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Effects of *Pongamia pinnata* (L.) Stem Bark Extract on Sperm Parameters and Epididymal Histoarchitecture in Male Wistar Rats

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ABSTRACT

Background: *Pongamia pinnata,* commonly referred to as Karanja, is a medicinal plant extensively employed in traditional systems of medicine for managing a variety of ailments.
Objective: This study aimed to investigate the antifertility potential of a 50% ethanolic stem bark extract of *P. pinnata* by assessing its effects on epididymal morphology, sperm characteristics, and reproductive performance in adult male Wistar rats.
Methods: Twenty-eight healthy adult male Wistar rats were randomly allocated into four experimental groups (n = 7 per group). Animals in the control group received only the vehicle, whereas the treatment groups were administered the 50% ethanolic stem bark extract orally at doses of 100, 200, and 300 mg/kg body weight daily for 60 consecutive days. Following the treatment period, epididymal weights were determined, and the tissues were processed for histopathological and histomorphometric evaluation. In addition, sperm parameters and fertility outcomes were assessed.
Results: Treatment with the stem bark extract produced a dose-dependent decline in epididymal weight, epithelial cell height of the cauda epididymis, and sperm indices, including sperm count, motility, and viability, when compared with the control group. Reproductive performance was also markedly impaired, as evidenced by significant reductions in fertility index and litter size. Histological examination further demonstrated degenerative changes in the epididymal tissue, indicating structural damage associated with extract administration.
Conclusion: Prolonged oral administration of the 50% ethanolic stem bark extract of *P. pinnata* adversely affected epididymal architecture and function, leading to compromised sperm quality and reduced fertility in male rats. These findings suggest that the stem bark extract possesses promising male antifertility activity and may serve as a potential source for the development of plant-derived male contraceptive agents.

Keywords: Antifertility; sperm parameter; *Pongamia pinnata;* epididymis; litter size

INTRODUCTION

The rapid growth of the global population has emerged as one of the most serious challenges of the modern era, particularly in developing countries. Uncontrolled population expansion places immense pressure on natural resources and infrastructure, adversely affecting economic development, social welfare, and environmental sustainability. It contributes to

several global problems, including poverty, malnutrition, resource depletion, environmental pollution, climate change, biodiversity loss, and social conflicts. As population growth continues to influence nearly every aspect of human development, the regulation of fertility through safe, effective, and acceptable contraceptive methods has become a major public health and socioeconomic priority. [1,2]

According to recent estimates, it reached approximately 8.2 billion in 2024 and is projected to increase to about 10.3 billion by the mid-2080s [3,4] Since the end of the Black Plague, the world's population has grown steadily due to improvements in healthcare, sanitation, agriculture, and living standards. [5] With a population of more than 1.4 billion, India is currently the most populated nation in the world. This continuous population growth has intensified concerns over resource availability, environmental sustainability, and socioeconomic development, emphasizing the need for effective population control strategies. [6]

Regulation of fertility has been practiced since ancient times, with medicinal plants and their extracts being widely used as natural contraceptives. Conversely, numerous synthetic contraceptive agents and environmental chemicals have been identified as endocrine-disrupting substances that can interfere with the normal functioning of the endocrine system. These agents may alter the synthesis, secretion, transport, metabolism, receptor binding, or biological action of endogenous hormones, thereby disturbing hormonal homeostasis. Such disruptions can have detrimental effects on multiple physiological processes, particularly reproductive, neurological, and metabolic functions, ultimately contributing to adverse health outcomes. [5] These concerns have increased interest in developing plant-based contraceptives that are effective, safe, reversible, and associated with minimal adverse effects. Such contraceptive options would be more widely accepted across different populations and could play an important role in controlling population growth. [6-8]

Several targets within the male reproductive system can be exploited for the development of novel male contraceptives. These include suppression of spermatogenesis in the testes, inhibition of sperm maturation in the epididymis, prevention of sperm transport, interference with sperm capacitation, and disruption of sperm functions required for successful fertilization. [4] Medicinal plants with antifertility potential may act through one or more of these mechanisms. In addition, many plant-derived compounds have been reported to reduce sperm count, motility, and viability, thereby impairing male fertility. Consequently, numerous medicinal plants have been investigated for their antifertility efficacy, with varying degrees of effectiveness. [9,10]

Medicinal plants have gained significant attention as potential sources of fertility-regulating agents due to their affordability, accessibility, and lower risk of adverse effects compared to synthetic contraceptives. As traditional medicine remains the primary healthcare system for nearly 70–80% of the global population, particularly in developing countries, phytochemical-rich medicinal plants offer promising candidates for the development of novel herbal contraceptives. [11,12]

Pongamia pinnata (Linn.) Pierre, commonly known as Karanja in Hindi and Indian beech in English, is a medium-sized glabrous tree belonging to the family Fabaceae. It is widely distributed in the tidal forests of India and has been extensively used in the traditional Indian system of medicine for the treatment of bronchitis, whooping cough, rheumatic arthritis, and diabetes. Numerous pharmacological studies have established that *P. pinnata* possesses a broad range of biological activities, including antioxidant, antimicrobial, antiparasitic, anti-inflammatory, anticonvulsant, antidiabetic, antihyperammonemic, cytotoxic, anthelmintic, insecticidal, and immunomodulatory effects. [13,14]

Phytochemical investigations have demonstrated that *P. pinnata* contains a wide variety of biologically active constituents. Among these, flavonoids and their derivatives, including flavones, flavans, and chalcones, are the most abundantly isolated compounds. In addition, the plant contains several other classes of phytochemicals, such as sesquiterpenes, diterpenes, triterpenes, steroids, amino acids, disaccharides, fatty acids, and ester compounds. [14,15] Furthermore, seven flavonoids—pongaflavone, karanjin, pongapin, pongachromene, 3,7-dimethoxy-3′,4′-methylenedioxyflavone, millettocalyxin C, and 3,3′,4′,7-tetramethoxyflavone have been isolated from the stem bark of *P. pinnata*. [15,16]

Although *P. pinnata* occupies a significant place in Ayurvedic medicine and traditional therapeutic practices, scientific evidence regarding its influence on male reproductive function remains limited. Therefore, the present investigation was undertaken to assess the contraceptive potential of the 50% ethanolic stem bark extract of *P. pinnata* in male rats.

MATERIAL AND METHODS

Plant material and Method of extraction

The stem bark of the plant was collected from the University of Rajasthan campus and its identity was confirmed at the Herbarium, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen was deposited in the Herbarium under accession number RUBL–20255. The collected stem bark was shade-dried and pulverized into a coarse powder. The powdered material was extracted with 50% ethanol using a Soxhlet extraction apparatus for 36 h at a temperature ranging from 60 to 80 °C. The extract was subsequently filtered and concentrated under reduced pressure at 40 °C to obtain a dry crude extract. The resulting brown viscous residue was suspended in an appropriate quantity of distilled water prior to oral administration in the experimental animals.

Experimental animals

Healthy adult male albino rats of the Wistar strain, with confirmed fertility, were selected for the present investigation. The animals were maintained in groups in polypropylene cages (12" × 10" × 8") under standard laboratory conditions, with a controlled temperature of 22 ± 3 °C and a photoperiod of 14 h light and 10 h dark. They had free access to drinking water and a standard laboratory pellet diet (Lipton India Pvt. Ltd.), which was occasionally supplemented with soaked grams and fresh leafy vegetables. All animals were housed and handled in accordance with the guidelines prescribed by the Indian National Science Academy (INSA, 1992, New Delhi, India) for the care and use of laboratory animals. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee, Department of Zoology, University of Rajasthan, Jaipur.

Experiment design

Male Wistar rats of comparable body weight (170–210 g) were randomly assigned into four experimental groups, with seven animals in each group. The treatment schedule was as follows:

Group I (Control): Animals received the vehicle (distilled water; 0.3 mL/rat/day) orally for 60 consecutive days.

Group II: For 60 days, animals were given the 50% ethanolic stem bark extract of P. pinnata orally at a dose of 100 mg/kg body weight/day, suspended in distilled water.

Group III: For 60 days, animals were given an oral dose of 200 mg/kg body weight/day of P. pinnata's 50% ethanolic stem bark extract suspended in distilled water.

Group IV: For 60 days in a row, animals were given an oral dose of 300 mg/kg body weight/day of P. pinnata's 50% ethanolic stem bark extract suspended in distilled water.

The animals were fasted for the whole night and put to death under ether anaesthesia twenty-four hours following the last treatment. Immediately, a heart puncture was

used to obtain blood samples for additional analysis.

Epididymal weight

After the trial was over, each animal's epididymides were removed and weighed separately. The tissues were carefully dissected free of surrounding fat and blood clots before weighing on a digital electronic balance. One half of each epididymis was immediately fixed in Bouin's fixative for subsequent histopathological evaluation.

Epithelial cell height

The height of the epithelial cells in the cauda epididymis was determined using an ocular micrometer under a light microscope at ×400 magnification. Measurements were taken from five different sections obtained from each animal, and the mean epithelial cell height was calculated.

Fertility index and litter size

The reproductive performance of control and treated animals was assessed by conducting mating trials. During the final five days of the treatment period, each male rat was housed overnight with two proven fertile parous female rats in the proestrous or estrous phase (male ratio, 1:2). Successful mating was confirmed by the detection of spermatozoa in vaginal smears collected the following morning, which was designated as Day 1 of pregnancy. Females with sperm-positive smears were separated and maintained individually until delivery. On the day of parturition, the total number of pups born to each female was recorded. Fertility index and litter size were used as indicators to evaluate the fertility and fecundity of the treated male rats.

Sperm parameters

Sperm motility and sperm count

Sperm motility and concentration were determined using the cauda epididymis. Approximately 100 mg of cauda epididymal tissue was finely minced with a sterile razor blade in 2 mL of prewarmed normal saline (0.9% NaCl, 37 °C). The resulting suspension was filtered through a nylon mesh to remove tissue debris. A drop of the well-mixed sperm suspension was placed in a Neubauer haemocytometer and covered with a coverslip. Sperm motility was expressed as the percentage of motile and immotile spermatozoa counted in different microscopic fields under a light microscope at × 100 magnifications. Sperm concentration was estimated using the standard haemocytometric method and expressed as million sperm/mL of suspension. [17]

Sperm viability

The eosin-nigrosin staining method was used to assess sperm viability. A microcentrifuge tube was filled with one drop of each of the 10% aqueous nigrosin solution and the 1% aqueous eosin Y solution. After adding a drop of well-mixed sperm suspension, the mixture was gently stirred. After applying the stained sample to a spotless glass slide, it was inspected at a magnification of ×400. A minimum of 200 spermatozoa were counted to determine the percentage of viable (unstained) and non-viable (red-stained) sperm cells. [18]

Histological study

For histopathological examination, epididymal tissues fixed in Bouin's solution were washed thoroughly with water to remove excess fixative. The tissues were subsequently dehydrated through ascending grades of alcohol, cleared in xylene, embedded in paraffin wax, and sectioned at a thickness of 5 µm. The sections were stained with hematoxylin and eosin (H&E) to provide adequate nuclear and cytoplasmic contrast. Histological changes were examined and photographed using a Nikon digital camera attached to a light microscope.

Statistical analysis

All experimental data were analyzed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Results are presented as mean ± SEM. Data were initially tested for homogeneity of variance. When the assumption of

homogeneity was satisfied, differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Epididymal weight

Oral administration of the 50% ethanolic stem bark extract of *P. pinnata* at doses of 100, 200, and 300 mg/kg body weight/day for 60 days resulted in a significant ($P < 0.05$) and dose-dependent reduction in the relative weight of the epididymides compared with the control group. Multiple comparison analysis further confirmed that the decrease in epididymal weight among the treatment groups was statistically significant ($P < 0.05$) (Figure 1).

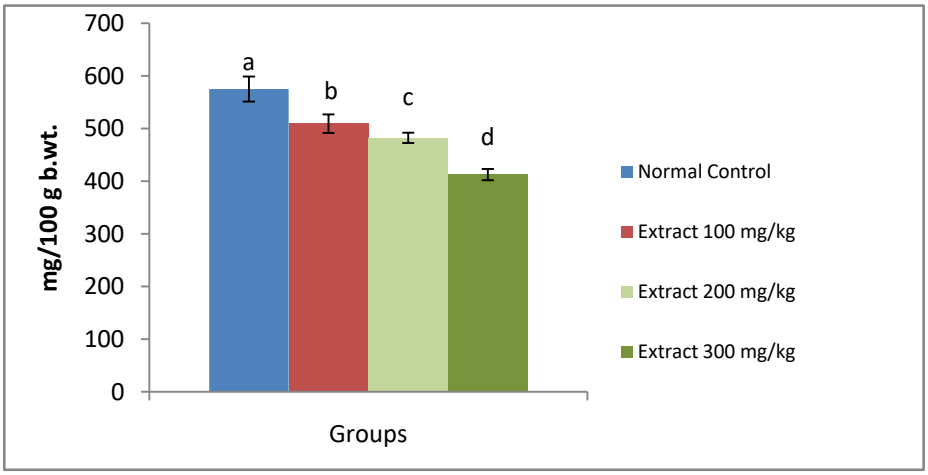

Figure 1: Effect of *P. pinnata* on testicular weight. *Tukey's multiple comparison approach indicates that values for a particular group in a row with the same letter as the superscript are not substantially different (at P<0.05).

Epithelial cell height

The mean epithelial cell height of the cauda epididymis was significantly ($P < 0.05$) reduced in rats treated with *P. pinnata* extract at doses of 100, 200, and 300 mg/kg body weight/day. The reduction was dose dependent, with progressively lower epithelial cell heights observed as the dose increased compared with the control animals (Figure 2).

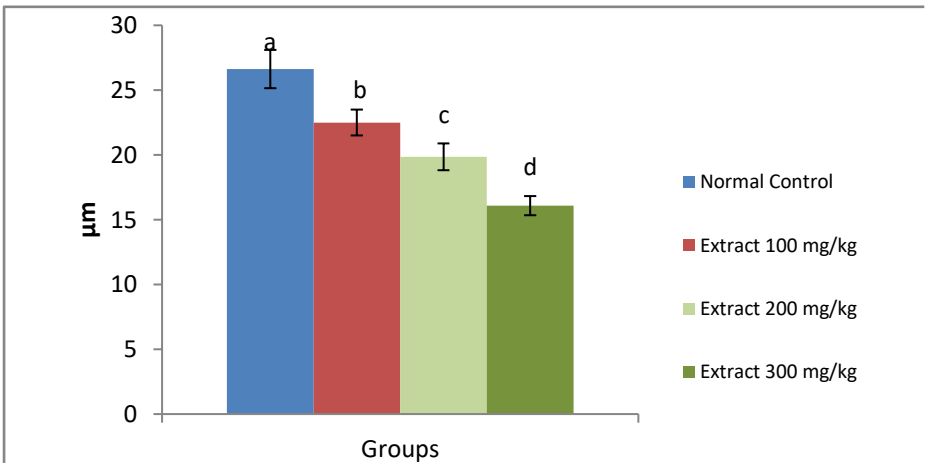

Figure 2: Effect of *P. pinnata* on epithelial cell height. *Tukey's multiple comparison approach indicates that values for a particular group in a row with the same letter as the superscript are not substantially different (at P<0.05).

Fertility index and litter size

The reproductive performance of the experimental animals is presented in figure 3 and 4. Treatment with the 50% ethanolic stem bark extract of *P. pinnata* at doses of 100, 200, and 300 mg/kg body weight/day produced a dose-dependent decline in fertility when compared with the control group. The percentage of pregnant females mated with treated males decreased to 57.14%, 42.86%, and 28.57%, respectively (Figure 3).

Similarly, the mean litter size showed a progressive reduction with increasing extract dose. The average litter size in the treatment groups was 6.87 ± 0.54, 6.00 ± 0.57, and 3.50 ± 0.49 pups, respectively, indicating a dose-dependent decline in reproductive capacity (Figure 4).

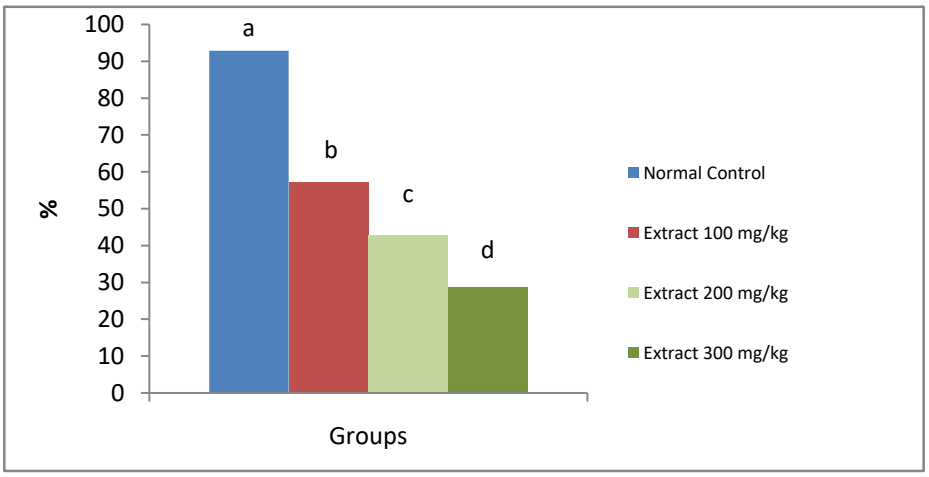

Figure 3: Effect of *P. pinnata* on fertility index. *Tukey's multiple comparison approach indicates that values for a particular group in a row with the same letter as the superscript are not substantially different (at P<0.05).

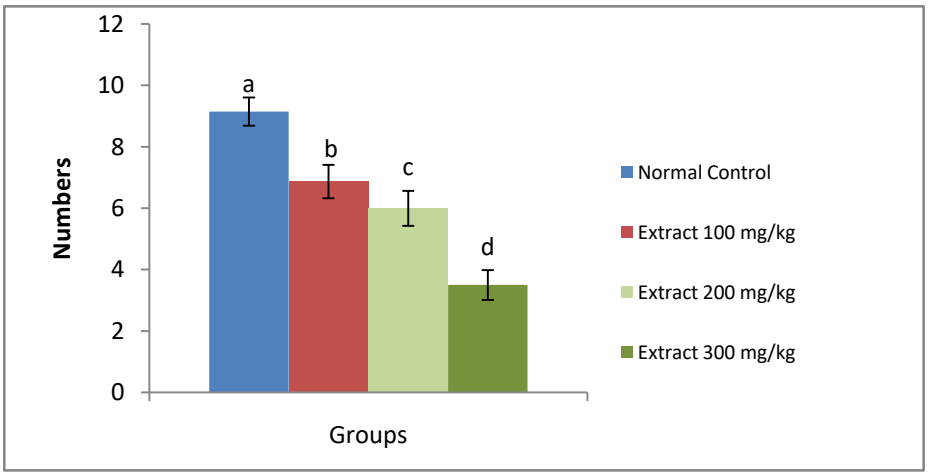

Figure 4: Effect of *P. pinnata* on litter size. *Tukey's multiple comparison approach indicates that values for a particular group in a row with the same letter as the superscript are not substantially different (at P<0.05).

Sperm parameters

The effects of the 50% ethanolic stem bark extract of *P. pinnata* on sperm characteristics, including sperm density, viability, and motility, were evaluated after 60 days of treatment and are presented in Table 1.

A significant (P < 0.05) and dose-dependent reduction in sperm density was observed in the cauda epididymis of rats administered *P. pinnata* extract at doses of 100, 200, and 300 mg/kg body weight/day compared with the control group. Statistical analysis also revealed significant differences among the three treatment groups. In animals receiving the highest dose (300 mg/kg body

weight/day), sperm count was reduced by 63.61% relative to the control group.

Treatment with *P. pinnata* extract produced a significant ($P < 0.05$) dose-dependent decline in the percentage of viable spermatozoa in the cauda epididymis. The reduction was significant not only in comparison with the control group but also among the low-, medium-, and high-dose treatment groups. At the highest administered dose (300 mg/kg body weight/day), sperm viability decreased to 34.29%.

The percentage of motile spermatozoa recovered from the cauda epididymis was significantly ($P < 0.05$) reduced following administration of *P. pinnata* extract at doses of 100, 200, and 300 mg/kg body weight/day for 60 days. The decline in sperm motility was dose dependent, with significant differences observed between the control and treatment groups as well as among the treated groups. Rats receiving the highest dose of the extract exhibited a mean sperm motility of 18.67 ± 1.63%.

Table 1: Effect of *P. pinnata* on sperm parameters

TREATMENT	Control	*Pongamia pinnata*		
PARAMETER		**100 mg/kg b.wt.**	**200 mg/kg b.wt.**	**300 mg/kg b.wt.**
Sperm density (million/ml)	44.74 ± 1.82 [a*]	31.69 ± 1.12 [b]	24.31 ±1.06 [c]	16.28 ±1.59 [d]
Sperm viability (%)	83.02 ± 1.45 [a]	61.71 ± 2.88 [b]	45.14 ±2.21 [c]	34.29 ±1.89 [d]
Sperm motility (%)	75.71 ± 2.11 [a]	39.57 ±1.88 [b]	29.71 ±1.93 [c]	18.67 ±1.63 [d]

Values represent mean ± SEM (n=7).

*Tukey's multiple comparison approach indicates that values for a particular group in a row followed by the same letter as superscript are not substantially different (at $P<0.05$).

Histopathology

Histological examination of the cauda epididymis in the control group revealed large, closely packed epididymal tubules separated by minimal intertubular connective tissue. The tubules were lined by tall columnar epithelial cells bearing well-developed stereocilia on their luminal surface, and the lumen was densely filled with spermatozoa (Figure 5A & 5B).

Rats treated with *P. pinnata* extract at a dose of 100 mg/kg body weight/day exhibited mild histopathological alterations in the cauda epididymis. A significant ($P < 0.05$) reduction in tubular diameter and epithelial cell height was observed compared with the control group. The luminal sperm population was decreased, and a slight increase in the intertubular connective tissue was also evident (Figure 5C & 5D).

Administration of the extract at 200 mg/kg body weight/day resulted in more pronounced histological changes. The epithelial lining of the cauda epididymis showed degenerative alterations accompanied by a reduction in epithelial cell height. The stereocilia appeared shortened and sparse, while the intertubular connective tissue was noticeably increased. In addition, the lumen contained fewer spermatozoa along with the presence of sperm debris when compared with the control group (Figure 5E &5 F).

In rats receiving the highest dose (300 mg/kg body weight/day), severe degenerative changes were evident in the cauda epididymis. The epithelial cells exhibited marked degeneration with a significant ($P < 0.05$) decrease in epithelial cell height. A considerable increase in intertubular connective tissue was observed, and the lumen contained only a few degenerating spermatozoa together with infiltration of leukocytes (Figure 5G & 5H).

Overall, the histopathological alterations induced by the 50% ethanolic stem bark extract of *P. pinnata* were dose dependent, with the most severe degenerative changes occurring in animals treated with the highest dose (300 mg/kg body weight/day).

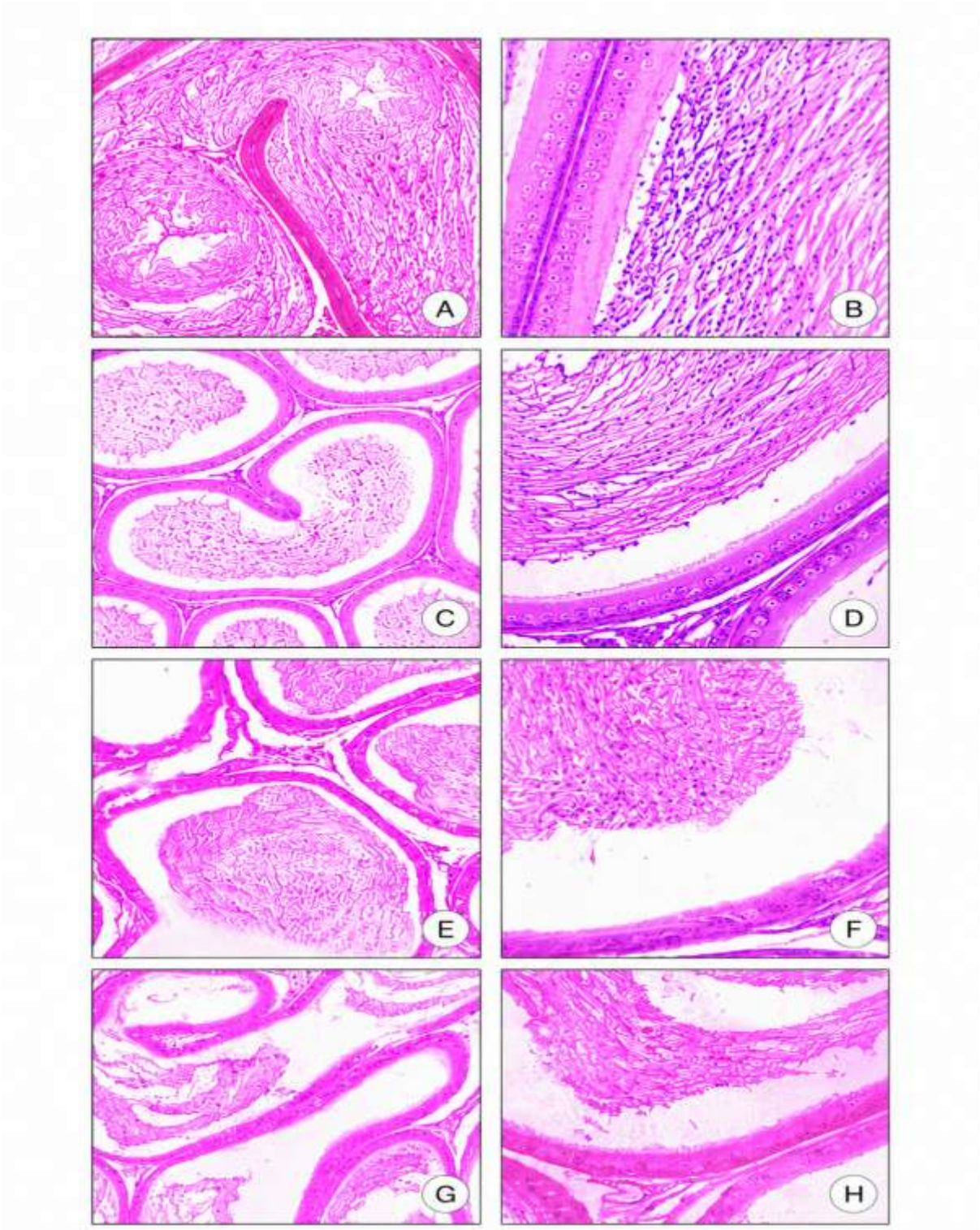

Figure 5: (A–H). Photomicrographs of the cauda epididymis of control and *Pongamia pinnata* stem bark extract-treated rats. **A–B:** Control, showing normal histoarchitecture and sperm-filled lumina. **C–D:** 100 mg/kg/day, showing mild degeneration with reduced tubular size, epithelial height, and sperm density. **E–F:** 200 mg/kg/day, showing moderate degeneration, shortened stereocilia, increased intertubular connective tissue, and reduced spermatozoa. **G–H:** 300 mg/kg/day, showing severe epithelial atrophy and marked depletion of luminal spermatozoa. **A, C, E, G:** H&E ×100; **B, D, F, H:** H&E ×400.

DISCUSSION

The present investigation demonstrated a significant dose-dependent reduction in the relative weight of the epididymis following administration of the 50% ethanolic stem bark extract of *P. pinnata*. The decrease in

epididymal weight may be attributed to a reduction in the number of spermatozoa present within the epididymal lumen, possibly resulting from diminished androgen availability. Since the epididymis requires androgen concentrations nearly ten times higher than those present in the circulation to maintain its normal structure and physiological functions, suppression of androgen supply can adversely affect epididymal integrity. [19] Similar reductions in epididymal weight have previously been reported in animals treated with various medicinal plant extracts possessing antifertility activity. [20,21]

The mating studies carried out in the present work revealed a significant, dose-dependent decline in both fertility rate and mean litter size in rats treated with *P. pinnata* extract. The reduced fertility observed in the treated animals may be associated with impaired fertilizing capacity of spermatozoa or failure of implantation resulting from defective sperm maturation. [22] Previous studies have established a close positive relationship between sperm concentration, sperm motility, and male fertility, indicating that deterioration in these parameters is directly reflected in reproductive performance.

Administration of the extract also produced marked adverse effects on sperm quality. Significant dose-dependent reductions were observed in sperm count, sperm motility, and sperm viability in the cauda epididymis of treated rats. These findings suggest that chronic exposure to the extract compromises epididymal function and ultimately reduces the fertilizing potential of spermatozoa.

The decline in epididymal sperm count may be explained by reduced testosterone levels, since both spermatogenesis in the testes and sperm maturation within the epididymis are highly dependent on androgenic stimulation. Consequently, the decreased sperm concentration observed in the present study is likely to have contributed to the reduction in fertility index and litter size. [23]

The reduction in serum testosterone following extract administration may have disrupted the biochemical environment of the epididymis, thereby impairing normal sperm maturation. This impaired maturation likely contributed to the observed decline in sperm motility and viability, ultimately resulting in reduced fertility. These findings are in agreement with previous studies reporting decreased sperm motility and viability following treatment with medicinal plant extracts exhibiting antiandrogenic, antispermatogenic, and antifertility activities. [20,24,25] Similarly, green-synthesized silver nanoparticles prepared using *Pongamia pinnata* significantly reduced sperm parameters and serum testosterone levels in male Wistar rats. [26]

The decrease in sperm motility and viability may additionally be related to the spermicidal activity of phytoconstituents present in *P. pinnata*. Bandivdekar and Moodbidri (2002) demonstrated potent spermicidal activity of *P. pinnata* seed oil, supporting the possibility that similar bioactive constituents may contribute to the present findings. [27] Furthermore, impairment of sperm motility may result from disruption of oxidative metabolism and cellular energy production, both of which are essential for normal sperm movement. [28,29]

Histopathological evaluation of the cauda epididymis further confirmed the adverse effects of *P. pinnata* extract on epididymal structure. Progressive, dose-dependent degenerative alterations were observed, including marked degeneration of the epithelial lining, reduction in epithelial cell height, shortening and loss of stereocilia, expansion of the intertubular connective tissue, and accumulation of sperm debris with markedly fewer spermatozoa within the lumen. These structural abnormalities are indicative of impaired epididymal function and are likely associated with insufficient androgenic support to the organ. It is possible that phytochemicals present in the extract inhibit androgen biosynthesis by Leydig cells, thereby creating an unfavourable epididymal environment for sperm maturation.

The normal structure and physiological activity of the epididymis are known to

depend on factors originating from the testes, particularly androgenic stimulation. [30] Reduced androgen availability has been shown to produce characteristic histological alterations, including decreased epithelial cell height, loss of microvilli or stereocilia, and epithelial vacuolization. [31,32] Moreover, the cauda epididymis synthesizes and secretes several proteins into the luminal fluid that play essential roles in sperm maturation, and the expression of these proteins is regulated by androgens. [19,33] Earlier investigations have further demonstrated that numerous epididymal proteins become downregulated following orchidectomy, emphasizing the critical role of testicular factors in maintaining epididymal function. [30]

The histological alterations observed in the present study are in close agreement with previous investigations reporting degeneration of the epididymal epithelium together with a marked reduction in luminal spermatozoa following treatment with different medicinal plant extracts that reduce androgen availability and exhibit antifertility activity. [21,34,35]

CONCLUSION

The findings of the present study demonstrate that chronic oral administration of the 50% ethanolic stem bark extract of *Pongamia pinnata* produces significant, dose-dependent adverse effects on epididymal structure and function in adult male Wistar rats. Treatment with the extract resulted in a marked reduction in epididymal weight, epithelial cell height, sperm count, motility, and viability, accompanied by decreased fertility index and litter size. Histopathological examination further revealed progressive degenerative changes in the cauda epididymis, indicating impairment of the epididymal microenvironment required for normal sperm maturation and storage. Collectively, these findings suggest that the phytoconstituents present in the stem bark extract of *P. pinnata*, acting either individually or synergistically, possess significant male antifertility potential. Further studies are warranted to isolate the active phytoconstituents, elucidate their precise mechanism of action, and evaluate their safety, reversibility, and efficacy before considering their development as a plant-based male contraceptive.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

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