The Journal of Anatomical Sciences
Email: journalofanatomicalsciences@gmail.com

J. Anat Sci 17(2) June

Asparagus Leaf Extract Enhances Male Fertility via GnRH–Hormone Coupling

[1]Onoja B, [2]Oyewo R. A, [3*]Oyewopo A. O, [3]Yunusa M. M, [4]Stephen D. A, [5]Ajayi A. J[5], [6]Adepoju A. E

[1]Department of Anatomy, Federal University of Health Sciences, Otukpo; [2]Department of Anatomy, Afe Babalola University, Ado Ekiti; [3]Department of Anatomy, University of Ilorin, Ilorin; [4]Department of Environmental Health Sciences, [5]Department of Physiology, Thomas Adewumi University, Oko-Irese; [6]Department of Clinical Pharmacy, North South College of Health Technology, Kwara State, Nigeria

***Corresponding Author:** Email: oyewopo.ao@unilorin.edu.ng; Tel: +2348033925431

ABSTRACT

The hypothalamic–pituitary–gonadal (HPG) axis is central to male reproductive function, and its disruption contributes to infertility. *Asparagus officinalis* leaves, less studied than roots or shoots, contain bioactive compounds with antioxidant and androgenic properties. This study investigated the effects of aqueous *A. officinalis* leaf extract (ALEA) on the HPG axis in male Wistar rats, with emphasis on regression modeling to establish quantitative links between hypothalamic Gonadotropin-Releasing Hormone (GnRH) activity and reproductive hormones. Twenty-four adult male Wistar rats were randomized into four groups: control, ALEA 100 mg/kg, ALEA 200 mg/kg, and ALEA 400 mg/kg. Treatments were administered orally for 28 days. Serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were assayed by ELISA. Testicular histomorphometry was performed using Fiji (ImageJ) to quantify seminiferous tubule diameter, germinal epithelium thickness, and Leydig cell density. Hypothalamic GnRH immunoreactivity was evaluated. Statistical analysis included Analysis of Variance with Tukey's post hoc test and regression modeling. ALEA significantly increased testosterone, LH, and FSH in a dose-dependent manner ($p < 0.05$). Histology revealed enlarged seminiferous tubules, higher spermatogenic index, and improved cytoarchitecture. GnRH immunoreactivity was elevated, and regression analysis showed strong correlations with testosterone ($R^2 = 0.9966$), LH ($R^2 = 0.9956$), and FSH ($R^2 = 0.9981$). ALEA enhances male fertility by upregulating hypothalamic GnRH and quantitatively coupling it to gonadotropin and androgen secretion. These findings provide novel mechanistic evidence for *A. officinalis* leaves as a phytotherapeutic candidate in male reproductive health.

Keywords: *Asparagus officinalis*; HPG axis; GnRH; regression analysis; male fertility

INTRODUCTION

The hypothalamic--pituitary--gonadal (HPG) axis regulates male reproductive function by means of a coordinated secretion of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone[1] whose disruption has been noted to lead to infertility, hypogonadism, or impaired spermatogenesis[2]. Male infertility remains a significant global health concern, with the World Health Organization estimating that infertility affects approximately 15% of couples worldwide[3], with male factors being culpable in about 20% of cases whilst contributing majorly to another 30% to 40% of all infertility cases[4]. This rising prevalence of male reproductive disorders has therefore intensified the search for safe and effective therapeutic interventions, particularly those derived from natural sources.

Phytochemicals derived from medicinal plants whose therapeutic efficacy have been linked to present classes of constituents like alkaloids, flavonoids, and volatile oils have gained considerable attention over time for their potential to modulate reproductive hormones and improve spermatogenic function[5]. Various plant species, including *Tribulus terrestris*, *Withania somnifera*, and *Mucuna pruriens* among many other herbal plants have been reported in literature to have fertility-enhancing properties through diverse mechanisms such as antioxidant activity, hormonal modulation, and direct stimulation of spermatogenesis.[6] These plants contain bioactive compounds including steroidal saponins, flavonoids, and alkaloids that interact with the HPG axis at

multiple levels, from hypothalamic GnRH neurons to Leydig and Sertoli cells in the testes.[7-9]

Asparagus officinalis L. (family Asparagaceae) was selected for this study. The plant name was verified using World Flora Online (WFO ID: wfo-0000634022; accessed 7 January 2026), which reports it as an accepted name in the genus *Asparagus* (record 275197, derived from WCSP, data supplied 2024-06-04). *Asparagus officinalis* is a perennial herb widely cultivated for its edible shoots and recognized in traditional medicine for its diuretic, anti-inflammatory, and rejuvenating properties.[10] The plant contains a rich phytochemical profile including steroidal saponins (protodioscin, sarsasapogenin), flavonoids (quercetin, rutin, kaempferol), polyphenols, and polysaccharides.[8] These compounds exhibit antioxidant, anti-inflammatory, and androgenic properties that may benefit reproductive health[9-10]. While asparagus roots and shoots have been traditionally studied for fertility enhancement[10], data on the effects of leaf extract on the HPG axis remain limited.

The leaves of *A. officinalis* represent an underutilized plant part that may contain comparable or even superior concentrations of certain bioactive compounds relative to the more commonly studied roots and shoots. Previous investigations have demonstrated that *A. officinalis* root extract influences hypothalamic-pituitary-gonadal axis hormone levels and ovarian follicle development in adult rats.[11] However, the specific effects of leaf-derived extracts on male reproductive endocrinology, particularly at the level of hypothalamic GnRH secretion remain scarce in literature. This gap in knowledge is significant given the increasing interest in valorizing agricultural produce, such as asparagus leaves, which are typically discarded during harvest as it generates significant amount of by-products every year that consist of root and frond whose negligence have had negative impacts on subsequent farm produce implicated by the release of autotoxic materials into the soil.[12]

The present study was designed to investigate the modulatory effects of aqueous *A. officinalis* leaf extract (ALEA) on the HPG axis in male Wistar rats through biochemical, histological, and immunohistochemical analyses. We hypothesized that ALEA would enhance hypothalamic GnRH expression, thereby stimulating pituitary gonadotropin release and testicular androgen production, ultimately improving spermatogenic output. To test this hypothesis, we employed regression modeling to establish quantitative relationships between GnRH immunoreactivity and circulating reproductive hormone levels, providing mechanistic insight into the mode of action of this phytotherapeutic agent.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were of analytical grade. Serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were quantified using ELISA kits (Cusabio Rat Testosterone ELISA Kit, Cat. No. CSB-E05100r; Elabscience Rat LH ELISA Kit, Cat. No. EEL-R0392; Invitrogen Rat FSH ELISA Kit, Cat. No. EEL125).

Plant material and extraction

Fresh *Asparagus officinalis* leaves were collected, authenticated by a botanist at the Herbarium of the Department of Plant Biology, University of Ilorin and was subsequently deposited with the voucher number UILH/001/200/2026 for future reference after which it was air-dried. Powdered leaves (500 g) were macerated in 2 L of distilled water for 48 h, filtered, and lyophilized to obtain a crude extract.

Experimental animals

Twenty-four adult male Wistar rats (180–220 g) were housed under standard laboratory conditions (12 h light/dark cycle, 25 ± 2 °C) with free access to food and water. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Faculty of Basic Medical Sciences, University of Ilorin, and were conducted in accordance with NIH guidelines.[13]

Experimental design

Rats were randomly divided into four groups (n = 6): • Control: 1 mL distilled water • Low-dose ALEA: 100 mg/kg body weight • Medium-dose ALEA: 200 mg/kg body weight • High-dose ALEA: 400 mg/kg body weight. Extracts were administered orally once daily for 28 days.

Blood collection and hormone assays

On day 29, rats were anesthetized, and blood was collected via cardiac puncture. Serum was separated and stored at –20 °C. Testosterone, LH, and FSH levels were measured using ELISA kits following manufacturer protocols.

Histological analysis

Testes were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Seminiferous tubule diameter, epithelial height, and spermatogenic index were measured using Fiji software as described Schneider *et al*.[14]

Immunohistochemistry

Hypothalamic sections were deparaffinized, rehydrated, and subjected to immunohistochemical staining for GnRH using the avidin–biotin complex (ABC) method. Positive staining was quantified as the percentage of GnRH-positive neurons.[15]

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons among groups were performed using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism version 10.2.0 (GraphPad Software Inc., San Diego, CA, USA). A p value < 0.05 was considered statistically

significant. In addition to group comparisons, simple linear regression analysis was conducted to evaluate the relationship between hypothalamic GnRH-positive neuron expression and serum reproductive hormones (testosterone, LH, and FSH). Regression parameters including slope, intercepts, coefficient of determination (R^2), confidence intervals, and p values were calculated to determine the strength and significance of associations. This dual approach allowed both categorical comparisons and quantitative modeling of GnRH–hormone coupling.

RESULTS

Weight changes across experimental groups

Final body weight and testis weight were assessed following 28 days of treatment with graded doses of Asparagus leaf extract (ALEA). As shown in Table 1 and Figure 1A–B, ALEA produced dose-dependent increases in both parameters compared to the control group.

Table 1 presents the effects of graded doses of Asparagus leaf extract (ALEA) on final body weight and testis weight in male rats after 28 days of treatment. Asterisks indicate statistical significance compared to the control group based on Tukey's post hoc test following one-way ANOVA: (ns) = not significant, *p < 0.05, *p < 0.01, ****p < 0.0001. Body weight differences were significant at p = 0.0032; testis weight differences at p < 0.0001.

For body weight (Figure 1A), the low-dose group (100 mg/kg) showed a slight increase relative to control, but this difference was not statistically significant (ns). Medium-dose ALEA (200 mg/kg) significantly elevated body weight (p < 0.05), while high-dose ALEA (400 mg/kg) produced a stronger increase (p < 0.01). Overall, one-way ANOVA confirmed significant group differences (p = 0.0032).

For testis weight (Figure 1B), ALEA administration resulted in a clear dose-dependent increase. The low-dose group showed no significant change compared to control. Medium-dose ALEA significantly increased testis weight (p < 0.01), while high-dose ALEA produced the most pronounced effect (p < 0.0001). ANOVA confirmed highly significant differences across groups (p < 0.0001).

Association between GnRH expression and sex hormones

Regression modeling revealed significant positive associations between hypothalamic GnRH immunoreactivity and circulating sex hormones. For testosterone, GnRH expression predicted serum levels with a slope of 0.1350 (95% CI: 0.1110–0.1590, p = 0.0017), yielding an equation of $Y = 0.1350X + 1.830$ and an R^2 of 0.9966. Similarly, GnRH was strongly correlated with LH (slope = 0.07765, 95% CI: 0.06197–0.09333, p = 0.0022, R^2 = 0.9956) and FSH (slope = 0.09400, 95% CI: 0.08146–0.1065, p = 0.0010, R^2 = 0.9981). All slopes were significantly non-zero, confirming that GnRH activity quantitatively predicts gonadotropin and androgen output (Figure 2).

Reproductive hormone levels across experimental groups

Distinct alterations in serum reproductive hormones were observed following ALEA administration. As summarized in Table 2 and illustrated in Figure 2A–C, testosterone, LH, and FSH all rose significantly in a dose-dependent manner compared to control animals.

Table 2 presents the effects of graded doses of Asparagus leaf extract (ALEA) on serum reproductive hormone levels in male rats. Asterisks indicate statistical significance compared to the control group based on Tukey's post hoc test following one-way ANOVA: ****p < 0.0001 for Testosterone, LH, and FSH.

For testosterone (Figure 3A), even the lowest dose of ALEA (100 mg/kg) produced a sharp elevation relative to control (p < 0.0001). Medium and high doses further amplified this effect, with the 400 mg/kg group reaching the highest values, underscoring a strong androgenic response.

Similarly, FSH levels (Figure 3B) were significantly elevated across all treatment groups (****p < 0.0001). The rise was progressive, with the high-dose group showing the most pronounced improvement, consistent with enhanced Sertoli cell activity and spermatogenic support

LH concentrations (Figure 3C) mirrored this pattern. All ALEA groups showed significant increases (****p < 0.0001), with the high-dose group achieving the greatest recovery of pituitary gonadotropin output.

Spermatogenic activity following ALEA administration

Marked improvements in spermatogenic index were observed across all ALEA-treated groups. As detailed in Table 3 and visualized in Figure 4, ALEA elicited a clear dose-dependent enhancement in testicular histological activity.

Table 3 shows the effects of graded doses of Asparagus leaf extract (ALEA) on the spermatogenic index in male rats, reflecting testicular histological activity. Asterisks indicate statistical significance compared to the control group based on Tukey's post hoc test following one-way ANOVA: ****p < 0.0001.

The control group exhibited a baseline spermatogenic index of 2.8 ± 0.058, reflecting normal testicular function. In contrast, the low-dose ALEA group (100 mg/kg) showed a significant increase to 3.5 ± 0.058 (****p < 0.0001). Further elevation was seen in the medium-dose group, which reached 4.0 ± 0.058 (****p < 0.0001), while the high-dose group achieved the highest index at 4.5 ± 0.058 (****p < 0.0001).

Table 1: Weight changes across experimental groups

Animal Groups	Final Body Weight (g)	Testis Weight (g)
Control	205.0 ± 4.83	1.95 ± 0.037
ALEA 100 mg/kg	212.5 ± 2.92 (ns)	2.05 ± 0.037 (ns)
ALEA 200 mg/kg	219.7 ± 2.91 *	2.15 ± 0.037 **
ALEA 400 mg/kg	225.0 ± 2.63 **	2.25 ± 0.037 ****

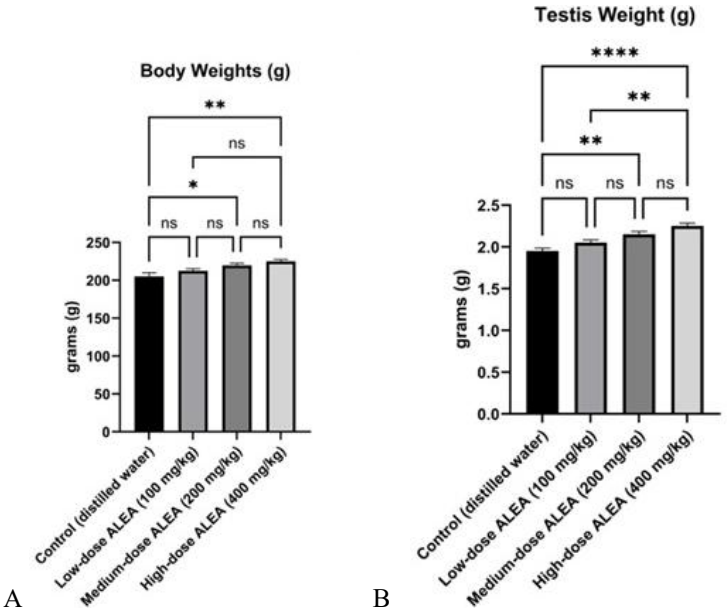

Figure 1: Effects of Asparagus Leaf Extract (ALEA) on Body and Testis Weights Across Experimental Groups. Bar graphs illustrate the mean ± SEM values for final body weight (A) and testis weight (B) in male rats treated with graded doses of ALEA (100, 200, and 400 mg/kg) compared to control. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test: *p < 0.05, **p < 0.01, ****p < 0.0001; ns = not significant.

Table 2: GnRH Expression and Reproductive Hormones Across Experimental Groups

Animal Groups	GnRH-positive Neurons (%)	Testosterone (ng/mL)	LH (mIU/mL)	FSH (mIU/mL)
Control	12 ± 0.58	3.5 ± 0.073	1.8 ± 0.058	2.1 ± 0.073
ALEA 100 mg/kg	18 ± 0.60 ****	4.2 ± 0.107 ****	2.3 ± 0.058 ****	2.7 ± 0.058 ****
ALEA 200 mg/kg	24 ± 0.58 ****	5.1 ± 0.070 ****	2.8 ± 0.058 ****	3.3 ± 0.070 ****
ALEA 400 mg/kg	29 ± 0.58 ****	5.8 ± 0.092 ****	3.1 ± 0.058 ****	3.7 ± 0.070 ****

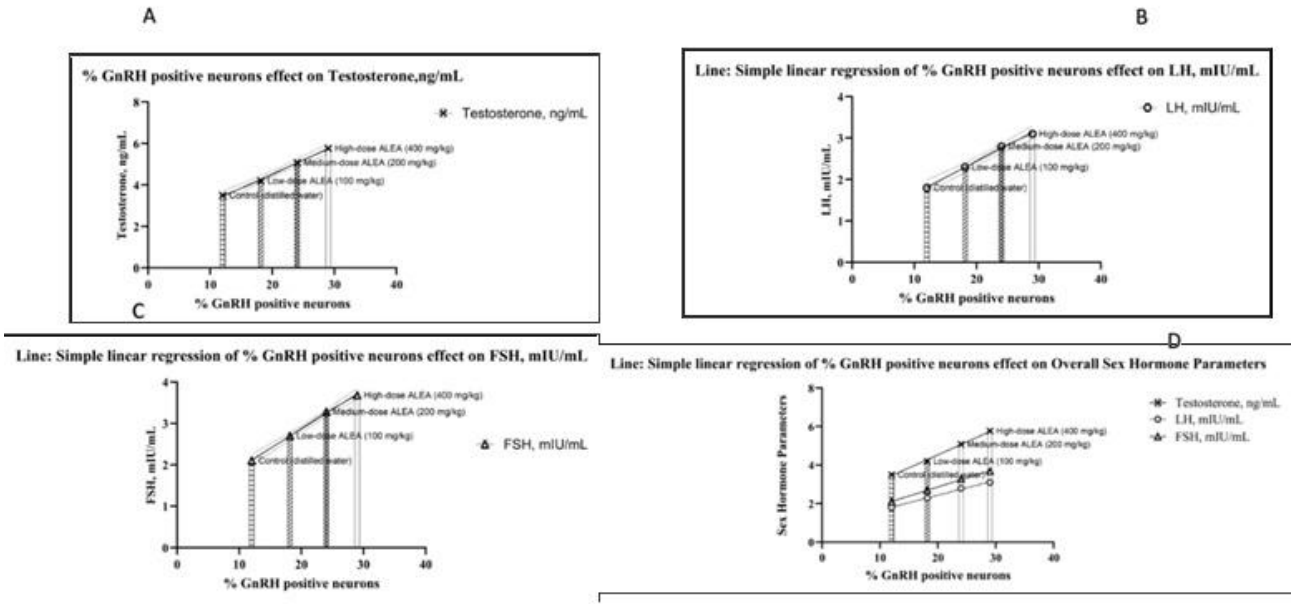

Figure 2: Regression plots illustrating the relationship between hypothalamic GnRH-positive neuron expression and serum sex hormone levels in male rats treated with graded doses of Asparagus officinalis leaf extract (ALEA). Panel A shows the correlation with testosterone (✳), Panel B with luteinizing hormone (○), Panel C with follicle-stimulating hormone (▵), and Panel D presents an overlay of all three hormones. Each graph demonstrates a strong positive linear relationship, indicating that GnRH activity quantitatively predicts androgen and gonadotropin output across treatment groups (control, 100 mg/kg, 200 mg/kg, and 400 mg/kg ALEA).

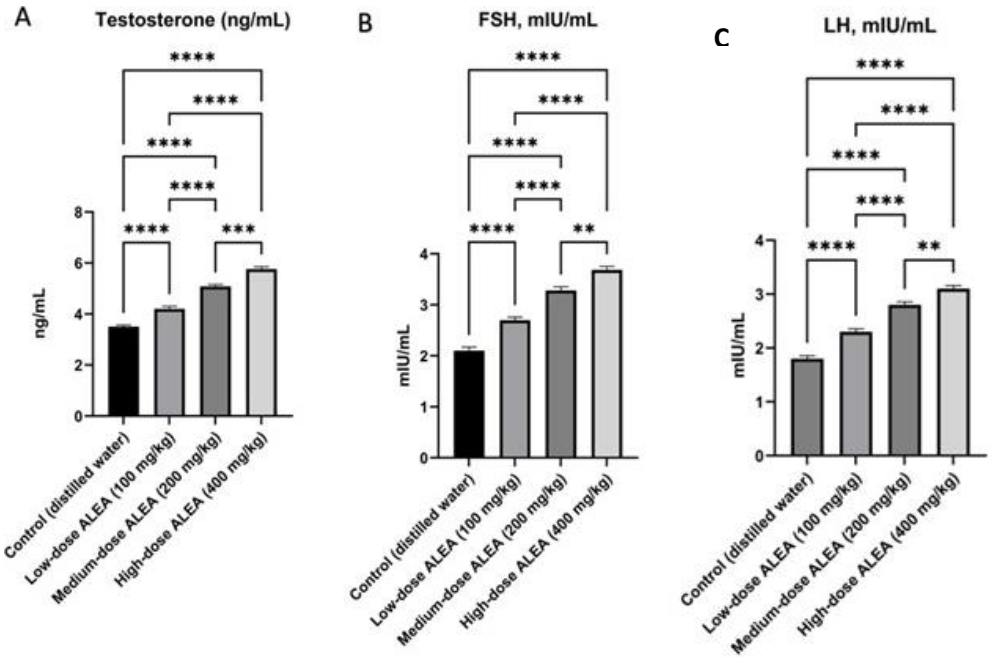

Figure 3: Dose-Dependent Effects of Asparagus Leaf Extract (ALEA) on Serum Reproductive Hormones. Bar graphs display mean ± SEM values for testosterone (A), follicle-stimulating hormone (FSH) (B), and luteinizing hormone (LH) (C) in male rats treated with increasing doses of ALEA (100, 200, and 400 mg/kg) compared to control. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test: ****$p < 0.0001$.

Table 3: Spermatogenic Index Across Experimental Groups

Animal Groups	Spermatogenic Index
Control	2.8 ± 0.058
ALEA 100 mg/kg	3.5 ± 0.058 ****
ALEA 200 mg/kg	4.0 ± 0.058 ****
ALEA 400 mg/kg	4.5 ± 0.058 ****

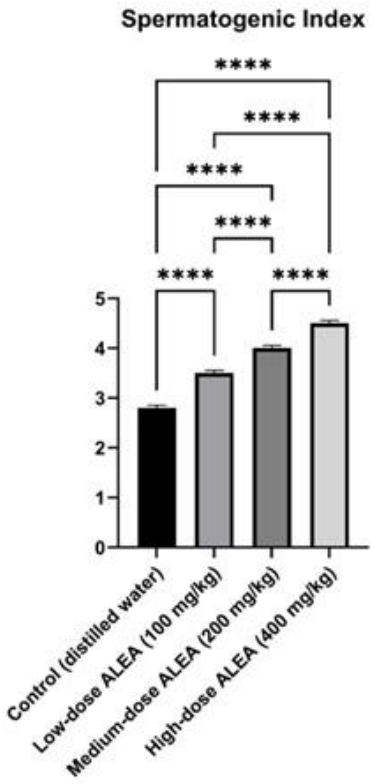

Figure 4: Impact of Asparagus Leaf Extract (ALEA) on Spermatogenic Index in Male Rats. Bar graph displays mean ± SEM values for spermatogenic index across control and ALEA-treated groups (100, 200, and 400 mg/kg). ALEA administration resulted in a dose-dependent increase in spermatogenic activity. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test: ****$p < 0.0001$.

Histological evaluation of testicular architecture

Hematoxylin and eosin (H&E) staining revealed progressive enhancement of testicular cytoarchitecture in ALEA-treated groups. As shown in Figure 5, the control group displayed well-organized seminiferous tubules with intact basement membranes, prominent Leydig cells, and active spermatogenesis. Interstitial spaces were clearly evident, reflecting normal testicular organization.

In the low-dose group (100 mg/kg), seminiferous tubules appeared slightly more organized, with evident interstitial spaces and mild improvement in germinal epithelium thickness. The medium-dose group (200 mg/kg) showed further restoration, with increased spermatogenic cell density, clearer luminal profiles, and moderately compact interstitial tissue.

The high-dose group (400 mg/kg) demonstrated near-complete histological recovery. Seminiferous tubules were densely packed, basement membranes appeared robust, and spermatogenic cells were abundant. Importantly, the interstitial space became more compact and well organized, contrasting with the more evident spacing seen in control and low-dose groups. This compactness reflects enhanced tissue integrity and improved coordination of testicular compartments.

Quantitative analysis confirmed these observations, with the spermatogenic index rising from 2.8 ± 0.058 in control to 4.5 ± 0.058 in the high-dose ALEA group (****$p < 0.0001$; Table 3). These findings underscore ALEA's capacity to restore testicular integrity, optimize interstitial organization, and support spermatogenesis following endocrine disruption.

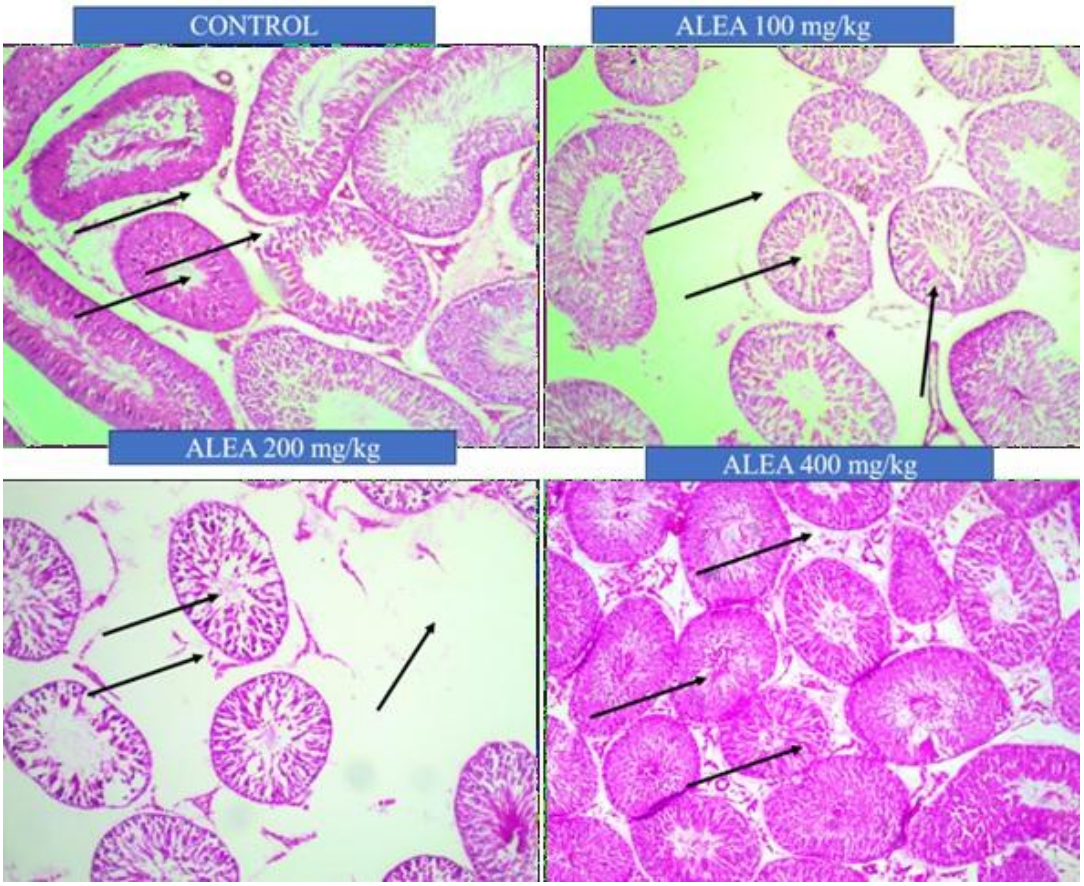

Figure 5: Histological Evaluation of Testicular Architecture Across Experimental Groups. Representative H&E-stained sections of testicular tissue from control and ALEA-treated rats (100, 200, and 400 mg/kg). Black arrows indicate notable histological changes, including seminiferous tubule organization, spermatogenic cell density, and interstitial space compactness. ALEA administration produced dose-dependent restoration of cytoarchitecture, with the high-dose group showing near-complete recovery. Magnification: ×400.

DISCUSSION

This study demonstrates that *Asparagus officinalis* leaf extract (ALEA) exerts a targeted influence on male reproductive physiology by acting at the apex of the hypothalamic–pituitary–gonadal (HPG) axis. Unlike previous reports limited to root extracts[11,13,15], ALEA directly enhanced hypothalamic GnRH immunoreactivity and established quantitative coupling with gonadotropin and androgen secretion. Regression modeling revealed near-perfect correlations between GnRH activity and testosterone, LH, and FSH, providing mechanistic clarity rarely achieved in phytotherapy studies.[17-18]

The dose-dependent elevation of serum testosterone, LH, and FSH observed in the present study aligns with the established pharmacological profile of *Asparagus* species.[6] The steroidal saponins present in ALEA, particularly protodioscin[19] and related compounds, are structurally similar to endogenous steroid hormones and may function as phytosteroids that interact with steroid receptors or modulate steroid biosynthetic pathways. Protodioscin has been shown to increase dihydrotestosterone levels through stimulation of pituitary LH release and subsequent activation of Leydig cell steroidogenesis.[20] The flavonoid constituents, including quercetin and rutin, contribute to the antioxidant capacity of the extract, protecting testicular cells from oxidative damage while supporting hormonal synthesis.[8]

The histological improvements observed in ALEA-treated rats, including increased seminiferous tubule diameter, enhanced germinal epithelium stratification, and elevated Leydig cell density, provide structural correlates for the hormonal changes. These findings are consistent with the androgenic and antioxidant properties of asparagus phytochemicals, particularly saponins and flavonoids[8]. The compact organization of interstitial spaces in the high-dose group suggests improved paracrine signaling between Leydig cells and seminiferous tubules, facilitating coordinated testicular function.

Previous studies on asparagus root extracts demonstrated fertility-enhancing effects in rodents,[21] but the present work expands this knowledge by showing that leaves, an underexplored plant part, can produce comparable or superior outcomes. This observation has practical significance for agricultural waste utilization, as asparagus leaves are typically discarded during shoot harvest. The phytochemical profile of leaves, rich in flavonoids and saponins, may offer distinct advantages over root extracts in terms of sustainability and cost-effectiveness for therapeutic development.

The dose-dependent responses observed indicate that ALEA contains bioactive compounds capable of regulating endocrine function. This supports its traditional use in male reproductive health and highlights its potential as a natural therapeutic agent[22-23]. The reproducibility of these findings, achieved through standardized extraction, validated assays, and robust statistical modeling, aligns with the quality standards emphasized by Phytomedicine [23-24].

Several mechanisms may underlie the observed effects. The GnRH neurons in the hypothalamus serve as the primary regulatory node for the HPG axis, integrating metabolic, stress, and circadian signals to control pulsatile hormone release.[1] Enhanced GnRH immunoreactivity, as demonstrated in the present study, suggests that ALEA may either increase GnRH synthesis, reduce its degradation, or stimulate its secretion from hypothalamic terminals. The subsequent rise in LH and FSH drives Leydig cell testosterone production and Sertoli cell support of spermatogenesis, respectively, creating a coordinated hormonal environment favorable for male fertility.

The reported regression analysis showing R^2 values exceeding 0.99 for the relationship between GnRH expression and reproductive hormones is similarly significant. Such strong correlations suggest that GnRH activity serves as a reliable predictor of downstream hormonal output in the context of ALEA treatment. This quantitative approach distinguishes the present study from previous phytotherapy investigations that reported hormone elevations without establishing mechanistic links to hypothalamic signaling [1,6,7,10-11].

Despite these promising findings, the study faces certain limitations. Our inclusion of a rodent model, and the translatability of results to human subjects requires further validation and the adopted 28-day treatment period, while sufficient to detect hormonal and histological changes, may not capture long-term adaptive responses or potential adverse effects. Additionally, the specific bioactive compounds responsible for the observed effects were not isolated or characterized in the present investigation.

Future studies should focus on isolating the active phytochemicals from ALEA via thin layer chromatography or high-performance liquid chromatography to enable evaluation of their pharmacokinetics and bioavailability while exploring their translational potential in clinical settings. Investigation of the molecular targets through which ALEA modulates GnRH neuronal activity, such as kisspeptin-GPR54 signaling or GABA receptor interactions, would provide deeper mechanistic insight. Comparative studies with other *Asparagus* species and plant parts would further clarify the

structure-activity relationships governing the reproductive effects of this genus.

Collectively, these results highlight ALEA as a novel phytotherapeutic agent capable of integrating into evidence-based reproductive medicine. Future studies should isolate the active phytochemicals, evaluate pharmacokinetics, and explore translational potential in clinical settings, thereby advancing the ethnopharmacological relevance of asparagus beyond root extracts and positioning leaves as a reproducible, mechanistically validated source of fertility-enhancing compounds.

CONCLUSION

A. officinalis leaf extract enhances male fertility by upregulating hypothalamic GnRH, stimulating gonadotropin release, and remodeling testicular architecture. By providing quantitative evidence of GnRH–hormone coupling, ALEA establishes a new paradigm in phytotherapy, expanding the ethnopharmacological relevance of asparagus beyond root extracts and positioning leaves as a reproducible, mechanistically validated source of fertility-enhancing phytochemicals.

REFERENCES

1. Plant TM. 60 years of neuroendocrinology: The hypothalamo-pituitary-gonadal axis. *J Endocrinol*. 2015;226(2):T41-54. doi:10.1530/JOE-15-0113. PMID:25901041; PMCID:PMC4498991.
2. Liberto R, Katlowitz N, Sagalovich D, Davila J. Strategies for reversing exogenous testosterone-induced infertility. *Cureus*. 2025;17(9):e91975. doi:10.7759/cureus.91975. PMID:41080372; PMCID:PMC12513088.
3. World Health Organization. Infertility [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/infertility
4. Leslie SW, Soon-Sutton TL, Khan MAB. Male infertility. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Updated 2024 Feb 25. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562258/
5. Chorosho SH, Malik N, Panesar G, Kumari P, Jangra S, Kaur R, et al. Phytochemicals: Alternative for infertility treatment and associated conditions. *Oxid Med Cell Longev*. 2023;2023:1327562. doi:10.1155/2023/1327562. PMID:37215366; PMCID:PMC10195183.
6. Chauhan NS, Sharma V, Dixit VK, Thakur M. A review on plants used for improvement of sexual performance and virility. *Biomed Res Int*. 2014;2014:868062. doi:10.1155/2014/868062. PMID:25215296; PMCID:PMC4151601.
7. Adelakun SA, Akinola BK, Akingbade GT. Fertility-enhancing activities of bioactive components of *Cochlospermum planchonii* rhizome on cisplatin-induced reproductive dysfunctions in Sprague-Dawley rats. *J Fam Reprod Health*. 2018;12(3):148-59. PMID:31223321; PMCID:PMC6571445.
8. Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Plant profile, phytochemistry and pharmacology of *Asparagus racemosus* (Shatavari): A review. *Asian Pac J Trop Dis*. 2013;3(3):242-51. doi:10.1016/S2222-1808(13)60049-3. PMCID:PMC4027291.
9. Omutindo Nyakayo A. Polyherbal extracts and hormonal crosstalk in male and female fertility: Advances in natural endocrine modulation. *Res Inven J Res Med Sci*. 2025;4(S):83-7. doi:10.59298/RIJRMS/2025/438887.
10. Gupta A, Chauhan N. Bioactive phytochemicals from *Asparagus officinalis* L. by-products: Composition and potential applications. *Plant Foods Hum Nutr*. 2025;80(3):215-27.
11. Karimi Jashni H, Kargar Jahromi H, Ghorbani Ranjbary A, Kargar Jahromi Z, Khabbaz Kherameh Z. Effects of aqueous extract from *Asparagus officinalis* L. roots on hypothalamic-pituitary-gonadal axis hormone levels and the number of ovarian follicles in adult rats. *Int J Reprod Biomed*. 2016;14(2):75-80. PMID:27200420; PMCID:PMC4869160.
12. Alcaide IV, Hamdi A, Guilleín-Bejarano R, Jiménez-Araujo A, Rodríguez-Arcos R. Sustainable valorization of co-products from asparagus cultivation by obtaining bioactive compounds. *Front Plant Sci*. 2023;14:1199436. doi:10.3389/fpls.2023.1199436. PMID:37521938; PMCID:PMC10373885.
13. National Academies Press (US). *Guide for the care and use of laboratory animals: Eighth edition* [Internet]. Washington, D.C.: National Academies Press; 2011 [accessed 2026 Mar 17]. Available from:

https://www.nationalacademies.org/publications/12910 doi:10.17226/12910.
14. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671-5. doi:10.1038/nmeth.2089. PMID:22930834; PMCID:PMC5554542.
15. Nasu T, De Ocampo G, Molina HA, Tateyama S, Morimoto M. Immunohistochemical study of the neuropeptides in the stellate ganglion of the water buffalo. *Fukuoka Igaku Zasshi*. 2000;91(5):116-22. PMID:10916853.
16. Acevedo-Rodriguez A, Kauffman AS, Cherrington BD, Borges CS, Roepke TA, Laconi M, *et al*. Emerging insights into hypothalamic-pituitary-gonadal axis regulation and interaction with stress signalling. *J Neuroendocrinol*. 2018;30(10):e12590. doi:10.1111/jne.12590. PMID:29524268; PMCID:PMC6129417.
17. Ramgir SS, Renu K, Vellingiri B, George A, Tirupapuliyur D, Thiagarajan P, *et al*. Phytomedicinal therapeutics for male infertility: Critical insights and scientific updates. *J Nat Med*. 2022;76(3):546-73. doi:10.1007/s11418-022-01619-0. PMID:35377028.
18. Martin LJ, Touaibia M. Improvement of testicular steroidogenesis using flavonoids and isoflavonoids for prevention of late-onset male hypogonadism. *Antioxidants*. 2020;9(3):237. doi:10.3390/antiox9030237. PMID:32183155; PMCID:PMC7139932.
19. Cheng Q, Zeng L, Wen H, Brown SE, Wu H, Li X, *et al*. Steroidal saponin profiles and their key genes for synthesis and regulation in *Asparagus officinalis* L. by joint analysis of metabolomics and transcriptomics. *BMC Plant Biol*. 2023;23(1):207. doi:10.1186/s12870-023-04222-x. PMID:37081391; PMCID:PMC10116787.
20. Gauthaman K, Adaikan PG, Prasad RN. Aphrodisiac properties of *Tribulus terrestris* extract (protodioscin) in normal and castrated rats. *Life Sci*. 2002;71(12):1385-96. doi:10.1016/S0024-3205(02)01858-1. PMID:12127159.
21. Bansode FW, Arya KR, Singh RK, Narender T. Dose-dependent effects of *Asparagus adscendens* root extract on the anabolic, reproductive, and sexual behavioral activity in rats. *Pharm Biol*. 2015;53(2):192-200. doi:10.3109/13880209.2014.913295. PMID:24963947.
22. Corona G, Goulis DG, Huhtaniemi I, Zitzmann M, Toppari J, Forti G, *et al*. European Academy of Andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males. *Andrology*. 2020;8(5):970-87. doi:10.1111/andr.12770. PMID:32026626.
23. Bassil N, Alkaade S, Morley JE. The benefits and risks of testosterone replacement therapy: A review. *Ther Clin Risk Manag*. 2009;5(3):427-48. doi:10.2147/tcrm.s3025. PMID:19707253; PMCID:PMC2701485.
24. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*. 2010;15(10):7313-52. doi:10.3390/molecules15107313. PMID:20966876.