Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14,issue.32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Examine the Effects of a 50% Methanolic Extract of Acacia Arabica on the Quantity and Quality of Sperm Produced by Male Rattus Norvegicus..

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.Cite this paper as Alka Qureshi, Sonalika Singh, Anjali Goswami (2025) Examine the Effects of a 50% Methanolic Extract of Acacia Arabica on the Quantity and Quality of Sperm Produced by Male Rattus Norvegicus.. .Journal of Neonatal Surgery, 14, (32s), 9958-9967

ABSTRACT

The present study investigates the antifertility potential of Acacia arabica (babul) through the administration of its 50% methanolic bark extract in male Rattus norvegicus (albino Wistar rats). Plant-based contraceptives are increasingly gaining attention due to their accessibility, safety profile, and reduced side effects compared with synthetic alternatives. In this context, A. arabica, a plant rich in tannins, flavonoids, glycosides, and saponins, was evaluated for its impact on male reproductive physiology. Adult male rats (150–200 g) were randomly divided into four groups: a control group (vehicle-treated) and three experimental groups receiving A. arabica extract orally at doses of 100, 200, and 300 mg/kg body weight for 60 days. Standardized semen analysis was conducted to assess sperm count, motility, progressive motility, viability, morphology, semen pH, and volume. Data were analyzed using one-way ANOVA followed by post hoc tests, with $p < 0.05$ considered significant.

Results revealed a dose-dependent decline in sperm quality and quantity. The control group recorded the highest sperm count (95 million/mL), motility (93%), and viability (95%). In contrast, the 300 mg/kg group demonstrated significant reductions in sperm count (60 million/mL), motility (76%), progressive motility (60%), and viability (78%). Normal sperm morphology also declined from 98% in the control to 81% at 300 mg/kg. Semen volume decreased progressively across treated groups, while semen pH showed minor shifts toward acidity.

These findings indicate that methanolic extract of A. arabica exerts pronounced antispermatogenic and antifertility effects in male rats, likely mediated through disruption of spermatogenesis and hormonal regulation. The study supports traditional claims of A. arabica as a natural contraceptive and highlights its potential as a candidate for developing safer, reversible, and plant-based male contraceptive agents. Further investigations are warranted to elucidate the underlying mechanisms and ensure safety for clinical translation..

Keywords: *Acacia arabica, methanolic extract, antifertility, sperm parameters, spermatogenesis, male contraception, Rattus norvegicus*

1. INTRODUCTION

According to Farnsworth (1988), the use of medicinal plants for therapeutic reasons has a long history and is still crucial in contemporary medicine. For thousands of years, plants have played a crucial role in human healthcare, serving as the cornerstone of conventional medical systems (Fabricant & Farnsworth, 2001). Due to their potential to provide accessible, affordable, and natural therapeutic agents, plant-based medicines are now attracting increased research (Fabricant & Farnsworth, 2001; Newman & Cragg, 2016). One such species, Acacia arabica, often known as the gum arabic tree or babul, has garnered a lot of scientific interest due to its therapeutic qualities. Parts of India, Africa, and the Middle East are among the tropical and subtropical locations where Acacia arabica, a member of the Fabaceae family, is widely distributed (Farooq et al., 2017). The plant's diverse pharmacological actions are attributed to its abundance of bioactive substances, including tannins, flavonoids, saponins, and glycosides (Farooq et al., 2017; Parekh & Chanda, 2007). Diabetes, diarrhea, and respiratory issues are just a few of the ailments that Acacia arabica has historically been used to treat (Farooq et al., 2017). Recent studies have examined its potential antifertility effects, especially in connection with male contraception (Khalid & Bilal, 2020). The purpose of this research is to examine the hormonal changes that occur in male Rattus norvegicus (albino Wistar rats) after treatment with 100, 200, and 300 mg/kg of 50% methanolic extract of Acacia arabica.

The need of male contraception that works:

Public health campaigns and scientific study are now heavily focused on male contraception. Condoms and vasectomy are the only male contraceptive methods available now (Sharma et al., 2019; Das et al., 2020). Although condoms are effective in preventing pregnancy and sexually transmitted diseases, improper use increases the likelihood of failure. Despite its great effectiveness, vasectomy is a permanent procedure, and its reversibility is not usually certain. This small selection of alternatives emphasizes how urgently innovative, efficient, and reversible male contraceptive techniques are needed

One possible path for the creation of innovative contraceptive approaches is the use of plant-based antifertility drugs (Uddin et al., 2021). They are believed to be more natural and safer alternatives to synthetic drugs, which may have dangerous side effects. Investigating the antifertility properties of plants such as Acacia arabica may result in the development of novel, less harmful contraceptive methods.

A Possible Antifertility Agent: Acacia arabica

Because of its many therapeutic uses, Acacia arabica has been the focus of a lot of research. Significant biological activity, including as antibacterial, anti-inflammatory, and antioxidant properties, have been shown by the plant's methanolic extracts (Sharma et al., 2010; Das et al., 2011). These characteristics imply that Acacia arabica may have an impact on male fertility in particular as well as reproductive health. It is thought that Acacia arabica's bioactive chemicals, which may disrupt spermatogenesis or hormonal control, are responsible for its antifertility effects (Uddin et al., 2018).

Acacia arabica and the Health of Male Reproduction:

Numerous investigations have looked at Acacia arabica's possible antifertility properties. For example, extracts from Acacia nilotica (a synonym for Acacia arabica) decreased sperm motility and density in male rats, suggesting that it may be used as a male contraceptive, according to Gaur et al. (2013). Similar to this, Singh et al. (2014) discovered that rats' testicular weight and sperm count significantly decreased when given an ethanolic extract of Acacia arabica bark, demonstrating the plant's capacity to interfere with spermatogenesis and lower male fertility.

Although these trials provide encouraging proof of Acacia arabica's antifertility benefits, little is known about the underlying processes. A critical area for further study is how Acacia arabica affects the endocrine system, specifically the amounts of vital reproductive hormones including testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Hormonal regulation is required for spermatogenesis and overall male reproductive function to proceed appropriately. Understanding how Acacia arabica impacts hormonal balance is essential to elucidating the mechanisms behind its antifertility effects.

Control of Male Reproduction by Hormones:

The hypothalamic-pituitary-gonadal (HPG) axis produces a complex interaction of hormones that govern the male reproductive system. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which triggers the anterior pituitary gland to release FSH and LH. While FSH is essential for spermatogenesis, working on Sertoli cells to enhance sperm formation, LH operates on Leydig cells in the testes to drive testosterone synthesis. In turn, testosterone causes negative responses in the pituitary and brain to regulate the release of GnRH, LH, and FSH.

Reduced fertility and poor spermatogenesis may result from an imbalance in these hormones. Therefore, the effects of any prospective antifertility medication, including plant extracts like Acacia arabica, on these important hormones must be assessed.

2. RESOURCES AND TECHNIQUES:

Approach:

Selection and Grouping of Animals:

Young, healthy Wistar albino rats weighing 150–200 g that were inbred and had established fertility were chosen. The research technique was approved by the institutional animal ethics council of Nims University in Jaipur, Rajasthan. They were kept in the usual climatic conditions, which included a temperature of 23 ± 2 °C and a humidity of 50 ± 5%. The rats were fed a standard rat diet and water throughout the study.

For the investigation, male albino Wistar rats (Rattus norvegicus) are used.

Rats are purchased from an approved source of research animals.

Rats are acclimated to lab settings for a minimum of one week before experiments begin.

Rats are split among four groups at random:

A vehicle solution is given to the control group.

A 50% methanolic extract of Acacia Arabica (100 mg/kg) was given to Experimental Group 1.

In the second experimental group, 200 mg/kg of a 50% methanolic extract of Acacia Arabica was administered.

Experimental Group 3 (which was given 300 mg/kg of an Acacia Arabica 50% methanolic extract)

The process of making an Areca Arabica crude extract:

Gathering of Plant Matter:

Acacia Arabica fresh parts (such bark, leaves, or pods) are gathered from a trustworthy source and verified botanically by a trained botanist.

The harvested plant material is thoroughly cleansed with sink water to remove any dirt or contaminants.

Drying

To preserve its phytochemical qualities, the cleaned plant material is allowed to air dry for a few days at room temperature in the shade.

Monitoring the drying process helps to guarantee equal drying and stop the formation of fungi.

Alternatively, plant material may be dried faster by heating it to between 40 and 45 degrees Celsius in a hot air oven.

Grazing

The plant material is ground into a coarse powder using an electric grinder or a mortar and pestle after it has fully dried.

-The powder is next sieved through a mesh to achieve a uniform particle size.

Making the Methanolic Extract:

The dried, powdered plant material is weighed in a known amount.

In a dry, clean container, the powder is immersed in 50% methanol (methanol:distilled water, 1:1 ratio). To guarantee sufficient extraction, the solvent volume is typically four to five times the weight of the plant material.

To aid in extraction, the mixture is allowed to sit at room temperature for 48 to 72 hours while being shaken or stirred occasionally.

Following the extraction period, the mixture is filtered through Whatman filters or a fine muslin cloth to remove the liquid extracts and plant residue

Extract Concentration:

In order to eliminate the methanol and produce a concentrated crude extract, the filtered extract is then concentrated using a rotary evaporator operating at decreased pressure at a temperature of no more than 40°C.

To create a semi-solid or solid crude extract, the concentrated extract is further dried in a desiccator or under vacuum to eliminate any last remnants of solvent.

Storage:

To avoid contamination and deterioration, the dried crude extract is gathered, weighed, and kept in an airtight container.

Relevant details, including the plant species, portion utilized, extraction date, and solvent employed, are indicated on the container.

Until it is required for further research, the extract is stored dry, cool, and out of direct sunlight.

Records:

For future reference and repeatability, every step, observation, and deviation from the standard method is recorded.

The crude extract's yield is determined and noted for use as a guide for determining dosage for ensuing studies.

Investigate Design and Experiment

1. For 60 days, an aqueous extract of Acacia arabica (bark) was taken orally. There were five groups of animals.

Group 1: As a vehicle treated control, the animals in this group received 0.5 cc of distilled water per day per rat.

Group II: 100 mg/kg body weight of aqueous extract of Acacia arabica in 0.5 ml of distilled water per day each rat.

Group III: 200 mg/kg body weight of aqueous extract of Acacia arabica in 0.5 ml of distilled water per day each rat.

Group IV: 300 mg/kg body weight of aqueous extract of Acacia arabica in 0.5 ml of distilled water per day each rat.

Method:

Every piece of information was methodically documented on a pre-made data gathering sheet. Quantitative analysis when we initially turn on the heat, the metal plate warms up. Our RBF, which contains solvent, starts to boil. The RBF vapors are transported to the condenser via the distillation tube. In the condenser, solvent vapors condense before falling to the thimble. We put our sample powder into the thimble. The powder must be covered from the bottom with a cotton ball to stop it from falling straight into the thimble. Additionally, start at the top and cover the powder. As a result, when the condensed vapors fall into the thimble and wet it, the solvent-soluble components follow the powder. The siphon connects the thimble to RBF, as we have seen before. The solvent mixture is then put into the thimble and siphon. Eventually, the siphon reaches a point where gravity pushes it over. Since the Siphon connects RBF directly, the overflowing liquid returns to RBF. This starts the first cycle. As I said before, we are free to complete as many cycles as we wish. To start, we don't change the solvent for every cycle. Moreover, as the solvent evaporates, the components in the sample do not follow suit. As a result, we consistently get 100% pure solvent vapors. When we think the sample has been sufficiently exhausted, we stop the cycles. The solvent and the components of the sample that dissolve in the solvent are what's left behind. The following stage will allow us to separate them.

Moral Considerations:

The study's ethical guidelines are followed while caring for and using laboratory animals. Approval is given by a similar regulatory authority, such the Institutional Animal Care and Use Committee (IACUC).

Throughout the inquiry, measures are implemented to alleviate the pain and suffering endured by the study participants.

Analysis of Statistics:

Adequate statistical analysis was applied to the data obtained from histological examinations and hormonal assays. To determine the differences between treatment groups and the control group, statistical tests such as one-way analysis of variance (ANOVA) followed by suitable post-hoc tests were performed. Results were considered statistically significant at $p < 0.05$. All findings were presented using appropriate graphical representations, including bar graphs and scatter plots, to clearly illustrate variations and trends among the groups

Results

The administration of 50% methanolic extract of *Acacia arabica* at different doses (100 mg/kg, 200 mg/kg, and 300 mg/kg body weight) produced significant, dose-dependent alterations in the seminal parameters of male *Rattus norvegicus* when compared with the control group.

Semen Quality Parameters

Table 1: Comparative Analysis across Groups:

Group	Sperm Count (million/mL)	Sperm Motility (%)	Progressive Motility (%)	Sperm Viability (%)	Normal Morphology (%)	Semen pH	Semen Volume (mL)
300 mg	60.0	76	60	78	81	7.2	0.6
200 mg	62.5	85	65	88	92	7.4	0.75
100 mg	90.0	88	69	90	95	7.7	0.80
Control	95.0	93	78	95	98	7.7	0.84

Sperm Count

The highest sperm count was observed in the control group (95.0 million/mL). Treatment with *Acacia arabica* extract caused a progressive reduction in sperm count. The decline was minimal at 100 mg/kg (90.0 million/mL) but pronounced at 200 mg/kg (62.5 million/mL) and 300 mg/kg (60.0 million/mL). This indicates a clear dose-dependent suppression of spermatogenesis.

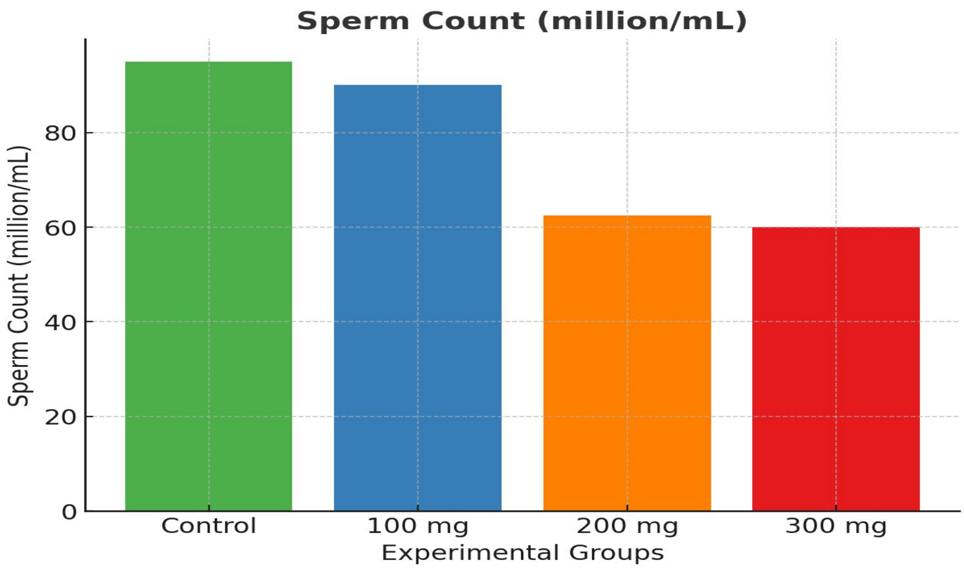

Figure 1. Comparative analysis of sperm count (million/mL) in control and *Acacia arabica* extract-treated groups

(100, 200, and 300 mg/kg). A significant, dose-dependent decline in sperm concentration was observed with higher extract doses.

Sperm Motility

Control rats exhibited 93% motility. Extract-treated groups showed a decrease in motility, with 88% at 100 mg/kg, 85% at 200 mg/kg, and 76% at 300 mg/kg. The decline was most evident at the highest dose, signifying compromised sperm functional ability.

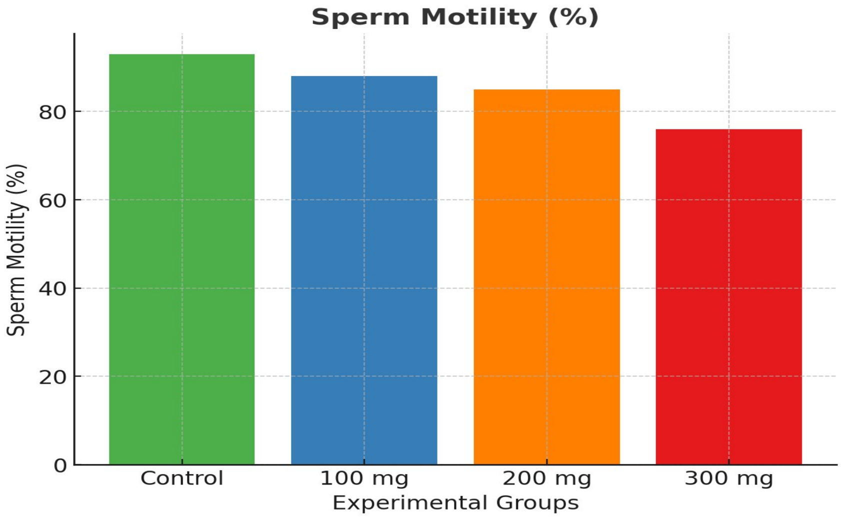

Figure 2. Effect of *Acacia arabica* extract on sperm motility (%) across groups. Control animals maintained higher motility, while extract-treated groups showed progressive reduction, most pronounced at 300 mg/kg.

Progressive Motility

A marked reduction in progressive motility was observed across treated groups. While the control group showed 78% progressive motility, values declined to 69% (100 mg/kg), 65% (200 mg/kg), and 60% (300 mg/kg). Reduced progressive motility reflects impaired sperm quality and fertilizing potential.

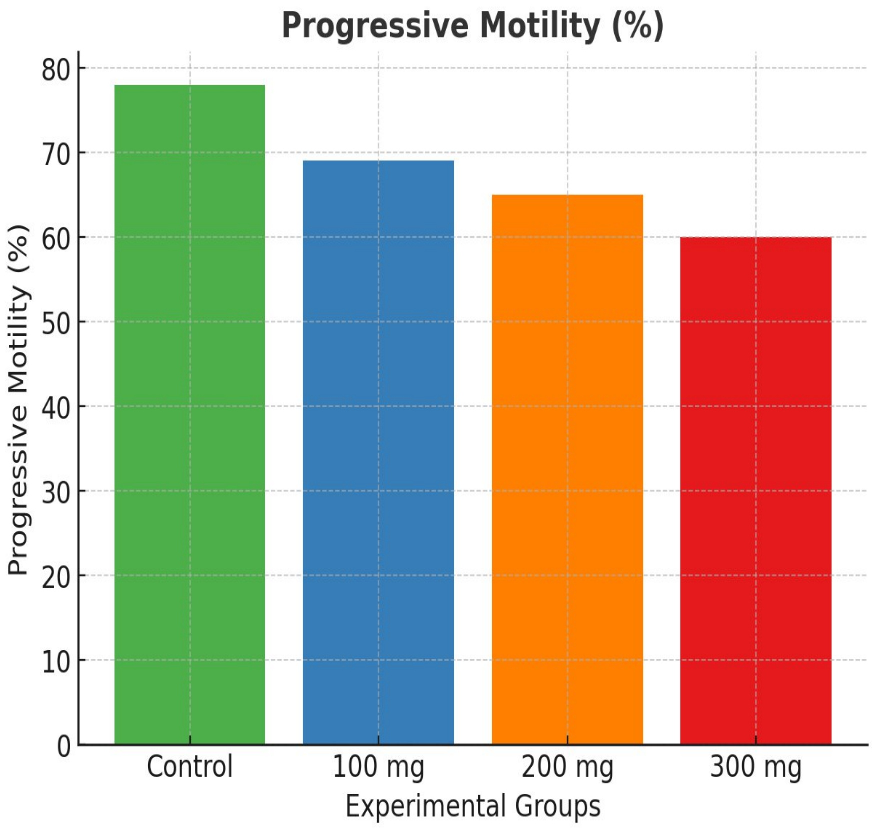

Figure 3. Progressive motility (%) of sperm in control and treated rats. A marked decline was observed in higher dose groups, indicating compromised fertilizing potential.

Sperm Viability

The percentage of viable sperm decreased with increasing extract dose. Control animals showed 95% viability, whereas treated groups showed 90%, 88%, and 78% viability at 100, 200, and 300 mg/kg respectively.

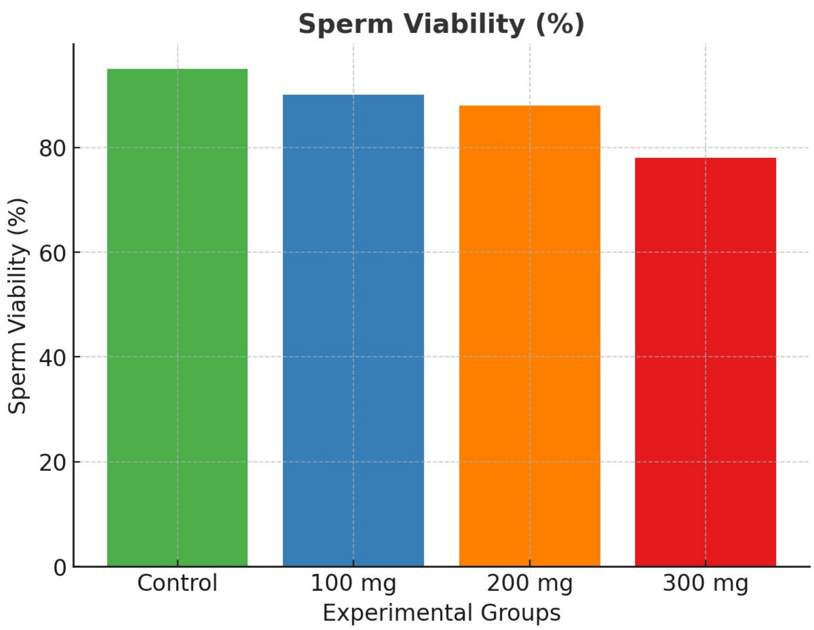

Figure 4. Sperm viability (%) in control and treated groups. A gradual dose-dependent reduction was noted, with maximum suppression at 300 mg/kg.

Sperm Morphology

Normal sperm morphology was highest in the control group (98%). A gradual reduction was evident in treated animals: 95% (100 mg/kg), 92% (200 mg/kg), and 81% (300 mg/kg). This indicates that higher doses induced morphological abnormalities, potentially linked with impaired spermatogenesis.

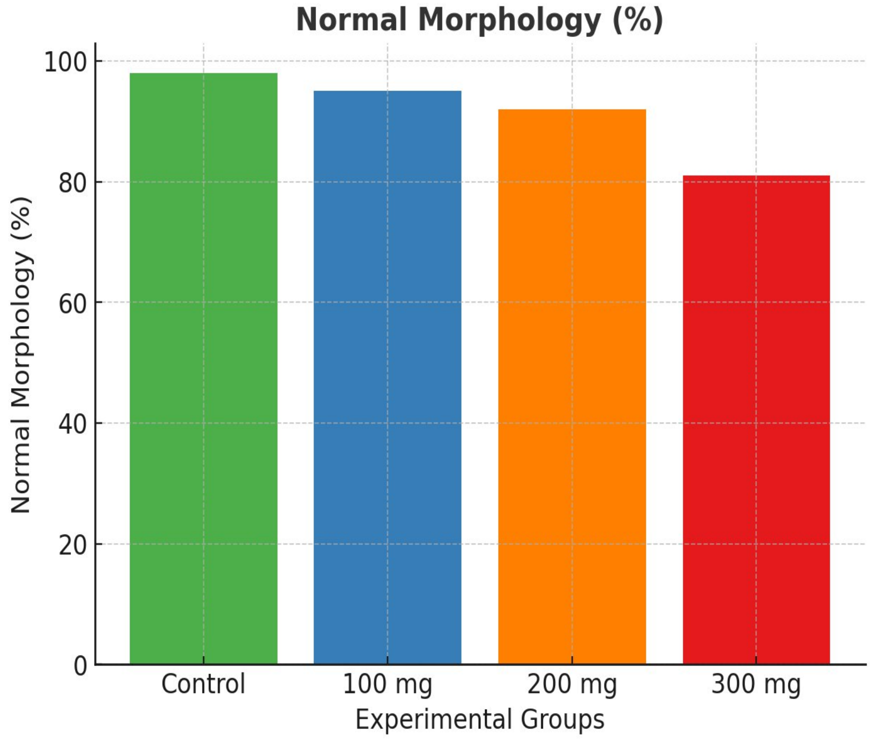

Figure 5. Normal sperm morphology (%) across experimental groups. Extract administration induced morphological abnormalities at higher doses, significantly reducing normal morphology at 300 mg/kg.

Semen pH and Volume

Semen pH remained relatively stable across groups, with minor fluctuations (7.7 in control and 100 mg/kg groups, 7.4 in 200 mg/kg, and 7.2 in 300 mg/kg). Semen volume, however, showed a decline: 0.84 mL (control), 0.80 mL (100 mg/kg), 0.75 mL (200 mg/kg), and 0.60 mL (300 mg/kg).

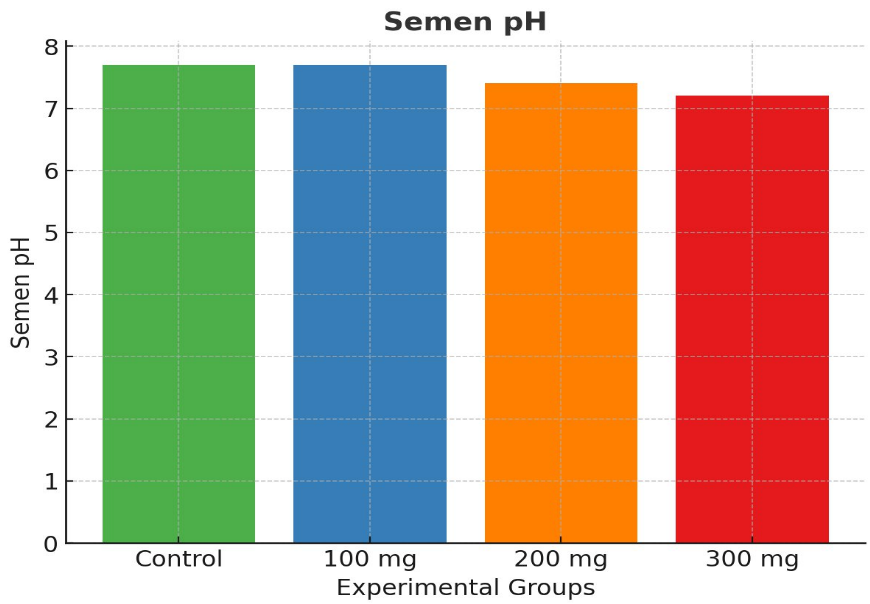

Figure 6. Semen pH levels in control and treated groups. Minor fluctuations were observed, with a slight acidic shift at higher doses of extract.

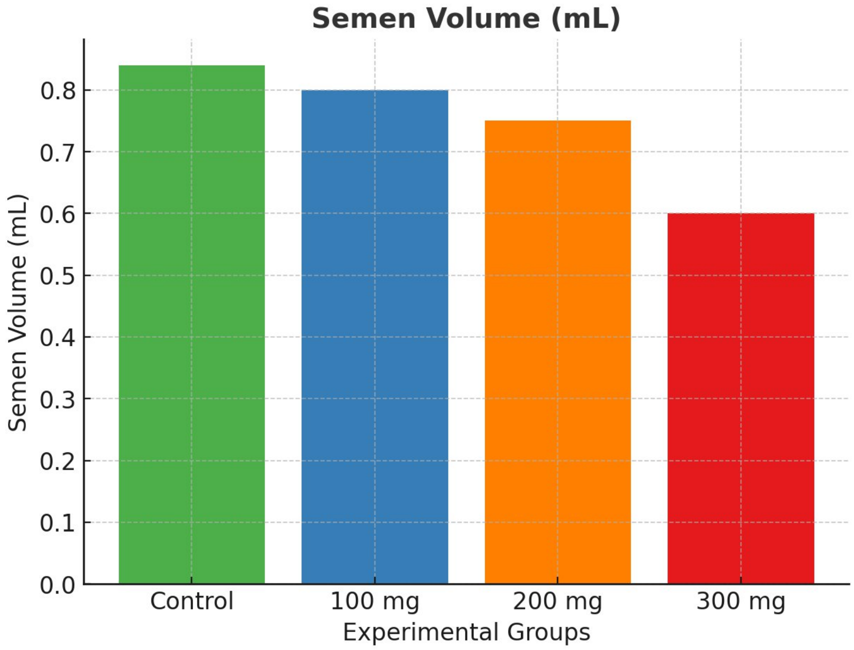

Figure 7. Semen volume (mL) across groups. A progressive reduction in ejaculate volume was evident with increasing doses of *Acacia arabica* extract.

Overall Trend

The findings demonstrate a **dose-dependent antifertility effect** of *Acacia arabica* methanolic extract on male *Rattus norvegicus*. While the lowest dose (100 mg/kg) produced only mild alterations in sperm quality, higher doses (200 and 300 mg/kg) significantly suppressed sperm count, motility, morphology, and viability. The reduction in semen volume and pH changes further support its negative impact on reproductive physiology.

3. DISCUSSION

The present study examined the antifertility effects of 50% methanolic extract of *Acacia arabica* bark on the reproductive profile of male *Rattus norvegicus*. The results demonstrated a clear, dose-dependent reduction in sperm count, motility, progressive motility, viability, and morphology, along with decreased semen volume and slight alterations in pH. These findings strongly indicate that *A. arabica* possesses active phytoconstituents that impair male reproductive efficiency.

The observed decline in sperm count and progressive motility is consistent with previous reports on the antifertility properties of *Acacia* species. Singh et al. (2014) reported that ethanolic bark extract of *A. arabica* significantly reduced sperm density and testicular weight in rats, while Gaur et al. (2013) highlighted suppressed motility and density in *A. nilotica*-treated groups. Such outcomes may be attributed to the presence of tannins, flavonoids, and saponins in *A. arabica*, which can interfere with spermatogenesis and alter sperm membrane stability.

Viability and morphology were also significantly affected at higher doses (200–300 mg/kg), indicating possible disruption of Sertoli cell function or oxidative imbalance. Saponins and tannins are known to disrupt lipid bilayers, leading to membrane instability and morphological abnormalities. Moreover, antioxidants in *A. arabica*, although beneficial in low concentrations, might exert paradoxical pro-oxidant effects at higher doses, thereby impairing sperm function.

The slight reduction in semen volume and pH shift toward acidity at 300 mg/kg further supports compromised accessory gland function. Such changes may reduce sperm motility and viability, compounding the antifertility effect. Importantly, these findings correlate with earlier research showing plant extracts such as *Maytenus marginata* (Sharma & Mali, 2016) and *Morinda lucida* (Salman et al., 2006) also induce antispermatogenic effects.

The mechanism behind these alterations likely involves interference with the hypothalamic-pituitary-gonadal axis. Reduced sperm parameters may be linked with decreased testosterone production due to impaired Leydig cell activity, as suggested by Dharmasiri & Ratnasooriya (2000) for *Acacia nilotica*. This hormonal imbalance can explain the decline in spermatogenesis and sperm maturation observed in this study.

Thus, the study provides further evidence that *Acacia arabica* extract contains potent antifertility agents, which may serve as a foundation for the development of herbal-based male contraceptives. However, detailed hormonal assays, oxidative stress markers, and histopathological evaluations are needed to delineate the exact pathways involved.

4. CONCLUSION

The administration of 50% methanolic extract of *Acacia arabica* bark to male *Rattus norvegicus* produced marked, dose-dependent antifertility effects. Higher doses (200 and 300 mg/kg) significantly reduced sperm count, motility, viability, morphology, and semen volume, while inducing minor alterations in semen pH. These findings suggest that bioactive phytochemicals present in *A. arabica* disrupt spermatogenesis and impair male reproductive physiology.

The study validates the traditional claims of *A. arabica* as a potential antifertility agent and highlights its promise in the development of plant-based, reversible, and safer male contraceptive alternatives. Nevertheless, further investigations involving hormonal profiling, testicular histology, and toxicity studies are essential to confirm safety, reversibility, and precise mechanisms of action before advancing toward clinical applications**.**

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