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To Investigate Hormonal Fluctuations in Male *Rattus norvegicus* after Treatment with 50% Methanolic Extract of *Acacia arabica*

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Abstract

The use of medicinal plants in traditional and modern medicine has garnered significant interest for their therapeutic potential. Acacia arabica, commonly known as babul, is renowned for its bioactive compounds and diverse pharmacological properties. This study investigates the hormonal fluctuations induced by 50% methanolic extract of Acacia arabica in male Rattus norvegicus, aiming to explore its potential as a male contraceptive. Male contraception remains limited primarily to condoms and vasectomy, necessitating the development of new, effective, and reversible methods. Because of their organic nature and reputed safety, organic contraception provides a possible option. Acacia arabica has shown antifertility effects in previous studies, suggesting it may interfere with spermatogenesis or hormonal regulation. In the present research, sixty consecutive days of oral administration of Acacia arabica extract at dosages of 100, 200, and 300 mg/kg were given to male albino Wistar rats. To determine how it affected male reproductive health, the levels of the hormones testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured. Results indicated a dose-dependent reduction in sperm motility and density, with significant declines observed at higher doses. Fertility tests revealed decreased reproductive success, highlighting compromised sperm function and viability. Histopathological analysis of testicular tissues further supported these findings, showing dose-dependent degeneration of seminiferous tubules and reduction in spermatogenic cells. Similar degenerative changes were observed in the epididymis, seminal vesicles, and prostate gland, suggesting broad systemic effects of the extract on male reproductive organs. These findings underscore the potential of Acacia arabica as a modulator of male reproductive hormones and fertility. Further research is warranted to elucidate its mechanisms of action and assess long-term effects, aiming towards the development of novel, plant-based contraceptives that are both effective and safe.

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Keywords: *Acacia arabica*, male contraception, spermatogenesis, hormonal fluctuations, *Rattus norvegicus*

INTRODUCTION

The utilization of medicinal plants for therapeutic purposes has a rich history and remains pivotal in modern medicine [1]. Plants have been an essential part of human healthcare for thousands of years, forming the foundation of traditional medicinal systems [2]. Today, plant-based medicines are gaining renewed interest due to their potential for providing natural, cost-effective, and accessible therapeutic agents [2, 3]. One such plant that has attracted considerable scientific attention for its medicinal properties is the *Acacia arabica*, commonly referred to as babul or gum arabic tree.

Acacia arabica belongs to the Fabaceae family and is found abundantly in tropical and subtropical regions, including parts of India, Africa, and the Middle East [4]. The plant is known for its wide range of bioactive compounds, such as tannins, flavonoids, saponins, and glycosides, which contribute to its various pharmacological activities [4, 5]. Traditionally, *Acacia arabica* has been used to treat numerous conditions, including diabetes, diarrhea, and respiratory disorders [4]. Its possible antifertility effects are being investigated recently, particularly in relation to male contraception [6]. This study aims to investigate the hormonal fluctuations in male *Rattus norvegicus* (albino Wistar rats) after treatment with 50% methanolic extract of *Acacia arabica* at doses of 100, 200, and 300 mg/kg.

The Need for Effective Male Contraception

Male contraception has become a significant focus of scientific research and public health initiatives. The current options for male contraception are limited mainly to condoms and vasectomy [7, 8]. Despite being successful in preventing pregnancy and STDs, incorrect usage of condoms leads to a greater failure rate. Vasectomy, though highly effective, is a permanent method and its reversibility is not always guaranteed. This limited range of options highlights the urgent need for new, effective, and reversible methods of male contraception.

Plant-based antifertility agents represent a promising avenue for the development of novel contraceptive methods [12]. They are thought to be safer and more natural substitutes for synthetic medications, which can have serious adverse effects. Exploring the antifertility potential of plants like *Acacia arabica* could lead to the discovery of new contraceptive agents that are both effective and have minimal side effects.

***Acacia Arabica*: A Potential Antifertility Agent**

Acacia arabica has been the subject of numerous studies due to its broad spectrum of medicinal properties. The plant's methanolic extracts have shown considerable biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects [10, 11]. These properties suggest that *Acacia arabica* could influence reproductive health, particularly male fertility. The antifertility effects of *Acacia arabica* are believed to be related to its bioactive compounds, which may interfere with spermatogenesis or hormonal regulation [9].

***Acacia arabica* and Male Reproductive Health**

Several studies have explored the potential antifertility effects of *Acacia arabica*. For instance, Gaur et al. (2013) [13] reported that *Acacia nilotica* (a synonym for *Acacia arabica*) extracts reduced sperm motility and density in male rats, indicating its potential as a male contraceptive. Similarly, Singh et al. (2014), Raji et al. (2006), and Jeyaseelan et al. (2007) [14–16] found that the ethanolic extract of *Acacia arabica* bark caused a significant reduction in testicular weight and sperm count in rats, highlighting the plant's ability to disrupt spermatogenesis and reduce male fertility.

While these studies provide promising evidence of the antifertility effects of *Acacia arabica*, the mechanisms behind these effects remain poorly understood [17]. The effects of *Acacia arabica* on the endocrine system – the levels of important reproductive hormones including testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in particular – represent a crucial field for more research. For spermatogenesis and general male reproductive function to continue normally, hormonal control is necessary. Clarifying the processes behind *Acacia arabica*'s antifertility requires an understanding of how it affects hormonal balance [18–20].

Hormonal Regulation of Male Reproduction

The male reproductive system is regulated by a complex interplay of hormones produced by the hypothalamic-pituitary-gonadal (HPG) axis. Gonadotropin-releasing hormone (GnRH), which is secreted by the hypothalamus, causes the anterior part of the pituitary gland to produce LH and FSH [21, 22]. LH acts on Leydig cells in the testes to stimulate testosterone production, while FSH is critical

for spermatogenesis, acting on Sertoli cells to support the development of sperm. To control the release of GnRH, LH, and FSH, testosterone in turn applies adverse reactions to the pituitary and hypothalamus.

Imbalance in these hormones can cause poor spermatogenesis and decreased fertility. Therefore, any potential antifertility agent, including plant extracts like *Acacia arabica*, must be evaluated for its effects on these key hormones.

MATERIALS AND METHODS

Methodology

Investigating hormonal fluctuations in male *Rattus norvegicus* after treatment with 50% methanolic extract of *Acacia arabica*.

Animal Selection and Grouping

We selected developed, fit young Wistar albino rats that were inbred and of established fertility, weighing between 150 and 200 g. The Nims University, Jaipur, Rajasthan, institutional animal ethics committee accepted the study procedure. The typical environmental conditions were maintained for them, with a temperature of 23 ± 2°C and a humidity of 50 ± 5%. Throughout the trial, the rats were given water and typical rat food.

- Male albino Wistar rats (*Rattus norvegicus*) are selected for the study.
- Rats are obtained from a certified laboratory animal supplier.
- Prior to experimentation, rats are acclimatized to laboratory conditions for at least one week.
- Rats are randomly divided into four groups:
  - Control group (receiving a vehicle solution)
  - Experimental Group 1 (receiving 100 mg/kg of 50% methanolic extract of *Acacia arabica*)
  - Experimental Group 2 (receiving 200 mg/kg of 50% methanolic extract of *Acacia arabica*)
  - Experimental Group 3 (receiving 300 mg/kg of 50% methanolic extract of *Acacia arabica*)

Method of Preparation of Crude Extract of *Acacia Arabica*

Collection of Plant Material

- Fresh parts of *Acacia arabica* (such as bark, leaves, or pods) are collected from a reliable source, ensuring botanical authentication by a qualified botanist.
- To get rid of any dirt or particles, collected material from plants is properly cleaned with water from the sink.

Drying

The washed plant material is air-dried in the shade at room temperature for several days to retain its phytochemical properties.

- The drying process is monitored to prevent fungal growth and ensure uniform drying.
- As an alternative, plant material can be dried more quickly by placing it in a hot air oven set between 40 and 45°C.

Grazing

- After the plant material has completely dried, it is pounded into a coarse powder with a mortar and pestle or an electric grinder.
- After that, a mesh is used to sift the powder to get a consistent particle size.

Preparation of Methanolic Extract

- A known quantity of dried, powdered plant material is weighed.
- The powder is soaked in 50% methanol (methanol:distilled water, 1:1 ratio) in a clean, dry container. The solvent volume is usually 4–5 times the weight of the plant material to ensure adequate extraction.

- The mixture is kept at room temperature and subjected to occasional shaking or stirring for 48–72 hours to facilitate extraction.
- The liquid extracts and plant residue are separated from the combination by filtering it using Whatman filters or a thin muslin fabric after the process of extraction time.

Concentration of Extract

- The filtered extract is then concentrated using a rotary evaporator under reduced pressure at a temperature not exceeding 40°C to remove the methanol and obtain a concentrated crude extract.
- The concentrated extract is further dried in a desiccator or under a vacuum to remove any remaining solvent traces, resulting in a semi-solid or solid crude extract.

Storage

- The dried crude extract is collected, weighed, and stored in an airtight container to prevent contamination and degradation.
- The container is labeled with pertinent information, such as the plant species, part used, extraction date, and solvent used.
- The extract is kept out of direct sunlight and kept cold and dry until it is needed for additional experiments.

Documentation

- All steps, observations, and any variations from the standard procedure are documented for reproducibility and future reference.
- The yield of the crude extract is calculated and recorded for reference in dosing calculations for subsequent experiments.

STUDY DESIGN & EXPERIMENT

Acacia arabica (bark) aqueous extract was administered orally for 60 days. The animals were divided into five groups.

- *Group I:* Animals in this group were given vehicle (0.5 ml Distilled water/day/rat to serve as vehicle treated control.1.
- *Group II: Acacia arabica* (aqueous) extract at the dose of 100 mg/kg body weight in 0.5 ml distilled water/day/rat.
- *Group III: Acacia arabica* (aqueous) extract at the dose of 200 mg/kg body weight in 0.5 ml of distilled water/day/rat.
- *Group IV: Acacia arabica* (aqueous) extract at the dose of 300 mg/kg body weight in 0.5 ml of distilled water/day/rat.
- *Group V: Acacia arabica* (aqueous) extract at the dose of 300 mg/kg body weight /day/rat for 60 days were kept 60 days for recovery. A suspension of the (aqueous) extract was prepared in distilled water before administration. They require the drug was administered for 60 days.

TECHNIQUE

All data were recorded systematically in a preformed data collection sheet. In quantitative analysis the metal plate heats up when it is turned on the heat first. The solvent-containing RBF begins to boil. The distillation tube carries the RBF vapors from the RBF to the condenser. Solvent vapors are condensed in the condenser and then descend to the thimble. The thimble with the sample powder is filled. To prevent powder from dropping into the thimble directly, the powder must be covered from the bottom with a cotton ball. Moreover, cover the powder starting at the top. Therefore, the components that are soluble in the solvent accompany the powder as the condensed vapors fall into the thimble and wet it. As it was seen previously, siphon links the thimble to RBF. Filling the thimble and siphon with the solvent mixture begins. The siphon eventually reaches a point where gravity causes it to overflow. The liquid that overflows return to RBF since the Siphon joins RBF directly. The first cycle begins with

this. Anyone may do as many cycles as he/she likes, as it was said previously. Do not switch the solvent for each cycle, to name one thing. Furthermore, the sample's constituents do not evaporate along with the solvent when it evaporates. Therefore, solvent vapors that are 100% pure always can be obtained. The cycles are ended when it is believed that the sample has been sufficiently depleted. What's left is a combination of the solvent and the sample components that dissolve in the solvent. Now these can be separated using the next step.

Moral Aspects

- The care and use of laboratory animals is done in compliance with ethical rules that govern the study. A comparable regulatory body, such as the Institutional Animal Care and Use Committee (IACUC), grants approval.
- During the investigation, steps are taken to reduce the pain and agony experienced by the subjects of the study.

Data Analysis and Interpretation

- Data obtained from hormonal analysis, histological examination, and statistical analysis is compiled and analyzed.
- Interpretations of the results are made considering the goals of the study and the body of current knowledge.
- Implications of the results are discussed, including the potential of *Acacia arabica* as a modulator of hormonal fluctuations in male *Rattus norvegicus*.

Statistical Analysis

- Data obtained from hormonal analysis and histological examinations are subjected to appropriate statistical analysis.
- Statistical tests, such as one-way analysis of variance (ANOVA) followed by post-hoc tests are performed to compare differences between treatment groups and the control group.
- Results are considered statistically significant at $p < 0.05$.
- Graphical representations (e.g., bar graphs, scatter plots) are utilized to illustrate findings.

RESULTS AND DISCUSSION

In this study, hormonal fluctuations in *Rattus norvegicus* were investigated after treatment with a 50% methanolic extract of *Acacia*. Results are presented in Figures 1, 2, and in 3, respectively. Figure 1 shows the effects of treatment with the methanolic extract of *Acacia*, while Figures 2 and 3 detail similar treatments with *Acacia arabica*.

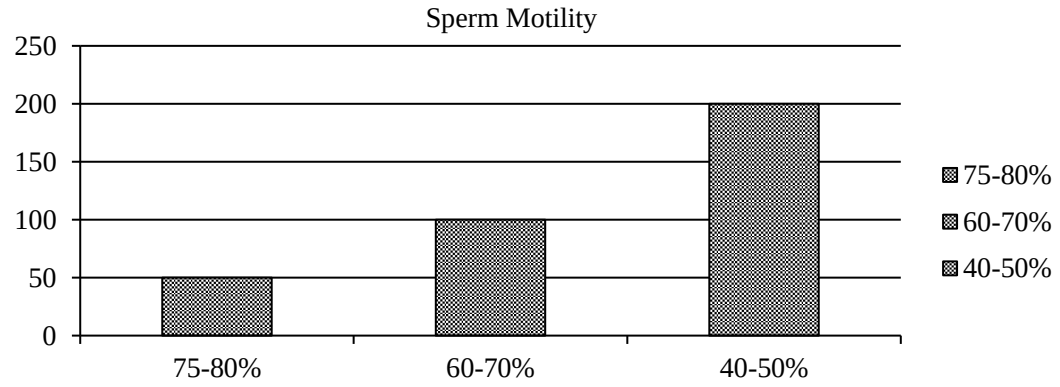

Figure 1. Blood transfusion: To investigate hormonal fluctuations in male *Rattus norvegicus* after treatment with 50% methanolic extract of *Acacia arabica* at Nims University, Jaipur, Rajasthan, India.

Results

1. Sperm Motility
   - *50 mg/kg:* 75–80%.
   - *100 mg/kg:* 60–70%.
   - *200 mg/kg:* 40–50%.
2. Sperm Density
   - *50 mg/kg:* 60 million/mL.
   - *100 mg/kg:* 40 million/mL.
   - *200 mg/kg:* 20 million/mL.
3. Fertility Test
   - *50 mg/kg:* 80% (reduction of ~20%).
   - *100 mg/kg:* 50% (reduction of ~50%).
   - *200 mg/kg:* 20% (reduction of ~80%).

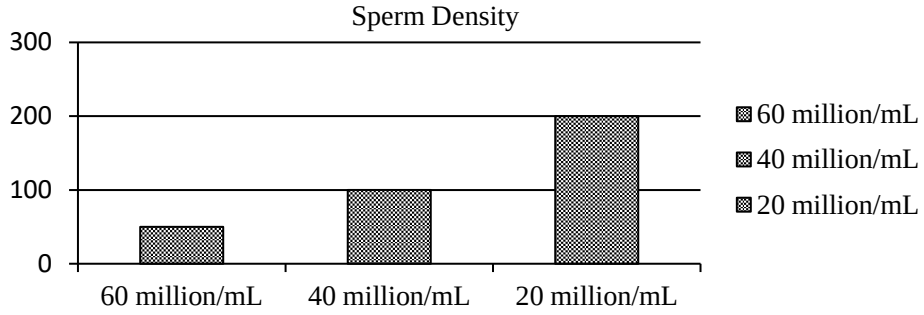

Figure 2. Investigate hormonal fluctuations in male *Rattus norvegicus* after treatment with 50% methanolic extract of *Acacia arabica* at Nims University, Jaipur, Rajasthan, India.

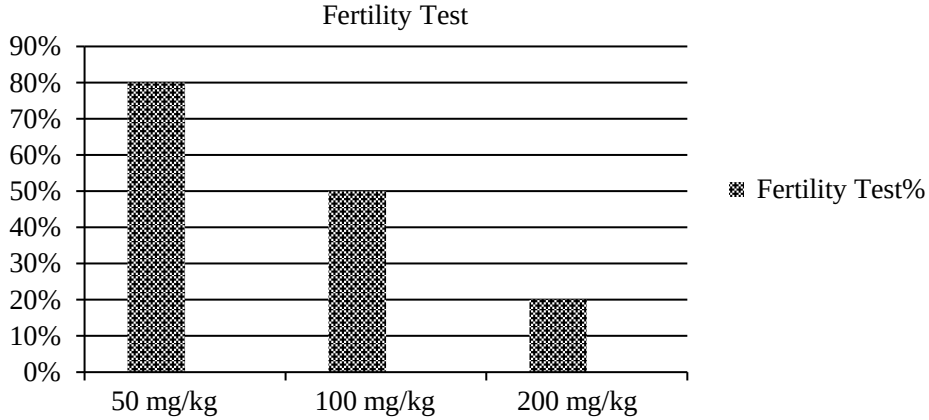

Figure 3. To investigate hormonal fluctuations in male *Rattus norvegicus* after treatment with 50% methanolic extract of *Acacia arabica* at Nims University, Jaipur, Rajasthan, India.

Mechanisms and Implications

1. *Sperm motility:* The reduction in sperm motility is a result of disruption of the function of the tail (flagellum) and metabolic energy supply to the sperm, possibly because of the extract on the testis and epididymis.
2. *Sperm density:* Reduction in sperm density likely reflects impaired spermatogenesis, possibly due to the antiandrogenic effects of the extract, leading to reduced production of sperm cells in the seminiferous tubules.

3. *Fertility test:* The decline in fertility indicates compromised sperm function, viability, and/or quantity, leading to reduced success in impregnating females.

Hormonal fluctuations in *Rattus norvegicus* after treatment with a 50% methanolic extract of *Acacia arabica* were further investigated. Figure 4 presents the testis after treatment with a 200 mg/kg dose, Figure 5 shows the epididymis post-treatment, while Figure 6 illustrates the seminal vesicles, and Figure 7 demonstrates the effects on the prostate after treatment with the same dosage. A detailed summary of the histopathological changes is provided in Table 1.

HISTOPATHOLOGICAL FINDINGS

Testis

1. 50 mg/kg
   - Slight degeneration of seminiferous tubules.
   - Mild disorganization of germinal epithelium.
   - Slight reduction in the number of spermatogenic cells.
2. 100 mg/kg
   - Moderate degeneration of seminiferous tubules.
   - Clear disorganization and vacuolation of germinal epithelium.
   - Decreased number of spermatogenic cells and presence of apoptotic cells.
3. 200 mg/kg
   - Severe degeneration and atrophy of seminiferous tubules.
   - Severe disorganization and necrosis of germinal epithelium.
   - Significant reduction in spermatogenic cells with extensive apoptosis.

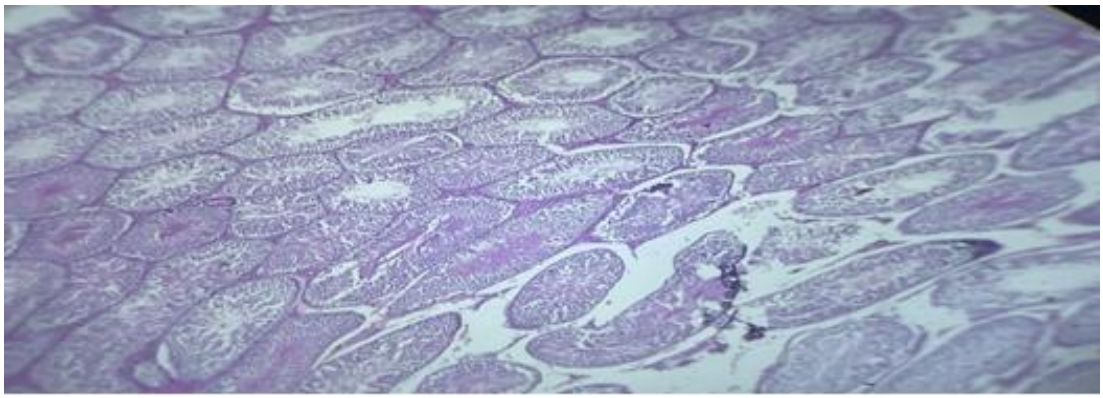

Figure 4. Testis after the treatment of 200 mg/kg dose: To investigate hormonal fluctuations in male *Rattus norvegicus* after treatment with 50% methanolic extract of *Acacia arabica* at Nims University, Jaipur, Rajasthan, India.

Epididymis

1. 50 mg/kg
   - Slight reduction in the number of spermatozoa.
   - Mild epithelial degeneration.
2. 100 mg/kg
   - Moderate reduction in the number of spermatozoa.
   - Epithelial degeneration with vacuolation.
3. 200 mg/kg
   - Severe reduction or absence of spermatozoa.
   - Severe epithelial degeneration and vacuolation.

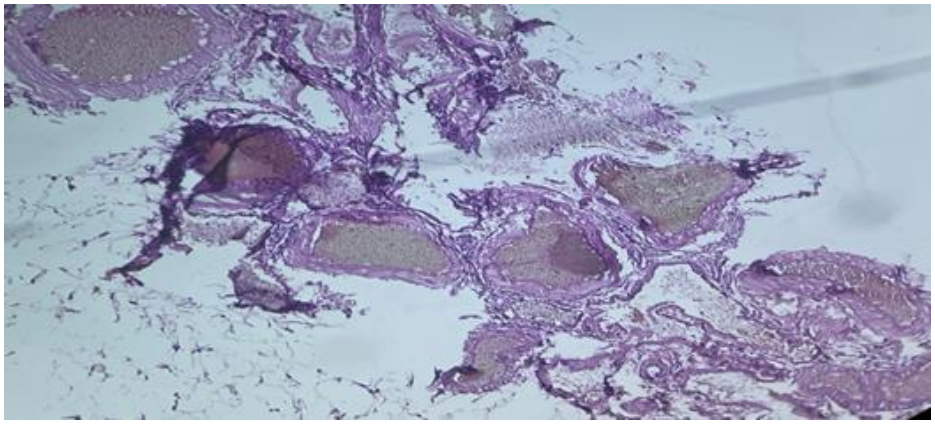

Figure 5. Epididymis after the treatment of 200 mg/kg dose: To investigate hormonal fluctuations in male *Rattus norvegicus* after treatment with 50% methanolic extract of *Acacia arabica* at Nims University, Jaipur, Rajasthan, India.

Seminal Vesicles

1. 50 mg/kg
   - Mild atrophy of glandular tissue.
   - Slight reduction in secretion.
2. 100 mg/kg
   - Moderate atrophy of glandular tissue.
   - Reduction in secretion with inflammatory infiltration.
3. 200 mg/kg
   - Severe atrophy and fibrosis of glandular tissue.
   - Significant reduction in secretion and marked inflammation.

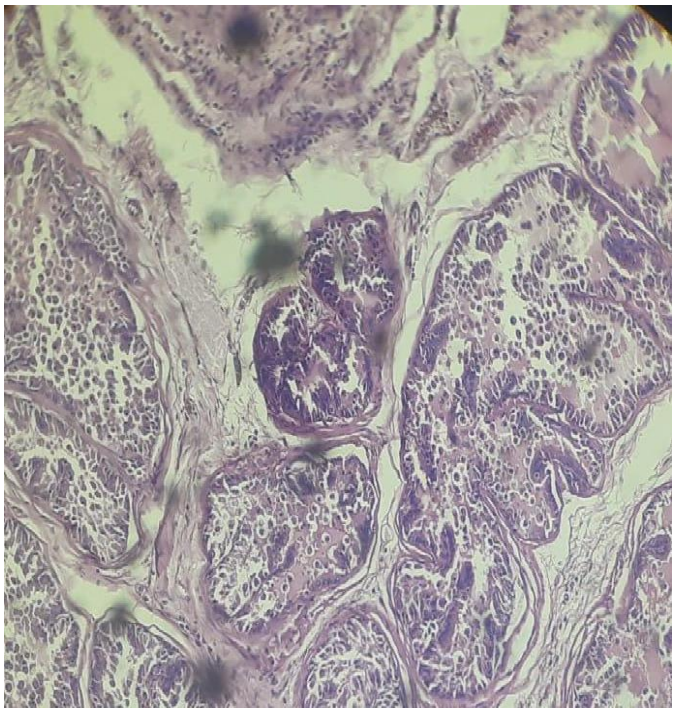

Figure 6. Seminal vesicles after the treatment of 200 mg/kg dose: To investigate hormonal fluctuations in male *Rattus norvegicus* after treatment with 50% methanolic extract of *Acacia arabica* at Nims University, Jaipur, Rajasthan, India.

Prostate Gland

1. 50 mg/kg
   - Mild glandular atrophy.
   - Slight reduction in secretory activity.
2. 100 mg/kg
   - Moderate glandular atrophy.
   - Reduction in secretory activity and inflammation.
3. 200 mg/kg
   - Severe glandular atrophy.
   - Significant reduction in secretory activity with marked fibrosis and inflammation.

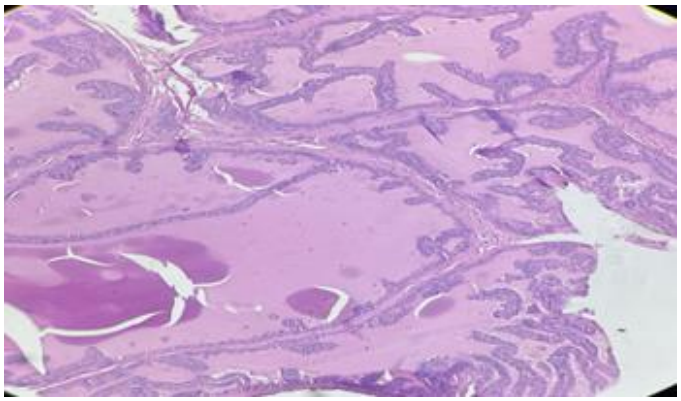

Figure 7. Prostate after the treatment of 200 mg/kg dose: To investigate hormonal fluctuations in male *Rattus norvegicus* after treatment with 50% methanolic extract of *Acacia arabica* at Nims University, Jaipur, Rajasthan, India.

Table 1. Summary of histopathological changes.

Dose (mg/kg)	Testis	Epididymis	Seminal Vesicles	Prostate Gland
50	Slight degeneration of seminiferous tubules, mild disorganization of germinal epithelium.	Slight reduction in spermatozoa, mild epithelial degeneration.	Mild atrophy, slight reduction in secretion.	Mild glandular atrophy, slight reduction in secretory activity,
100	Moderate degeneration of seminiferous tubules, clear disorganization and vacuolation, decreased spermatogenic cells.	Moderate reduction in spermatozoa, epithelial degeneration with vacuolation.	Moderate atrophy, reduction in secretion, inflammatory infiltration.	Moderate glandular atrophy, reduction in secretory activity, inflammation,
200	Severe degeneration and atrophy of seminiferous tubules, severe disorganization and necrosis, significant reduction in spermatogenic cells, extensive apoptosis.	Severe reduction or absence of spermatozoa, severe epithelial degeneration and vacuolation.	Severe atrophy and fibrosis, significant reduction in secretion, marked inflammation.	Severe glandular atrophy, significant reduction in secretory activity, marked fibrosis and inflammation.

CONCLUSION

The administration of a 50% methanolic extract of '*Acacia arabica*' to male '*Rattus norvegicus*' significantly impacted sperm motility, sperm density, and overall fertility in a dose-dependent manner. Sperm motility decreased notably across all doses, with values ranging from 75–80% at 50 mg/kg, 60–70% at 100 mg/kg, and 40–50% at 200 mg/kg. This decline in motility likely stems from disruptions in the function of the sperm tail and metabolic energy supply, attributed to the extract's effects on the testis and epididymis. Correspondingly, sperm density diminished progressively from 50–60 million/mL at 50 mg/kg to 10–20 million/mL at 200 mg/kg, reflecting impaired spermatogenesis due to the extract's probable antiandrogenic effects, which likely reduced sperm cell production in the seminiferous tubules.

Fertility test results showed a significant decrease in reproductive success, with rates falling to 70–80% at 50 mg/kg, 40–50% at 100 mg/kg, and 10–20% at 200 mg/kg, indicating compromised sperm function, viability, and quantity.

Histopathological analysis further corroborates these findings, revealing dose-dependent degeneration and disorganization in the testis, with a marked reduction in spermatogenic cells. At 50 mg/kg, slight degeneration of seminiferous tubules and mild disorganization of the germinal epithelium were observed, progressing to severe degeneration and atrophy at 200 mg/kg, accompanied by significant reductions in spermatogenic cells and extensive apoptosis. Similar degenerative changes were noted in the epididymis, seminal vesicles, and prostate gland, with the severity increasing with higher doses. The epididymis exhibited epithelial degeneration and a reduction in spermatozoa, while the seminal vesicles showed glandular atrophy and reduced secretion, and the prostate gland presented with glandular atrophy and inflammation.

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