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Histomorphological Changes in the Ovary and Uterine Horn of Female Wistar Rats Following 28-Day Oral Administration of *Acacia nilotica* Pod Extract

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ABSTRACT

Acacia nilotica pods are extensively used in Northern Nigeria for treating reproductive disorders, yet their morphological effects on female reproductive organs remain poorly characterized. This study evaluated dose-dependent histological changes in ovarian and uterine tissues following repeated oral administration. Fifty adult female Wistar rats were divided into five groups (n=10): control (normal saline), three treatment groups (125, 250, 500 mg/kg for 28 days), and one recovery group (500 mg/kg for 28 days followed by 14-day withdrawal). Ovaries and uterine horns were examined histologically using hematoxylin-eosin staining with semi-quantitative follicular assessment. Ovarian and uterine horn weights remained unchanged across groups ($p>0.05$), except for modest uterine horn index elevation at 250 and 500 mg/kg ($p<0.05$). Uterine architecture was preserved in all groups. Ovarian histology revealed dose-dependent increases in atretic follicle density (control: 2.4±0.6 vs. 500 mg/kg: 8.2±1.1 per high-power field; $p<0.01$), with reduced mature follicle counts. Recovery animals showed partial reversal (5.1±0.9 atretic follicles/HPF). Corpus luteum and developing follicles persisted across groups. Aqueous *Acacia nilotica* pod extract induces reversible, dose-dependent ovarian follicular atresia without affecting uterine morphology, suggesting selective reproductive toxicity requiring caution in traditional use.

Keywords: Follicular atresia, ovarian histology, medicinal plant toxicity, uterine morphology

INTRODUCTION

The mammalian ovary undergoes dynamic morphological and functional changes throughout reproductive life, characterized by coordinated follicular recruitment, growth, ovulation, and corpus luteum formation, processes that are tightly regulated by endocrine and paracrine signaling pathways[1]. Follicular atresia, the physiological degenerative process affecting the majority of developing follicles, plays an essential role in maintaining ovarian homeostasis and preserving the finite ovarian reserve; however, this process can be pathologically accelerated by environmental toxins, pharmacologic agents, or plant-derived xenobiotics, potentially resulting in impaired fertility or premature ovarian dysfunction[2]. In contrast to the ovary, the uterus, comprising the endometrial epithelium, supporting stroma, and smooth muscle myometrium, generally maintains structural integrity under normal physiological conditions, with morphological alterations typically occurring only in response to hormonal fluctuations, inflammatory processes, or exposure to toxic substances that disrupt reproductive tissue architecture[3]. Because both organs function as integrated components of the female reproductive axis, toxicological insults affecting either ovarian folliculogenesis or uterine endometrial structure may compromise reproductive capacity.

Acacia nilotica (L.) pods are widely consumed in Northern Nigeria as components of traditional medicinal preparations used for the management of reproductive disorders, including vaginal infections, menstrual irregularities, and other gynecological complaints[4]. The frequent use of these preparations, often without dosage standardization or long-term safety evaluation, raises concerns regarding potential subclinical or cumulative reproductive toxicity. Despite the extensive ethnomedicinal reliance on this plant, systematic experimental studies examining its direct histomorphological effects on female reproductive organs remain limited.

Previous phytochemical investigations have identified saponins, tannins, and flavonoids as major bioactive constituents of Acacia nilotica pods[4,5]. These classes of compounds have been reported in other plant species to influence reproductive physiology and, in some cases, to exert anti-fertility or gonadotoxic effects through mechanisms involving follicular degeneration, endocrine disruption, oxidative stress, or interference with steroidogenesis[6,7]. Such findings underscore the importance of controlled experimental evaluation of ovarian and uterine tissue responses following exposure to the extract.

Accordingly, the present study aimed to characterize dose-dependent histomorphological changes in ovarian and uterine tissues following 28-day oral administration of aqueous Acacia nilotica pod extract in female Wistar rats, with additional assessment of tissue recovery and reversibility after a 14-day withdrawal period. We hypothesized that chronic exposure would induce follicular degeneration and endometrial alterations proportional to the administered dose.

MATERIALS AND METHODS

Plant material and extraction

Acacia nilotica pods were collected in July 2023 from Forestry Quarters, Maiduguri, Borno State, Nigeria (11°50′N, 13°09′E). The plant was authenticated by a taxonomist in the Department of Biological Sciences, University of Maiduguri (voucher specimen: UNIMAID/BIO/2023/047). Air-dried pods were pulverized and extracted via the Soxhlet method (500g powder in 1L distilled water, 48h at 40°C). The aqueous extract was concentrated, yielding 200g (40% w/w) and stored at 4°C.

Phytochemical screening

Standard qualitative tests were performed to detect alkaloids, flavonoids, saponins, tannins, cardiac glycosides, terpenoids, and carbohydrates[5,8]. Qualitative phytochemical analysis was performed using standard colorimetric methods. Alkaloids were detected using Mayer's and Dragendorff's reagents; flavonoids by the alkaline reagent test; saponins by the frothing test; tannins by ferric chloride reaction; cardiac glycosides by Keller–Killiani test; terpenoids by Salkowski reaction; and carbohydrates by Molisch's test, following established procedures[5,8].

Animals and experimental design

Fifty nulliparous female Wistar rats (90–100g, 8–10 weeks old) were obtained from the University of Maiduguri animal house. Animals were housed in polypropylene cages (5–10 per cage) under natural photoperiod with standard rodent chow and water ad libitum. After 14-day acclimatization, rats were randomly allocated to five groups (n=10): Group I (control, normal saline), Groups II–IV (125, 250, 500 mg/kg extract daily for 28 days), and Group V (500 mg/kg for 28 days plus 14-day withdrawal). All procedures followed international guidelines for laboratory animal use[9].

Ethical approval

This study was approved by the Department of Human Anatomy Postgraduate Ethics Committee, University of Maiduguri (Approval No. UM/HA/PGR13.14-07703). All experimental procedures were conducted in accordance with institutional guidelines and the National Research Council Guide for the Care and Use of Laboratory Animals (8th edition).

Tissue collection and processing

Animals were humanely euthanized by cervical dislocation performed by trained personnel in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (8th edition). This method is an accepted humane technique for small laboratory rodents and was selected to ensure rapid loss of consciousness while avoiding potential chemical interference with reproductive tissue morphology.

Ovaries (bilateral) and uterine horns were excised, weighed (Mettler Toledo AB204-S analytical balance, ±0.1mg precision), and organ indices calculated as (organ weight/final body weight) × 100. Tissues were fixed in Heidenhain's Susa fixative for 24 hours, dehydrated through graded alcohols, cleared in xylene, and embedded in paraffin wax. Sections (5 µm) were cut using a rotary microtome (Leica RM2125 RTS), mounted on glass slides, and stained with hematoxylin-eosin (H&E).

Histological assessment

Ovarian sections were examined at ×100 and ×400 magnification (Olympus CX23 microscope) by a blinded histopathologist. Five non-overlapping high-power fields (HPF, ×400) per ovary were assessed for follicle counts categorized as: primordial, primary, secondary, tertiary (antral), atretic (characterized by pyknotic granulosa cells, oocyte degeneration, and zona pellucida fragmentation), and corpus luteum. Uterine sections were evaluated for epithelial integrity, stromal cellularity, and myometrial architecture. Representative photomicrographs were captured using an Olympus DP22 digital camera.

Statistical analysis

Data were expressed as mean ± SEM. One-way ANOVA with Tukey's post-hoc test was used for group comparisons (GraphPad Prism 9.0). Statistical significance was set at $p<0.05$.

RESULTS

Phytochemical constituents

Qualitative screening confirmed the presence of flavonoids, cardiac glycosides, terpenoids, tannins, saponin glycosides, and carbohydrates; alkaloids and anthraquinones were absent (Table 1).

Organ weights and indices

No significant differences in bilateral ovarian weights were observed across groups ($p>0.05$). Uterine horn index was comparable to control at 125 mg/kg but increased significantly at 250 mg/kg (0.734±0.137 vs. 0.412±0.061, $p<0.05$) and 500 mg/kg (0.687±0.090, $p<0.05$). The recovery group showed intermediate values (0.519±0.039), suggesting partial normalization (Table 2).

Ovarian histomorphology and quantitative follicle assessment

Control ovaries demonstrated normal cortical architecture with follicles at various developmental stages and prominent corpora lutea. Atretic follicle density averaged 2.4±0.6 per HPF, consistent with physiological follicular turnover (Figure 1A).

Treatment groups exhibited dose-dependent increases in follicular atresia: 125 mg/kg (4.8±0.7/HPF, $p<0.05$), 250 mg/kg (6.5±0.9/HPF, $p<0.01$), and 500 mg/kg (8.2±1.1/HPF, $p<0.01$ vs. control). Atretic follicles showed characteristic pyknotic granulosa cells, oocyte degeneration, and zona pellucida fragmentation. Mature tertiary follicles were reduced in treated groups, while primordial and primary follicles remained relatively preserved. Corpora lutea were present across all groups, indicating preserved ovulatory capacity despite increased atresia (Figures 1 I–IV).

The recovery group (500 mg/kg + 14-day withdrawal) showed partial reversal with atretic follicle density of 5.1±0.9/HPF ($p<0.05$ vs. non-recovery 500 mg/kg group; $p>0.05$ vs. 250 mg/kg group), demonstrating incomplete but significant regenerative capacity (Figure 1 IV).

Uterine horn histology

Uterine horn sections from all treatment groups showed intact columnar epithelium with normal nuclear-cytoplasmic ratios, preserved endometrial stroma with typical vascular distribution, and regular myometrial smooth muscle architecture. No erosion, hyperplasia, inflammation or glandular abnormalities were detected at any dose level or in the recovery group (Figure 2).

Table 1: Phytochemical constituents of aqueous Acacia nilotica pod extract

Phytochemical	Result
Flavonoids	+
Cardiac glycosides	+
Terpenoids	+
Tannins	+
Saponin glycosides	+
Carbohydrates	+
Alkaloids	-
Anthraquinones	-

(+) Detected qualitatively; (–) Not detected.

Table 2: Organ indices of rats treated with aqueous Acacia nilotica pod extract for 28 days

Group	Left ovary	Right ovary	Uterine horn
Control	0.079±0.039	0.098±0.032	0.412±0.061
125 mg/kg	0.046±0.004	0.042±0.003	0.393±0.026
250 mg/kg	0.097±0.017	0.087±0.014	0.734±0.137*
500 mg/kg	0.190±0.007	0.213±0.009	0.687±0.090*
500 mg/kg + Recovery	0.181±0.013	0.185±0.016	0.519±0.039

* Significant at $p<0.05$

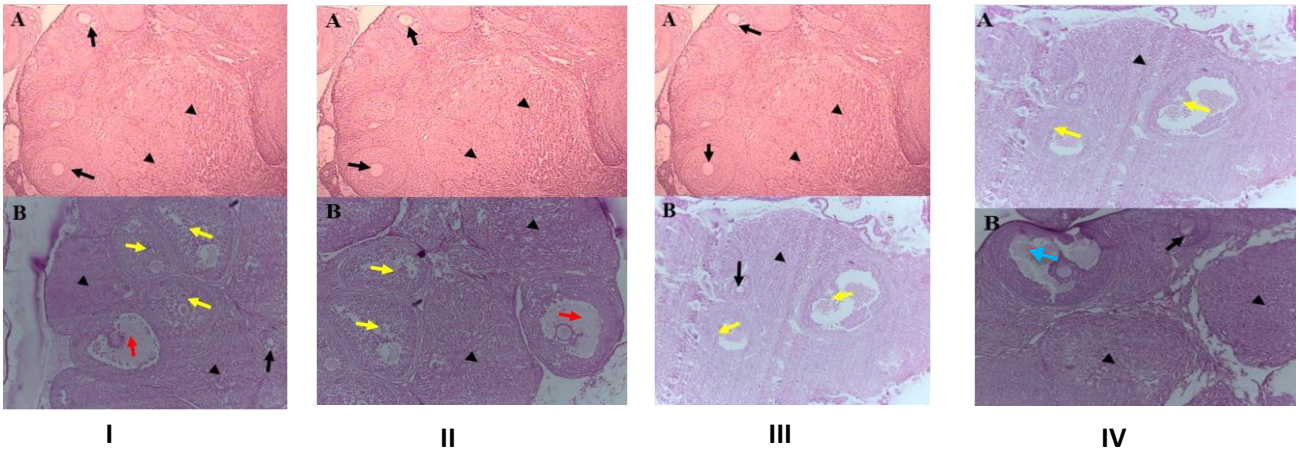

Figure 1: Dose-Dependent Ovarian Changes (Composite). Representative photomicrographs of rat ovaries (H&E, ×100). (I) The control and 125 mg/kg group showing increased atretic follicles (yellow arrows) with preserved developing follicles. (II) The control and 250 mg/kg group demonstrating marked follicular degeneration. (III) The control and 500 mg/kg group showing extensive atresia with reduced mature follicles. (IV) The 500 mg/kg group and recovery demonstrating incomplete but significant regenerative capacity (blue arrows). Scale bar = 100 µm.

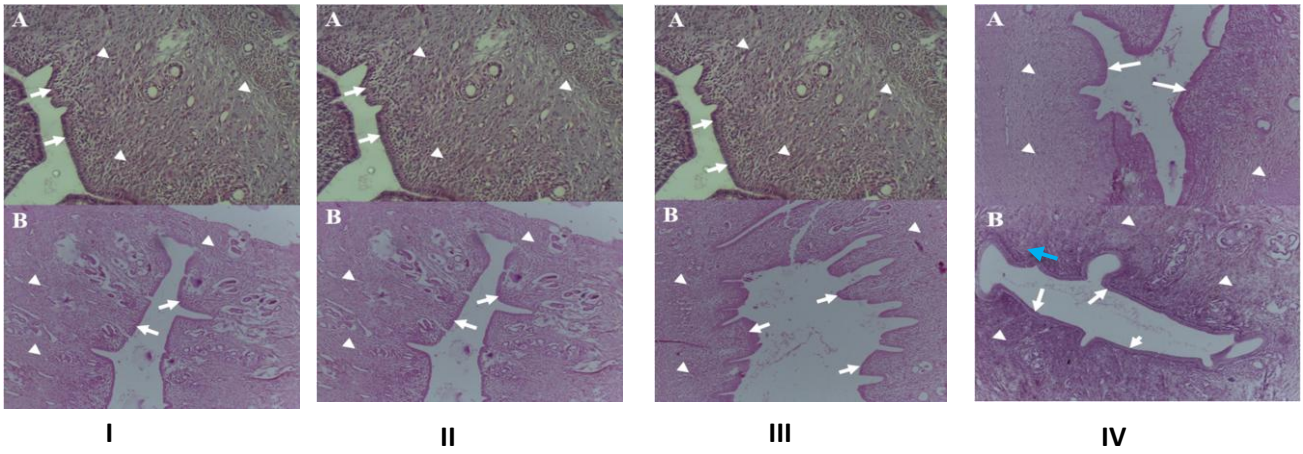

Figure 2: Dose-Dependent Uterine Horn Changes (Composite). Representative photomicrographs of rat uterine horn (H&E, ×100) showing uterine epithelium (white arrow) and connective tissue (white arrowheads). No pathological changes were observed across all treatment groups. (I) Control and 125 mg/kg group. (II) Control and 250 mg/kg group. (III) Control and 500 mg/kg group. (IV) 500 mg/kg group and the recovery group. Scale bar = 100 μm.

DISCUSSION

This study demonstrates tissue-specific reproductive toxicity of aqueous *Acacia nilotica* pod extract, characterized by dose-dependent ovarian follicular atresia with preserved uterine architecture. The quantitative assessment of follicular degeneration increased from 2.4±0.6 atretic follicles per HPF in untreated rats to 8.2±1.1 atretic follicles per HPF in rats receiving the highest dose (500 mg/kg), providing objective evidence of ovotoxicity, while the absence of uterine pathology indicates differential organ sensitivity.

The modest elevation in uterine horn index at higher doses without corresponding histological abnormalities likely reflects transient vascular congestion or mild edema rather than pathological hypertrophy. Organ weight changes are early toxicity indicators[10], but the histological preservation of endometrial and myometrial integrity argues against structural toxicity to the uterus. Follicular atresia is a physiological process eliminating 99% of mammalian oocytes[11]. However, the 3.4-fold increase in atretic follicle density at 500 mg/kg exceeds normal turnover rates, indicating pathological acceleration. The morphological hallmarks, pyknotic granulosa cells, oocyte fragmentation, and zona pellucida disruption, are consistent with apoptotic atresia[12].

Saponins, identified in this extract, are established ovotoxins that disrupt steroidogenesis by interfering with cholesterol trafficking and mitochondrial membrane integrity[7,13]. Tannins may exacerbate this through protein precipitation and oxidative stress induction[14]. The preservation of primordial and primary follicles suggests that toxicity targets actively developing follicles with higher metabolic demands, while quiescent follicles remain relatively protected.

The 38% reduction in atretic follicle density after a 14-day withdrawal period (from 8.2 to 5.1/HPF) demonstrates partial ovarian recovery. This regenerative response likely reflects clearance of the toxic agent with subsequent resumption of normal folliculogenesis, recruitment of previously quiescent primordial follicles, and restoration of gonadotropin-responsive follicular development[16]. However, the incomplete recovery suggests either irreversible damage to a subset of follicles or that the recovery duration was insufficient. Extended withdrawal studies are therefore warranted to determine whether full normalization can be achieved.

The striking contrast between ovarian toxicity and uterine preservation may reflect tissue-specific differences in xenobiotic metabolism, as the ovary's high cytochrome P450 activity may generate reactive metabolites[17], as well as differences in blood flow dynamics, with ovarian permeability to circulating toxins exceeding uterine uptake, and in cellular proliferation rates, since actively dividing granulosa cells are more vulnerable than the more differentiated uterine epithelium. This pattern of selectivity parallels observations reported for other plant ovotoxins, including gossypol and Tripterygium wilfordii extracts[18,19].

Acacia nilotica pods are consumed extensively in Northern Nigeria, often without dosage standardization or professional guidance. Extrapolating rodent doses to human equivalents (using body surface area normalization), the 500 mg/kg rat dose approximates 80 mg/kg in humans[20],

equivalent to 4.8g daily for a 60kg individual. Traditional preparations often exceed this dose, particularly when consumed chronically for reproductive complaints. Moreover, traditional formulations are not standardized, and the concentration of active phytochemicals can vary substantially with plant origin, harvesting conditions, preparation technique, and duration of storage, resulting in unpredictable systemic exposure. The demonstrated follicular toxicity, even with partial reversibility, raises concerns about subfertility risk in reproductive-age women. Healthcare providers in endemic regions should counsel patients on potential ovarian effects and advocate for regulated herbal product standardization.

This study employed semi-quantitative follicle counting rather than stereological analysis, which limits precision. Future work should incorporate design-based stereology for unbiased follicle quantification. Additionally, molecular markers of apoptosis (TUNEL, caspase-3) and cell proliferation (Ki-67) would strengthen mechanistic understanding. The 28-day exposure duration models subacute toxicity but does not capture chronic use patterns common in traditional medicine; extended studies (90-day, multigenerational) are needed. Finally, fertility outcome assessments (mating trials, litter size) would translate morphological findings into functional reproductive endpoints.

CONCLUSION

Repeated oral administration of aqueous *Acacia nilotica* pod extract induces dose-dependent ovarian follicular atresia without affecting uterine histology, with partial reversibility upon treatment cessation. These findings mandate caution in the unregulated traditional use of this plant for reproductive disorders, particularly among women of childbearing age.

Conflict of interest: The authors have no conflicts of interest to declare.

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Authors' contribution:

FBJ: Research conception and design, data collection, laboratory analysis, data analysis, manuscript drafting, and revision. **MU:** Research supervision, study design input, data interpretation, critical manuscript revision, and final approval.

REFERENCES

1. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. Endocr Rev. 2000;21(2):200-14.
2. Tilly JL. Commuting the death sentence: how oocytes strive to survive. Nat Rev Mol Cell Biol. 2001;2(11):838-48.
3. Jabbour HN, Kelly RW, Fraser HM, Critchley HOD. Endocrine regulation of menstruation. Endocr Rev. 2006;27(1):17-46.
4. Alli LA, Adesokan AA, Salawu AO. Antimalarial activity of fractions of aqueous extract of Acacia nilotica root. J Intercult Ethnopharmacol. 2016;5(2):180-5.
5. Sofowora A. Medicinal plants and traditional medicine in Africa. 3rd ed. Ibadan: Spectrum Books; 2008.
6. Malini T, Vanithakumari G. Rat toxicity studies with β-sitosterol. J Ethnopharmacol. 1990;28(2):221-34.
7. Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: a review. Br J Nutr. 2002;88(6):587-605.
8. Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders Publishers; 2002. p. 42-44, 221-29, 246-49, 304-06, 331-32, 391-93.
9. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals [Internet]. 8th ed. Washington (DC): National Academies Press; 2011 [cited 2026 Jan 31]. Available from: https://www.nap.edu/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth
10. Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, *et al*. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicol Pathol. 2007;35(5):742-50. doi:10.1080/01926230701595292.

11. Kaipia A, Hsueh AJ. Regulation of ovarian follicle atresia. Annu Rev Physiol. 1997;59:349-63.

12. Hussein MR. Apoptosis in the ovary: molecular mechanisms. Hum Reprod Update. 2005;11(2):162-77. doi: 10.1093/humupd/dmi001. Epub 2005 Feb 10. PMID: 15705959.

13. Li X, Liu Y, Wang N, Liu Y, Wang S, Wang H, *et al*. Synthesis and discovery of 18β-glycyrrhetinic acid derivatives inhibiting cancer stem cell properties in ovarian cancer cells. RSC Adv. 2019;9(47):27294-304. doi:10.1039/C9RA04608J.

14. Adamczyk B, Simon J, Kitunen V, Adamczyk S, Smolander A. Tannins and their complex interaction with different organic nitrogen compounds and enzymes: old paradigms versus recent advances. ChemistryOpen. 2017;6(5):610-14.

15. Welt CK, Schneyer AL. Differential regulation of inhibin B and inhibin A by follicle-stimulating hormone and local growth factors in human granulosa cells from small antral follicles. J Clin Endocrinol Metab. 2001;86(1):330-36.

16. Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, *et al*. Oocyte-specific deletion of *Pten* causes premature activation of the primordial follicle pool. Science. 2008;319(5863):611-13.

17. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in humans. Cancer Lett. 2005;227(2):115-24.

18. Gadelha ICN, Fonseca NBS, Oloris SCS, Melo MM, Soto-Blanco B. Gossypol toxicity from cottonseed products. Sci World J. 2014;2014:231635. doi:10.1155/2014/231635.

19. Tang W, Eisenbrand G. *Glycyrrhiza glabra* L. In: Tang W, Eisenbrand G. Chinese drugs of plant origin: chemistry, pharmacology, and use in traditional and modern medicine. Berlin: Springer-Verlag; 1992. p. 849-854.

20. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008;22(3):659-61.