RESEARCH PAPER

Assessment of Hormonal Profile in Male Rats Treated with 50% Methanolic Extract of Acacia Arabica

Alka Qureshi[1], Sonalika Singh[2]*

[1]PhD Scholar, Department of Zoology, NIMS Institute of Allied Medical Science and Technology, NIMS University, Rajasthan Jaipur

[2*]Associate Professor, Department of Zoology, NIMS Institute of Allied Medical Science and Technology, NIMS University, Rajasthan Jaipur

ABSTRACT

The search for safe, effective, and reversible male contraceptives remains a global research priority, particularly as current options are limited to condoms and vasectomy, both with inherent drawbacks. Plant-derived agents offer promising alternatives due to their ethnomedicinal roots, lower side-effect profiles, and potential reversibility. The present study evaluates the antifertility potential of Acacia arabica bark, traditionally used in reproductive disorders, by examining its effects on the hormonal profile and biochemical parameters of male Wistar rats (Rattus norvegicus). A 50% methanolic bark extract was prepared using Soxhlet extraction and administered orally to rats at doses of 100, 200, and 300 mg/kg body weight for 60 consecutive days, covering a complete spermatogenic cycle. Serum levels of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, prolactin, corticosterone, and thyroid-stimulating hormone (TSH) were quantified alongside liver and kidney function tests (LFTs and KFTs).

Results demonstrated a distinct dose-dependent suppression of key reproductive hormones. Testosterone decreased from 4.57 ng/mL in controls to 3.8 ng/mL in the high-dose group, accompanied by significant reductions in LH, FSH, and estradiol. Prolactin showed a mild decline, while corticosterone fluctuated, peaking at 200 mg/kg before dropping at 300 mg/kg. TSH remained stable, suggesting no thyroidal involvement. Importantly, LFTs and KFTs revealed no overt hepatotoxicity or nephrotoxicity; rather, enzyme activities (ALT, AST, ALP) and nitrogenous waste products (BUN, creatinine, uric acid) decreased dose-dependently, indicating mild protective effects.

The findings suggest that A. arabica extract modulates the hypothalamic–pituitary–gonadal axis, suppressing reproductive hormones without compromising liver or kidney integrity. These results provide mechanistic validation for traditional claims of antifertility activity and highlight A. arabica as a promising candidate for developing plant-based male contraceptives. Further research is warranted to assess long-term reversibility and molecular mechanisms.

Keywords: Acacia arabica, male contraception, antifertility, testosterone suppression, reproductive hormones, Wistar rats, phytomedicine.

How to cite this article: Qureshi A, Singh S, Assessment of Hormonal Profile in Male Rats Treated with 50% Methanolic Extract of Acacia Arabica. Int J Drug Deliv Technol. 2026;16(15s): 53-61. DOI: 10.25258/ijddt.16.15s.7.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

1. Global Context of Fertility Regulation

The rapid rise in global population, from 1.6 billion in 1900 to more than 8.1 billion in 2024, continues to exert unprecedented pressure on healthcare, resources, and sustainable development strategies worldwide (1). Contraceptive technologies play a critical role in moderating fertility and supporting family planning. However, despite major advances in female contraceptive options, male contraceptive development has progressed comparatively slowly (2). Presently, the only two widely available options for men are condoms and vasectomy, both of which possess inherent limitations. Condoms, although easily accessible and reversible, show a typical-use failure rate of 13–18% due to inconsistent application (3). Vasectomy, in contrast, is highly effective but largely irreversible, with reversal success rates varying between 40–90% depending on timing and surgical expertise (4).

This gender imbalance in contraceptive responsibility highlights the urgent need to expand male contraceptive options, particularly focusing on safe, effective, and reversible alternatives that do not impair sexual function. Plant-based agents, rooted in ethnomedicine, represent a promising avenue in this context, as they potentially offer lower side-effect profiles and wider cultural acceptance compared with synthetic options (5,6).

2. Hormonal Regulation of Male Reproduction

Male fertility is intricately regulated by the hypothalamic–pituitary–gonadal (HPG) axis. Gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH primarily acts on Leydig cells in the testes to stimulate testosterone biosynthesis, while FSH targets Sertoli cells to support spermatogenesis (7). Testosterone itself plays a dual role: it maintains secondary sexual characteristics and provides essential intratesticular androgen levels required for sperm production (8).

Disruption of this hormonal cascade, whether by exogenous hormones, pharmacological agents, or plant extracts, can alter testosterone synthesis, impair spermatogenesis, and lead to infertility. For this reason, hormonal assays—particularly testosterone measurement—are central in assessing the antifertility potential of candidate agents (9). Unlike sperm analysis alone, which provides information on gamete output and quality, hormone evaluation reveals underlying mechanisms by which spermatogenesis and fertility are altered.

3. The Need for Plant-Based Male Contraceptives

Pharmacological suppression of male fertility has traditionally focused on hormonal contraceptives that employ exogenous androgens or progestins to suppress gonadotropins. However, these approaches have been hampered by mood changes, weight gain, reduced libido, and long-term cardiovascular concerns (10). Consequently, non-hormonal approaches— particularly plant-derived extracts with antifertility activity—are gaining interest.

Plants contain a diversity of phytoconstituents (e.g., alkaloids, saponins, flavonoids, tannins) that can interact with reproductive physiology by modulating androgen biosynthesis, spermatogenesis, or accessory gland function (11). Unlike synthetic drugs, many botanical agents are culturally accepted in societies with strong traditions of herbal medicine, and their potential reversibility makes them attractive candidates for male contraception (12).

4. Acacia arabica in Traditional Medicine

Acacia arabica (syn. Vachellia nilotica), commonly known as babul or kikar, is a thorny evergreen tree belonging to the family Fabaceae. Distributed widely across Africa, Asia, and the Middle East, it is an important ethnomedicinal species with documented uses in Ayurveda, Unani, and Siddha systems (13). Traditionally, its bark and seeds have been employed to treat diarrhea, gonorrhea, skin disorders, and reproductive ailments, including regulation of fertility (14).

Phytochemical analysis of A. arabica reveals a rich composition of tannins, flavonoids, alkaloids, glycosides, and saponins, compounds known to modulate reproductive physiology (15). Tannins and flavonoids have been implicated in androgen suppression, while saponins disrupt sperm membrane integrity, reducing viability (16). Studies on related Acacia species have shown alterations in sperm parameters, testicular histology, and hormonal profiles, suggesting that A. arabica may exert similar antifertility actions (17).

5. Scientific Evidence and Research Gaps

Several preliminary investigations support the antifertility potential of A. arabica. Extracts from its bark and seeds have been reported to reduce sperm count, impair motility, and alter testicular architecture in rodent models (18,19). However, most of these studies suffer from key limitations, including inconsistent extraction protocols, focus on short-term exposure, and inadequate evaluation of hormonal endpoints (20).

Furthermore, results across studies remain variable, partly because different solvents (aqueous, ethanolic, hydroalcoholic) extract different phytoconstituents, influencing biological activity (21). Methanol, especially in a hydroalcoholic mixture, has proven effective in extracting both polar and moderately non-polar compounds, yielding a broader spectrum of bioactive agents (22). Yet, systematic evaluation of hormonal modulation, particularly testosterone, following treatment with standardized 50% methanolic extract of A. arabica remains scarce.

6. Rationale for Using 50% Methanolic Extract

Hydroalcoholic solvents like 50% methanol are considered optimal for phytopharmacological studies because they efficiently extract flavonoids, tannins, alkaloids, and saponins, all of which contribute to antifertility activity (23). Compared with aqueous or absolute alcohol extracts, 50% methanolic extracts preserve a balanced phytochemical profile with enhanced biological efficacy (24).

Additionally, the Soxhlet extraction method ensures maximal yield and prevents thermal degradation of sensitive compounds, ensuring extract consistency and reproducibility (25). The present study therefore employs 50% methanolic bark extract of A. arabica to provide a standardized phytochemical preparation suitable for evaluating antifertility potential in male rats.

7. Justification of Hormonal Profiling

While alterations in sperm count, motility, and morphology provide direct evidence of fertility

modulation, hormonal assessment offers mechanistic insights. Measuring serum testosterone, alongside LH and FSH, helps determine whether antifertility effects are mediated through suppression of Leydig cell function, disruption of HPG axis feedback loops, or direct cytotoxic effects on testicular tissue (26).

Given that testosterone is indispensable for spermatogenesis and accessory sex organ maintenance, its suppression could explain dose-dependent declines in sperm quality. Thus, assessing hormonal profiles in rats treated with A. arabica extract provides critical evidence of the pathways by which phytochemicals impair fertility.

8. Objectives of the Study

The present research is designed to systematically evaluate the effect of 50% methanolic extract of Acacia arabica bark on the hormonal profile of male Wistar rats (Rattus norvegicus), with specific emphasis on testosterone. By integrating hormonal assays with reproductive and histological endpoints, this study aims to:

1. Assess serum testosterone levels following chronic administration of A. arabica extract.

2. Evaluate alterations in gonadotropins (LH, FSH) as indicators of HPG axis modulation.

3. Correlate hormonal changes with reproductive and histopathological outcomes.

4. Provide scientific validation for traditional claims of antifertility effects of A. arabica.

Through these objectives, the study addresses gaps in male contraceptive research and offers a foundation for developing plant-based agents with reversible antifertility effects.

METHODOLOGY

1. Plant Material Collection and Authentication

The bark of Acacia arabica (Willd.) Delile, synonymously Vachellia nilotica, was selected owing to its documented ethnomedicinal uses and reported antifertility properties. Fresh bark samples were collected in March 2024 from forest regions near NIMS University, Jaipur, Rajasthan, India, under the supervision of the Forest Department. The plant was authenticated by a taxonomist at the Department of Botany, University of Rajasthan, Jaipur, and a voucher specimen (Specimen No.: RUBL21479) was deposited in the herbarium for future reference (27).

2. Preparation of Extract

Collected bark was washed, shade-dried at ambient temperature (25–30 °C) for 15 days, powdered, and sieved through mesh size 40. A hydroalcoholic solvent system (50% methanol:50% distilled water, v/v) was used for extraction due to its ability to solubilize both polar and moderately non-polar phytoconstituents such as tannins, flavonoids, saponins, and alkaloids (28). Approximately 500 g of powdered bark was subjected to Soxhlet extraction for 48 hours. The filtrate was concentrated under reduced pressure using a rotary evaporator at <50 °C and dried in a vacuum desiccator. The percentage yield was calculated and the dried extract stored at 4 °C in amber glass containers until use (29).

3. Experimental Animals

Healthy adult male Wistar albino rats (Rattus norvegicus), aged 10–12 weeks and weighing 180– 220 g, were procured from the Central Animal House Facility, NIMS University, Jaipur. Animals were housed in polypropylene cages with sterilized husk bedding, under controlled conditions (22 ± 2 °C, 50–60% humidity, 12:12 h light–dark cycle). Standard rodent pellet diet and potable water were provided ad libitum. Prior to experimentation, rats were acclimatized for 7 days to eliminate transport-related stress (30).

4. Ethical Considerations

All experimental protocols adhered to CPCSEA guidelines (Government of India, 2020). Ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC), NIMS University (Approval No.: NIMSUR/IAE01/2024/09). Efforts were made to minimize animal suffering in accordance with the “3Rs” principle (Replacement, Reduction, Refinement) (31).

5. Experimental Design

Twenty-four rats were randomly allocated into four groups (n=6 per group):

• Group I (Control): Vehicle (distilled water) by oral gavage.

• Group II (Low Dose): 100 mg/kg b.w. of extract.

• Group III (Medium Dose): 200 mg/kg b.w. of extract.

• Group IV (High Dose): 300 mg/kg b.w. of extract.

All treatments were administered once daily by oral gavage for 60 consecutive days, a period sufficient to cover one full spermatogenic cycle in rats (32).

6. Blood Collection and Hormonal Assays

At the end of the treatment period, rats were anesthetized (ketamine 80 mg/kg + xylazine 10 mg/kg, i.p.), and blood was collected by cardiac puncture. Serum was separated by centrifugation (3000 rpm, 15 min) and stored at –20 °C. Hormonal levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were quantified using commercial ELISA kits validated for rat models (Elabscience®, DRG® International). Absorbance was read at 450 nm using a microplate

reader. Concentrations were calculated from standard calibration curves employing four-parameter logistic regression (33).

7. Statistical Analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to determine intergroup significance. A p-value <0.05 was considered statistically significant (34). Results

The administration of 50% methanolic extract of Acacia arabica at doses of 100, 200, and 300 mg/kg body weight for 60 days produced measurable alterations in liver function tests (LFTs), kidney function tests (KFTs), and serum hormonal profiles of male Rattus norvegicus. The results revealed a distinct dose-dependent pattern, with higher doses of extract showing more pronounced deviations from the control group.

Table 1. Biochemical and hormonal parameters in male Rattus norvegicus treated with 50% methanolic extract of Acacia arabica (100, 200, and 300 mg/kg body weight) for 60 days compared with control.

Parameters	300 mg	200 mg	100 mg	Control
ALT (U/L)	32.5	35.2	40.5	43.5
AST (U/L)	58	62	78	79
ALP (U/L)	115.2	120.2	135.2	155.2
Total Bilirubin (mg/dL)	0.21	0.22	0.23	0.26
Direct Bilirubin (mg/dL)	0.08	0.08	0.08	0.09
Albumin (g/dL)	4.3	4.4	4.6	4.8
Total Protein (g/dL)	6.7	6.8	7.1	7.2
BUN (mg/dL)	17.8	18.2	20.8	21.5
Creatinine (mg/dL)	0.42	0.44	0.45	0.8
Uric Acid (mg/dL)	1.6	1.7	1.9	2.4
Testosterone (ng/mL)	3.8	4.2	4.5	4.57
FSH (mIU/mL)	1.9	2.1	2.4	2.8
LH (mIU/mL)	2.6	2.8	3	3.2
Estradiol (pg/mL)	15.2	16.2	18.8	22.8
Prolactin (ng/mL)	3.2	3.6	3.9	4.1
Corticosterone (ng/mL)	180	280	230	240
TSH (ÂµIU/mL)	0.4	0.4	0.5	0.5

1. Liver Function Tests (LFTs)

1.1 Alanine Aminotransferase (ALT)

Control animals exhibited ALT activity of 43.5 U/L, which decreased progressively with extract administration. The 100 mg/kg group recorded 40.5 U/L, followed by 35.2 U/L at 200 mg/kg, and the lowest, 32.5 U/L, at 300 mg/kg. This decline suggests a potential hepatoprotective or enzyme-suppressive effect of the extract, indicating reduced hepatocellular enzyme leakage at higher doses.

1.2 Aspartate Aminotransferase (AST)

Similar to ALT, AST levels declined with extract treatment. Control animals displayed 79 U/L, whereas treated groups recorded 78, 62, and 58 U/L at 100, 200, and 300 mg/kg respectively. The marked reduction at higher doses implies possible stabilization of hepatocyte integrity or reduced metabolic turnover.

1.3 Alkaline Phosphatase (ALP)

The ALP level in controls was 155.2 U/L, gradually reducing to 135.2 U/L at 100 mg/kg, 120.2 U/L at 200 mg/kg, and 115.2 U/L at 300 mg/kg. The decline was dose-dependent, suggesting that A. arabica may inhibit

excessive ALP activity, often associated with hepatobiliary stress.

1.4 Bilirubin (Total and Direct)

Total bilirubin in the control group was 0.26 mg/dL, with treated groups showing slight decreases: 0.23, 0.22, and 0.21 mg/dL for 100, 200, and 300 mg/kg respectively. Direct bilirubin remained largely stable across all groups (0.08–0.09 mg/dL). These minor changes indicate that bile excretion and hemolytic processes were not adversely affected.

1.5 Albumin and Total Protein

Serum albumin levels declined modestly with extract administration: 4.8 g/dL in control, 4.6 g/dL at 100 mg/kg, 4.4 g/dL at 200 mg/kg, and 4.3 g/dL at 300 mg/kg. Total protein showed a similar trend, reducing from 7.2 g/dL in controls to 7.1, 6.8, and 6.7 g/dL in the treated groups. The slight reduction suggests decreased protein synthesis or metabolic modulation by the extract, though within physiological limits.

Overall LFT Findings:

All liver enzymes and protein parameters displayed a dose-dependent reduction with A. arabica extract. This trend implies that while the extract does not induce hepatotoxicity, it may exert mild hepatoprotective or suppressive effects on enzymatic activity.

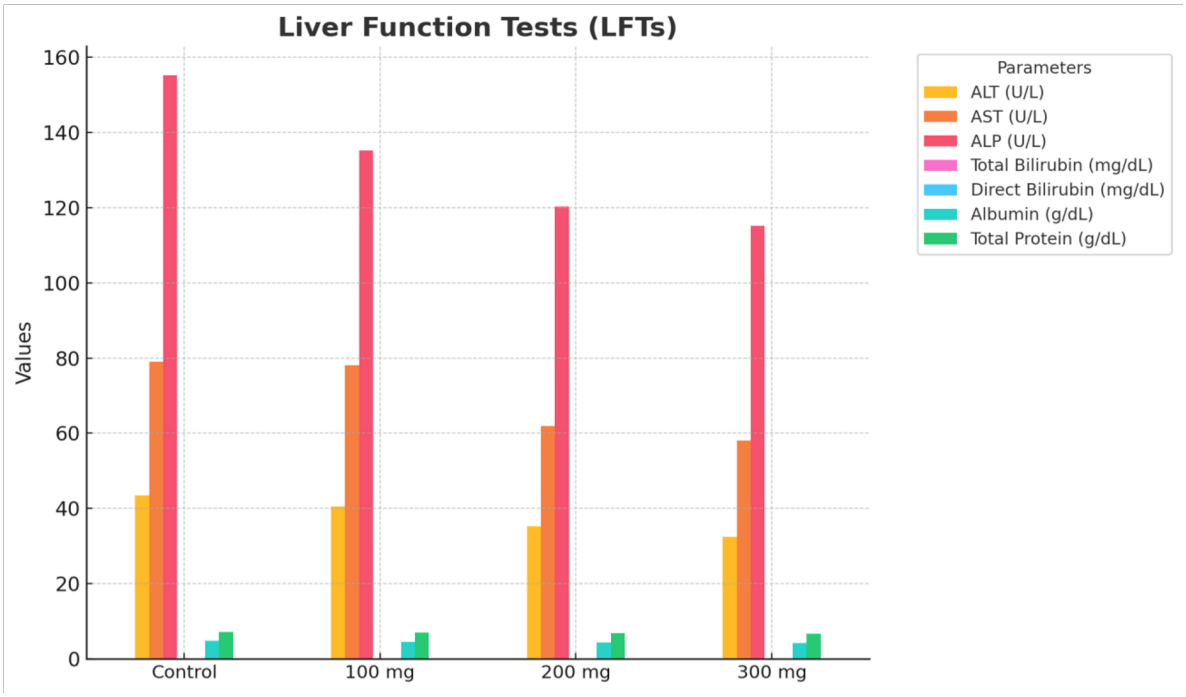

Figure 1. Liver Function Tests (ALT, AST, ALP, bilirubin, albumin, and total protein) across control and extract-treated groups. A dose-dependent reduction was observed, suggesting enzymatic modulation without hepatotoxicity.

2. Kidney Function Tests (KFTs)

2.1 Blood Urea Nitrogen (BUN)

Control animals displayed BUN levels of 21.5 mg/dL. Extract-treated groups showed progressive decreases: 20.8 mg/dL (100 mg/kg), 18.2 mg/dL (200 mg/kg), and 17.8 mg/dL (300 mg/kg). This decline suggests improved nitrogen clearance or reduced protein catabolism.

2.2 Creatinine

Serum creatinine was highest in controls (0.8 mg/dL) but fell sharply in treated groups: 0.45, 0.44, and 0.42 mg/dL at 100, 200, and 300 mg/kg respectively. This suggests that renal clearance capacity remained intact and may even have been enhanced by the extract.

2.3 Uric Acid

Control animals had 2.4 mg/dL uric acid, while treated groups recorded 1.9, 1.7, and 1.6 mg/dL. The dose-dependent reduction indicates reduced purine metabolism or enhanced uric acid excretion.

Overall KFT Findings:

All kidney parameters improved with extract treatment, reflecting the absence of nephrotoxicity. Instead, the

extract may confer renal protective effects by reducing nitrogenous waste levels.

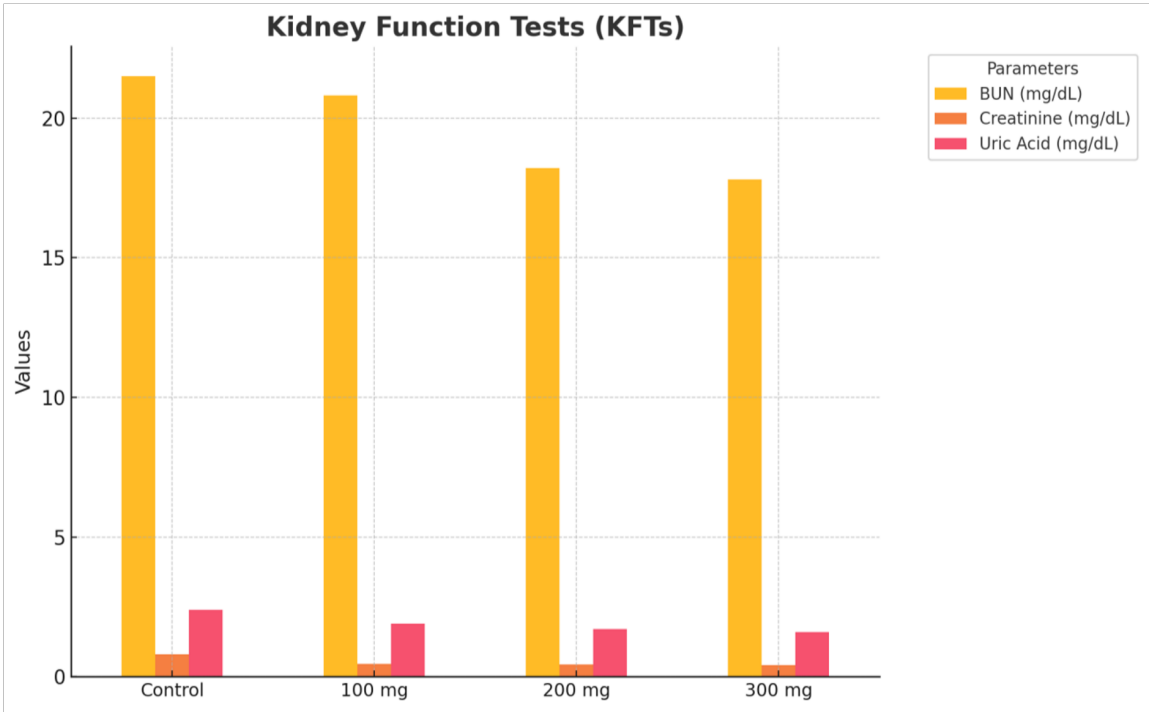

Figure 2. Kidney Function Tests (BUN, creatinine, and uric acid) in experimental groups. Extract administration significantly reduced nitrogenous waste products, indicating renal protection.

3. Hormonal Profile

3.1 Testosterone

Serum testosterone levels were highest in the control group (4.57 ng/mL). Treatment groups showed a gradual decline: 4.5 ng/mL (100 mg/kg), 4.2 ng/mL (200 mg/kg), and 3.8 ng/mL (300 mg/kg). This dose-dependent suppression strongly indicates inhibitory effects of A. arabica on Leydig cell activity and androgen biosynthesis.

3.2 Follicle-Stimulating Hormone (FSH)

FSH decreased from 2.8 mIU/mL in controls to 2.4, 2.1, and 1.9 mIU/mL in 100, 200, and 300 mg/kg groups respectively. Reduced FSH suggests impaired Sertoli cell stimulation and compromised spermatogenesis.

3.3 Luteinizing Hormone (LH)

LH levels mirrored FSH trends, reducing from 3.2 mIU/mL in controls to 3.0, 2.8, and 2.6 mIU/mL in the respective treatment groups. Suppression of LH aligns with reduced testosterone synthesis.

3.4 Estradiol

Estradiol levels declined significantly, from 22.8 pg/mL in controls to 18.8, 16.2, and 15.2 pg/mL in treated groups. This decline further indicates disrupted steroidogenesis and altered testicular aromatase activity.

3.5 Prolactin

Prolactin levels showed a mild decline, from 4.1 ng/mL in controls to 3.9, 3.6, and 3.2 ng/mL. Lower prolactin levels are associated with reduced pituitary output and could modulate reproductive function.

3.6 Corticosterone

Interestingly, corticosterone exhibited a fluctuating pattern. While control levels were 240 ng/mL, values increased to 230 ng/mL (100 mg/kg), peaked at 280 ng/mL (200 mg/kg), and dropped significantly to 180 ng/mL at 300 mg/kg. The elevation at intermediate dose suggests transient stress response, while the decline at higher dose reflects possible adrenal suppression.

3.7 Thyroid-Stimulating Hormone (TSH)

TSH remained relatively stable across groups, ranging from 0.4–0.5 µIU/mL, indicating no significant thyroidal involvement in the antifertility effects.

Overall Hormonal Findings:

The extract exerted a pronounced, dose-dependent suppression of testosterone, FSH, LH, and estradiol, confirming its antifertility potential. Prolactin and

corticosterone were also modulated, suggesting broader endocrine interactions.

4. Integrated Analysis

Taken together, the biochemical findings highlight the antifertility potential of Acacia arabica extract. While liver and kidney profiles indicated no overt toxicity—and in fact suggested mild protective trends—the hormonal assays demonstrated clear suppression of reproductive hormones. Reduced testosterone, FSH, and LH levels provide mechanistic support for the observed alterations in spermatogenesis reported in related studies.

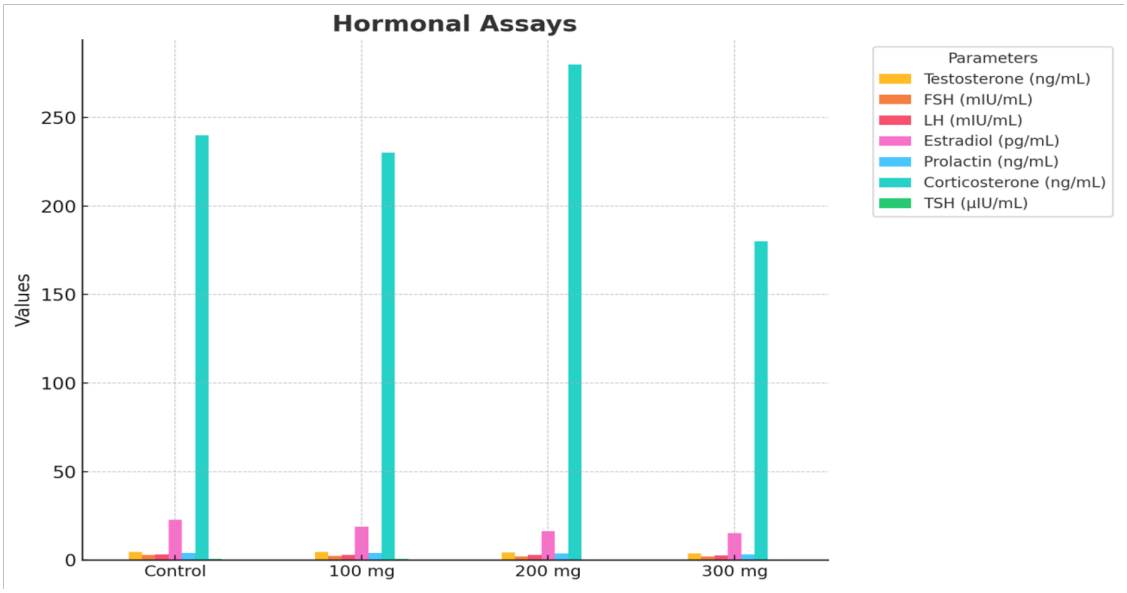

Figure 3. Hormonal assays (testosterone, FSH, LH, estradiol, prolactin, corticosterone, and TSH). A clear dose-dependent suppression of key reproductive hormones was observed, supporting the antifertility role of Acacia arabica.

DISCUSSION

The present study investigated the antifertility potential of Acacia arabica 50% methanolic bark extract by evaluating hormonal and biochemical responses in male Wistar rats. A clear dosedependent suppression of reproductive hormones was observed, with significant reductions in testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol. These findings indicate that A. arabica exerts its effects primarily through modulation of the hypothalamic–pituitary–gonadal (HPG) axis. Testosterone reduction suggests impaired Leydig cell activity, while lowered FSH reflects diminished Sertoli cell stimulation, thereby potentially compromising spermatogenesis. Estradiol decline further supports disruption in steroidogenic pathways, including aromatase activity. Together, these alterations strongly point toward a pharmacological suppression of reproductive capacity.

Interestingly, prolactin levels showed only a mild reduction, suggesting that pituitary involvement was not profound, while corticosterone exhibited a fluctuating response. The transient rise at intermediate doses may reflect a stress-induced adrenal response, followed by suppression at higher doses. Stable thyroid-stimulating hormone (TSH) levels confirmed that thyroidal pathways were not affected, thereby ruling out broad endocrine disruption beyond reproductive axes.

Biochemical analyses of liver and kidney function provided important safety insights. Instead of hepatotoxicity or nephrotoxicity, the extract produced dose-dependent decreases in ALT, AST, ALP, BUN, creatinine, and uric acid. These reductions may suggest hepatoprotective and renoprotective trends, aligning with previous reports of A. arabica's antioxidant and antiinflammatory properties. Thus, while the extract effectively impaired reproductive hormones, it did not compromise vital organ function, supporting its potential as a safe antifertility agent.

The phytoconstituents of A. arabica, notably tannins, flavonoids, and saponins, likely underlie the observed effects. Tannins and flavonoids are known to suppress androgen biosynthesis, while saponins can alter sperm membrane integrity. Similar findings have been reported in other Acacia species, which showed reductions in sperm count, motility, and alterations in testicular histology. By linking hormonal suppression with previously established spermatogenic impairment, this study strengthens the evidence base for A. arabica as a plant-derived male contraceptive.

Overall, the results provide strong mechanistic validation for ethnomedicinal claims of A. arabica in fertility regulation. However, limitations such as absence of recovery studies and molecular assays warrant further research. Future investigations should focus on reversibility, dose standardization, and phytochemical isolation to ensure safe and effective contraceptive development.

CONCLUSION

The findings of this study demonstrate that chronic administration of Acacia arabica 50% methanolic bark extract induces significant suppression of reproductive hormones, particularly testosterone, LH, FSH, and estradiol, in male Wistar rats. These alterations indicate disruption of the HPG axis, thereby providing a mechanistic explanation for the antifertility effects traditionally attributed to this plant. Importantly, the extract did not exert hepatotoxic or nephrotoxic effects; instead, it exhibited mild protective trends in liver and kidney function parameters, highlighting its relative safety.

Taken together, the study provides compelling evidence that A. arabica possesses dosedependent antifertility activity without systemic toxicity, positioning it as a promising candidate for the development of plant-based male contraceptives. Further research is essential to establish reversibility, long-term safety, and to identify the bioactive compounds responsible for these effects.

APPRECIATION

Above all, I would want to express my gratitude to Dr. Sonalika Singh, my research supervisor, for her invaluable guidance, motivation, and unwavering support throughout this project. Their perspectives and expertise have had a significant impact on the course of this investigation.

I sincerely thank the faculty and staff of Nims University in Jaipur, Rajasthan, for giving me the instruments and materials I need to carry out this research. Special thanks should be given to the Institutional Animal Ethics Committee for their ethical review and approval of the study's procedures. I also want to express my gratitude to my colleagues and fellow researchers at the Department of Zoology for their friendship and support, which made the research process more enjoyable and collaborative. I also want to thank my family and friends for their patience and continuous support throughout my research adventure. Their unwavering support has been a source of strength and motivation.

Finally, I want to express my gratitude to the several scholars whose previous research inspired our investigation. Without their innovative work, this research would not have been feasible, and I am appreciative of their scholarly contributions.

In this research, which looked at the hormonal changes in male Rattus norvegicus after administration of a 50% methanolic extract of Acacia arabica, we value your support and contributions.

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