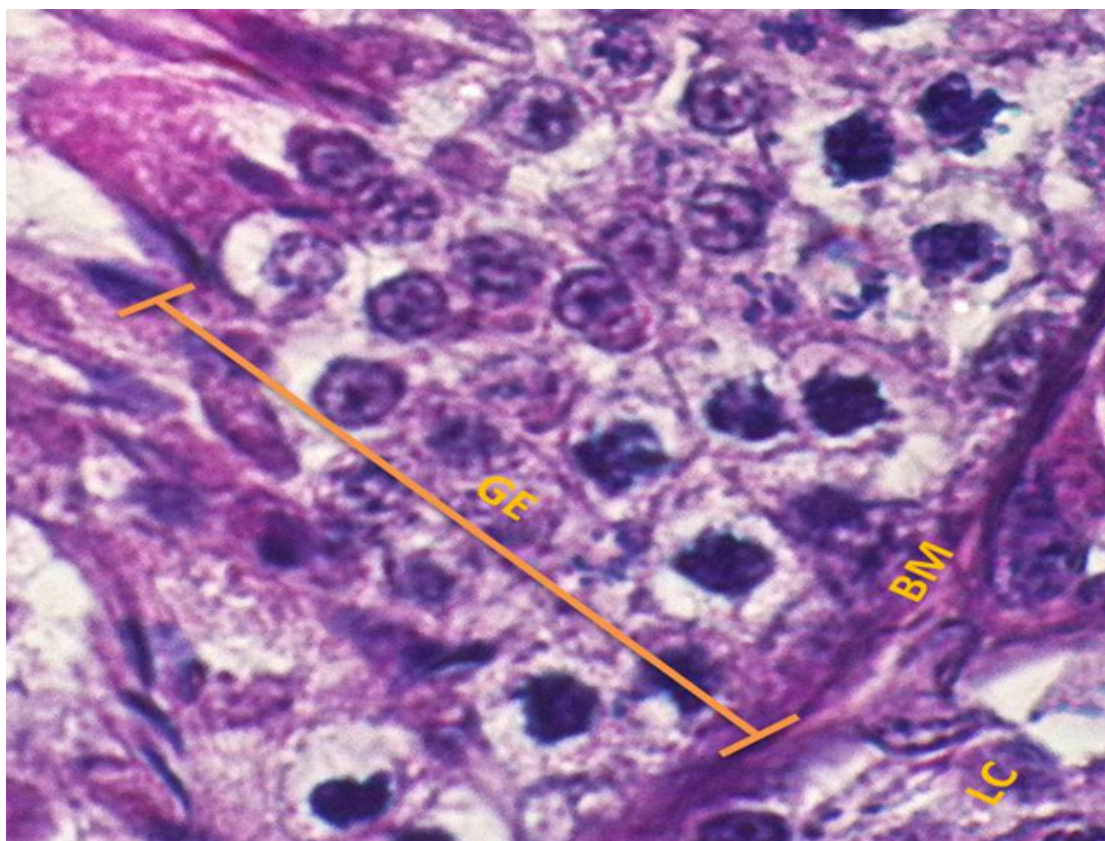


Figure 4: Histological section of testes exhibited spermatogenic hyperplasia, characterized by dilated seminiferous tubules, and abundant luminal spermatozoa anchored to Sertoli cells, H&E stain 600x (400mg/kg group).

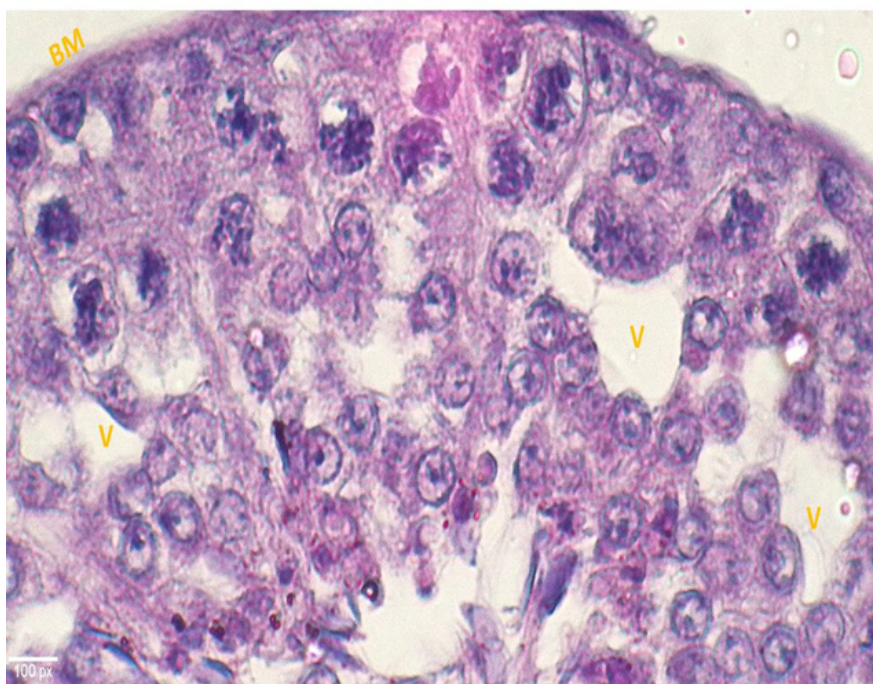


Figure 5: Histological section of testes displayed germinal epithelial degeneration, evidenced by extensive vacuolization, apoptotic figures, and a pronounced reduction in mature spermatozoa, H&E stain 600x (Control group).

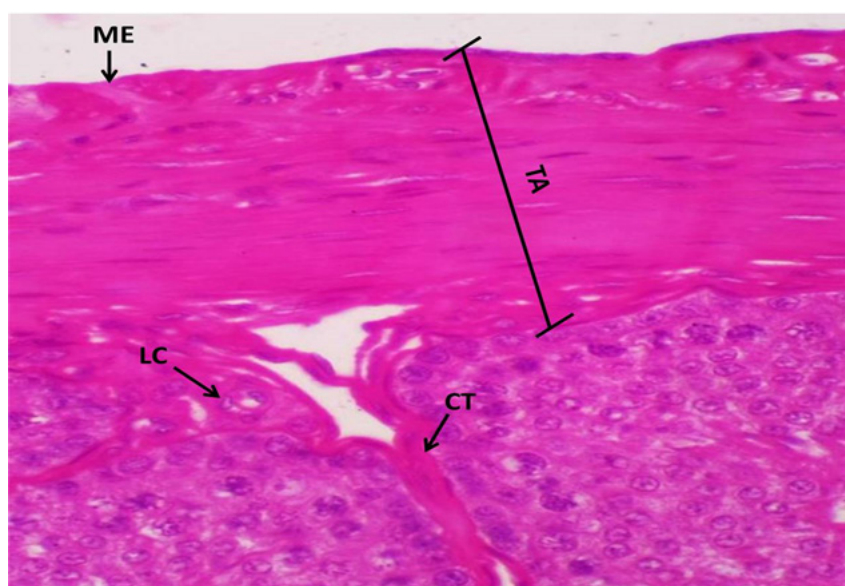


Figure 6: Histological architecture of testes characterized by a dense fibrous capsule composed of connective tissue bundles (CT); tunica albuginea (TA) covered by mesothelium (ME), H&E stain 400x (400mg/kg group).

Quantitative histological analysis shown that treatment significantly altered the thickness of the dense, regular connective tissue capsule (*Tunica albuginea*). The thickness of the capsule was $113.33 \pm 2.42 \mu\text{m}$ in the control group, but was significantly elevated to $147.96 \pm 2.58 \mu\text{m}$ and $210.26 \pm 2.08 \mu\text{m}$ in the second and third treated groups, respectively, as seen in Table 2 and Figure 6.

Qualitative examination of the treated testes revealed distinct septal branching and a higher density and greater number of germinal epithelial cells across various stages of spermatogenesis within the seminiferous tubules. In

the testis parenchyma, which is largely composed of convoluted seminiferous tubules, treated groups also exhibited a statistically significant increase in seminiferous tubule diameter (μm) in comparison with control animals, as seen in Table 2 and Figure 7. Furthermore, the study demonstrated a significant increase in the germinal epithelium thickness (μm) and the mean number of Sertoli cells per seminiferous tubule in the treated groups, as seen in Table 2. Notably, the second experimental group also showed a qualitative predominance of adipose tissue in the interstitial spaces, as seen in Figure 8.

Table 2: Effect of Asparagus extract in different concentrations on histological parameters of testes.

Parameters	Groups		
	Control	400 mg/kg	800mg/kg
Capsule thickness (μm)	113.33 $\pm$ 2.42 ^c	147.96 $\pm$ 2.58 ^b	210.26 $\pm$ 2.08 ^a
Diameter of seminiferous tubule (μm)	232.40 $\pm$ 2.36 ^a	212.56 $\pm$ 2.25 ^b	214.89 $\pm$ 1.44 ^b
Germinal epithelium thickness (μm)	73.02 $\pm$ 1.34 ^c	84.35 $\pm$ 1.25 ^b	89.76 $\pm$ 1.45 ^a
Mean number of Sertoli cells/80 seminiferous tubule	247.00 $\pm$ 4.21 ^c	312.89 $\pm$ 3.80 ^a	255.06 $\pm$ 4.00 ^b

Values are presented as mean $\pm$ SEM for 10 rabbits (n=10). Different superscript letters in a row refer to significant difference between the treatments ($P \leq 0.05$).

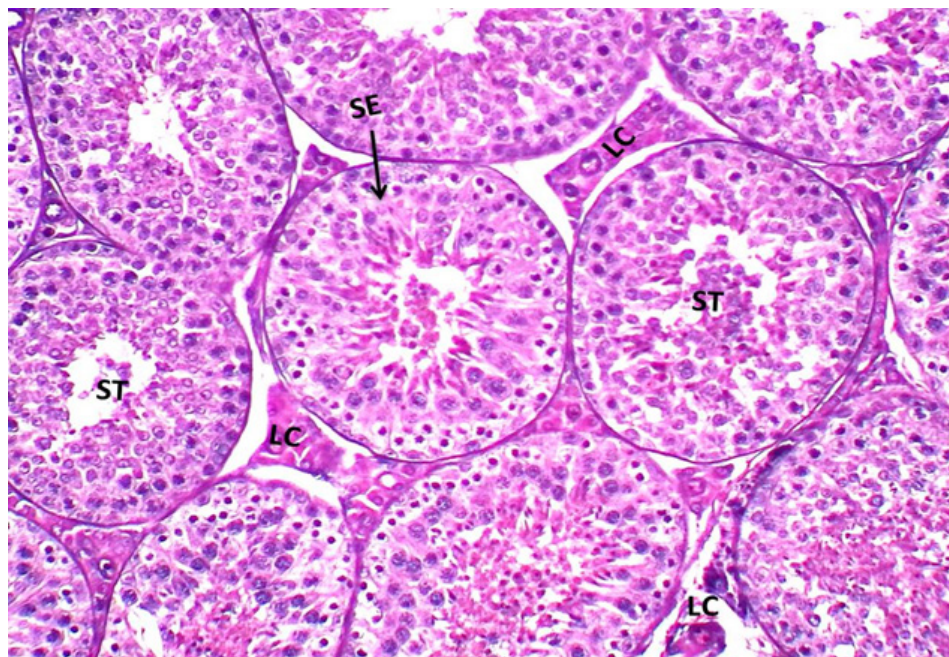


Figure 7: The testicular parenchyma formed by convoluted seminiferous tubules, the germinal epithelium of seminiferous tubule and Sertoli cells H&E stain 200x (800mg/kg group).

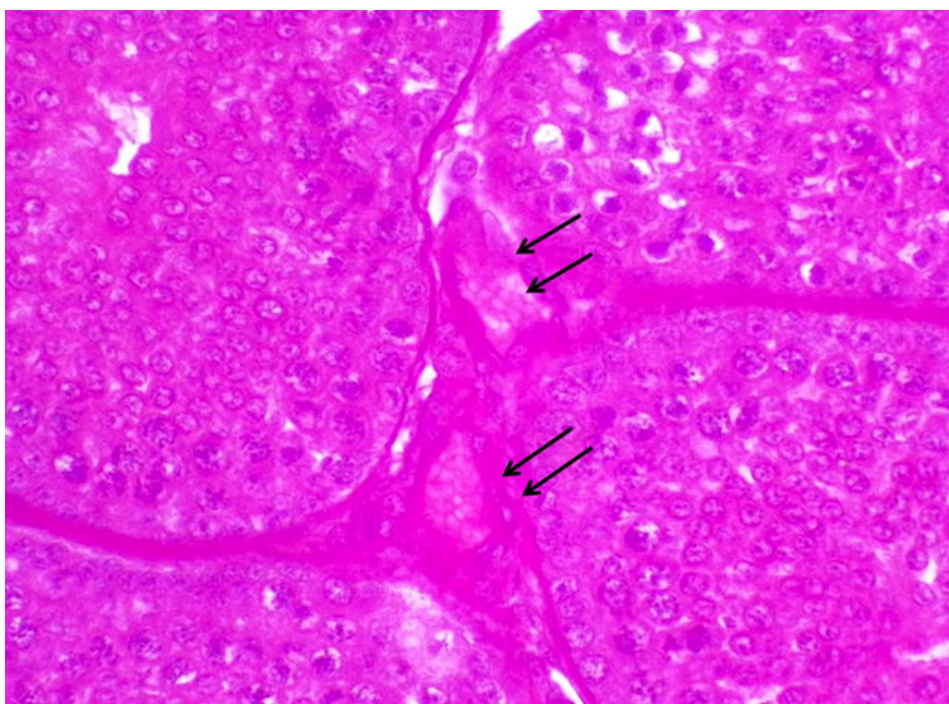


Figure 8: Transverse section of the testis from the 800 mg/kg *Asparagus officinalis* extract-treated group showing adipose tissue distributed within the interstitial spaces among seminiferous tubules. H&E stain 400x (400mg/kg group).

Asparagus officinalis extract administration in doses of 400 mg and 800 mg led to a significant increase in body weight, epididymal and testicular weight, and the testicular diameter and length of treated rabbits compared to the control. Increases suggest the presence of androgenic and spermatogenic activity of the extract, which may be responsible for enhanced male reproductive activity (Kumar *et al.*, 2010). This rise in body weight reported in treated animals is consistent with reports recording the occurrence of biologically active molecules, including saponins, flavonoids, and steroidal glycosides, in *A. officinalis*, commonly known to enhance metabolic activities and stimulate maximum nutrient uptake (Hafizur *et al.*, 2012).

The observed testicular and epididymal weights increase which indicates to stimulation of spermatogenic and steroidogenic activities, possibly refereed by elevated testosterone secretion or enhanced gonadotropin signaling. Comparable outcomes were reported by Gauthaman *et al.* (2002), who demonstrated that certain herbal preparations with aphrodisiac potential increased testicular mass and sperm production in rats. Similarly, Sharma *et al.* (2009) documented that *Asparagus racemosus* enhanced male reproductive performance through modulation of the hypothalamic–pituitary–gonadal (HPG) axis, resulting in elevated secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Additionally, the enlargement in testicular length and diameter among treated rabbits may refers to seminiferous tubule hypertrophy and increased in Leydig cell function, both of which are essential for efficient testosterone biosynthesis (De Soya, 2007).

The increase in epididymal weight indicates enhanced sperm maturation and storage, as the epididymis is essential for sperm functional development (Akbarsha *et al.*, 2015). *Asparagus* plant extracts rich in antioxidant which is improve testicular morphology by reducing oxidative stress, a major contributor to impaired spermatogenesis (Sengupta, 2018). *Asparagus* species, rich in polyphenols and ascorbic acid, may protect testicular cells from lipid peroxidation and DNA damage, supporting spermatogenesis (Gupta *et al.*, 2013).

Asparagus officinalis extract effects likely involve multiple mechanisms: (1) Antioxidant activity: quercetin, rutin, and glutathione scavenge free radicals, safeguarding Sertoli and Leydig cells (Kumar *et al.*, 2010); (2) Hormonal modulation: Bioactive compounds may stimulate GnRH release, enhancing testosterone synthesis (Mishra, 2016); (3) Improved testicular microcirculation: enhanced pampiniform plexus function supports thermoregulation

and nutrient delivery, promoting spermatogenesis (Setchell, 1998).

The histological profile of the control group testes in the present study delineates the canonical architecture essential for mammalian spermatogenesis. The presence of a robust tunica albuginea, comprised of dense irregular connective tissue, and its projecting septa inward which is create parenchyma, a structural motif conserved across species that provides mechanical integrity and subdivides the testis into functional units (lobules) (Ross and Pawlina, 2018). Within these lobules, the seminiferous tubules serve as the exclusive site for spermatogenesis, a fact well documented in lagomorphs and other mammals (Hess and de Franca, 2008). The interstitial compartment, bounded by a thin layer of loose connective tissue and characterizes by polygonal (triangular or quadrilateral) profiles, is not merely passive stromal filler. It has large surface area, for paracrine signaling and is critically specialized to harbor Leydig cells, the primary source of testosterone, alongside the vascular and neural supplies (Davidoff *et al.*, 2009; Aumüller, 2009). The integrity of interstitial space is important for steroidogenesis, as the endocrine output of Leydig cells directly governs spermatogenic efficiency and the maintenance of secondary sexual characteristics (Sharpe, 1994). Consequently, the control group histoarchitecture observed is confirms an intact morphologically and functionally competent state, providing a baseline to experimental or pathological alterations can be assessed.

The quantitative data demonstrated a dose-dependent and significant thickening of the tunica albuginea, increasing from 113.33 μm in the control group to 210.26 μm in the high-dose group, providing clear histological evidence of treatment-induced testicular fibrosis. This is remodeling of the dense connective tissue capsule is consistent with the activation of fibroblasts and excessive deposition of collagen, a process widely recognized to be primarily mediated by the pro-fibrotic cytokine TGF- β 1 (Meng *et al.*, 2016). The capsular fibrosis may be compromise testicular function by increasing tissue stiffness and altering biomechanical properties, as fibrosis in other organs is known to disrupt normal tissue compliance and function (Henderson *et al.*, 2020).

The hyper-stimulation of testicular parenchyma architecture as result to treatment induced which observed through the qualitative and quantitative collectively, so the increased branching of trabeculae, expansion of seminiferous tubule diameter, and thickening of the germinal epithelium all point towards an enhanced development of the spermatogenic compartments. This is further supported by the significant increase in Sertoli cell count, which is provide the essential structural and

nutritional support for germ cell development, and their population is a key determinant of spermatogenic output (Sharpe *et al.*, 2003).

The concurrent enlargement of tubules and epithelium suggests a potential increase in the spermatogenic activity or a disruption of the normal spermatogenic cycle, leading to tubular dilation. However, the notable presence of adipose tissue in the interstitium of the second group introduces a conflicting pathological element, as adipocyte infiltration is often associated with endocrine disruption and impaired testicular function (Sengupta, 2018). This combination of hypertrophic changes alongside ectopic fat deposition creates a complex picture, suggesting that the treatment may be simultaneously stimulating tubular components while potentially disrupting the metabolic and endocrine homeostasis of the testicular interstitial environment, which is vital for Leydig cell function and steroidogenesis (O'Hara and Smith, 2015).

CONCLUSION

Administration of *A. officinalis* extract at doses of 400 mg/kg and 800 mg/kg produced positive effects in male rabbits, including increases in body weight, testicular and epididymal weights, and testicular dimensions. Histological improvements were also observed, specifically in the thickness of the tunica albuginea, the diameter of the seminiferous tubules, the condition of the germinal epithelium, and the number of Sertoli cells. These beneficial effects are likely mediated through antioxidant activity, endocrine regulation, and enhanced testicular microcirculation. Collectively, the findings indicate that aqueous extract of asparagus can improve testicular structure and function, positioning it as a potential natural agent to support male fertility. However, further studies are required to elucidate its mechanisms of action and to establish its long-term safety profile.

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NOVELTY STATEMENT

This study offers the first detailed histomorphological and morphometric characterization of the positive modulatory effects of *Asparagus officinalis* extract on the rabbit testis (*Oryctolagus cuniculus*). Moving beyond basic functional

data, this research uncovers precise structural enhancements such as significant increases in germinal epithelium thickness, tunica albuginea hypertrophy, and Sertoli cell proliferation. Furthermore, it documents distinct tissue level adaptations, such as interstitial adipose deposition at high doses, providing an essential structural framework for utilizing this botanical extract to safely enhance male reproductive capacity

AUTHOR'S CONTRIBUTION

L.O.H. and M.A.A. conceived and designed the study. I.A.A. and M.A.A. performed the experiments, collected the samples, and carried out the laboratory analysis. L.O.H. and I.A.A. analysed the data and interpreted the results. I.A.A. drafted the initial manuscript. L.O.H. and M.A.A. critically reviewed and edited the manuscript for important intellectual content. All authors read and approved the final manuscript.

ETHICAL STATEMENT

Experimental commencement was preceded by formal approval from the local Animal Care and Use Committee at the University of Baghdad's College of Veterinary Medicine (Approval Reference: P.G/2083, 28 October 2024).

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The authors declare that they have not received fund.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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