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Multivitamin Attenuation of Reproductive Hormone Suppression by *Azadirachta Indica* (Neem) Leaf Extract in Female *Rattus norvegicus* Rats

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Abstract

Neem (*Azadirachta indica*) is a widely used medicinal plant known for its therapeutic value, particularly in areas with limited access to modern healthcare. However, studies indicate that neem may affect fertility by altering reproductive hormones. Its antifertility effects are mainly linked to compounds such as azadirachtin, nimbin, nimbidin, and nimbosterol, which can disrupt the hypothalamic–pituitary–gonadal axis and influence hormones like FSH, LH, and Oestrogen. Although these effects are documented, the role of multivitamin supplementation in moderating neem-induced hormonal changes remains unclear and requires further study. This study investigated the dose-dependent impact of neem leaf extract on reproductive hormones in female albino rats and assessed whether multivitamins could mitigate these effects. Mature neem leaves were collected from CITAM Kisumu and the University of Eldoret. Forty female albino rats sourced from Maseno University were acclimated for one month under controlled conditions (22–25°C, 12-hour light/dark cycle). Twenty-four healthy, sexually mature rats were randomly assigned to eight treatment groups. Ethanol-extracted neem preparations were administered orally, with or without multivitamins, for 28 days. Post-treatment, blood samples were analyzed for follicle-stimulating hormone (FSH), luteinizing hormone (LH), and Oestrogen using ELISA at Moi Teaching and Referral Hospital. Data were analyzed using one-way ANOVA, Tukey's post hoc test, and regression analysis, with significance set at $p<0.05$. Results showed dose-dependent suppression of reproductive hormones by neem. FSH decreased from 8.27±0.35 ng/mL in controls to 3.27±0.21 ng/mL in the highest neem dose (100 mg/kg), similar to the contraceptive group (3.03±0.15 ng/mL). LH declined from 6.17±0.35 ng/mL in controls to 2.90±0.20 ng/mL (Neem100), versus 2.57±0.21 ng/mL in the contraceptive group. Oestrogen levels fell from 52.59±2.55 pg/mL in controls to

13.53±1.25 pg/mL in Neem100, while controls and contraceptives had comparable levels (50.97±1.36 pg/mL). Multivitamin supplementation partially reversed these suppressive effects, increasing FSH to 5.87±0.15 ng/mL, LH to 5.13±0.15 ng/mL, and Oestrogen to 54.33±1.17 pg/mL in the Neem100+MV group. One-way ANOVA revealed highly significant differences among treatment groups for FSH ($F_{0.05(4,10)} = 181.67$, $p<0.0001$), LH ($F_{0.05(4,10)} = 88.24$, $p<0.0001$), and Oestrogen ($F_{0.05(4,10)} = 306.53$, $p<0.0001$). Correlation analysis showed a very strong positive correlation between FSH and LH ($r=0.992$, $p<0.0001$), while Oestrogen exhibited weak, non-significant correlations with FSH and LH. In conclusion, neem leaf extract exerts potent contraceptive effects through dose-dependent suppression of key reproductive hormones, while multivitamin supplementation partially counteracts these effects. These findings emphasize the need for cautious use of neem-based products among women of reproductive age and highlight the importance of understanding interactions between herbal remedies and nutritional supplements. Further research is warranted to clarify the underlying mechanisms and long-term implications of such interactions.

Keywords: Antifertility, Dose-dependent Effects, FSH, LH, Oestrogen, Hormonal Modulation

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Introduction

Medicinal plants have long been central to traditional healing practices and continue to provide valuable leads for modern drug development. Globally, more than 25,000 higher plant species are recognized for their medicinal properties, and according to the World Health Organization (WHO, 2022), over 80% of people in developing nations still rely on traditional medicine as their main form of healthcare. The continued use of plant-based therapies is largely linked to their affordability, accessibility, and perceived safety, as well as their proven ability to manage a wide range of chronic and infectious diseases (Fehér et al., 2020). Growing challenges such as antibiotic resistance, adverse effects of synthetic drugs, and poor access to medical care in many rural areas have further renewed global interest in herbal medicine (Abdallah et al., 2023; Izah et al., 2023).

In Africa, particularly across sub-Saharan regions, medicinal plants remain

deeply woven into community healthcare and cultural practices. The continent hosts an estimated 45,000 plant species, with more than 5,000 known for their medicinal uses (Moyo, Aremu & Van Staden, 2015).

In Kenya, traditional herbal medicine continues to play an important role in both rural and peri-urban areas where access to formal healthcare is often limited (Chebii, Muthee & Kiemo, 2020). Among the many plants used, *Azadirachta indica* A. Juss. (neem) stands out for its wide therapeutic range and cultural value. Commonly called "*Muarobaini*" in Kiswahili—meaning "the tree of forty cures" neem remains one of the most trusted natural remedies in many Kenyan communities for treating a variety of ailments. Neem belongs to the Meliaceae family and is native to the Indian subcontinent, but it has become widely distributed in tropical regions such as Australia, the Americas, and sub-Saharan Africa (Adhikari, 2022).

In East Africa, neem is commonly referred to as "Muarubaini" in Kiswahili, meaning "the tree of forty cures," reflecting its reputation as a panacea (Winterbottom, 2021). Other regional names include "Dogonyaro" in Nigeria (Orisakwe, Orish & Nwanaforo, 2020), "Margosa" during colonial times, and "Nim" in Hindi (Khanpara & Jadeja, 2022). In Kenya, it is widely cultivated in arid and semi-arid areas for its medicinal, environmental, and economic benefits. In Kenya, traditional medicine holds particular importance in rural and peri-urban settings (Chebii, Muthee, & Kiemo, 2020). Commonly used herbal remedies include *Solanum incanum* (Sodom apple), *Croton megalocarpus*, *Aloe secundiflora* and *Moringa oleifera*, which address a wide range of ailments including infections and reproductive health challenges. Among these, *Azadirachta indica* A. Juss., commonly known as neem, is especially notable for its versatility and cultural significance (Wylie & Merrell, 2022).

Neem (*Azadirachta indica*) contains a diverse range of biologically active compounds, including azadirachtin, nimbin, quercetin, salannin, and limonoids (Gupta et al., 2019). These compounds are responsible for many of the plant's well-recognized therapeutic actions, such as antibacterial, antifungal, anti-inflammatory, antioxidant, and hepatoprotective effects (Sarah et al., 2019). Traditionally, neem has been widely used in the treatment of malaria, skin infections, gastrointestinal disorders, and diabetes (Gupta et al., 2019). In addition to its general medicinal uses, neem has attracted interest for its ability to influence fertility. Studies indicate that extracts from neem leaves can suppress the estrous cycle, inhibit ovulation, and interfere with embryo implantation, often resulting in early pregnancy loss (Patil et al., 2021; Suryawanshi, 2011). These antifertility effects are believed to arise from disruptions of the hypothalamic–pituitary–gonadal axis, leading to altered secretion of reproductive hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and Oestrogen (Njoga et al., 2022). Furthermore, neem's active ingredients are thought to induce oxidative stress and tissue damage within the ovaries and uterus, thereby contributing to endocrine dysfunction (Patil et al., 2021).

Despite these insights, limited research has explored whether nutritional supplements might mitigate neem-induced reproductive toxicity. Multivitamins rich in antioxidant components particularly vitamins A, C, and E, and minerals such as zinc and selenium have been shown to stabilize hormonal balance and protect reproductive tissues against oxidative injury (Wróblewski, Wróblewska & Sobiesiak, 2024). This is especially important in regions like Kenya, where

herbal remedies and over-the-counter supplements are frequently used together without professional guidance.

Given neem's longstanding medicinal value and the growing consumption of multivitamins, it is important to clarify how varying doses of *A. indica* extract affect reproductive hormones and whether multivitamin supplementation can modify these responses. Therefore, this study aimed to evaluate the dose-dependent effects of *A. indica* leaf extract on key female reproductive hormones; FSH, LH, and Oestrogen and to determine the potential modulatory influence of multivitamin supplementation on these hormonal changes in a female albino rat model.

Methodology

Research Design

This study followed a controlled laboratory experiment to investigate how Neem s influences key reproductive hormones such as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Oestrogen in female albino rats. It also examined whether adding multivitamin supplements could help counteract any antifertility effects. The controlled setup allowed all variables to be carefully monitored, ensuring that the findings were accurate and could be replicated.

Study Area

The research was carried out in the Biotechnology and Microbiology Laboratories in the School of Natural and Applied Sciences at the MMUST situated along the Kakamega-Webuye road in Kenya's Western region (Figure 1). The region experiences a temperate climate, with annual rainfall ranging between 900 and 1,200 mm and temperatures from 18°C to 26°C. Known for its fertile farmland and agricultural activities mainly maize, bananas and dairy the semi-urban setting also provides access to modern laboratory facilities. Although the albino rats were initially bred at Maseno University, all experimental work and sample collection were conducted at the MMUST.

Study Population

The study used 40 female albino rats (*Rattus norvegicus*), 8–10 weeks old and weighing 180–250 g, sourced from Maseno University's School of physical and biological sciences in the department of zoology. They were transported under humane conditions to Masinde Muliro University of Science and Technology (MMUST) animal house at the school of medicine in the department of anatomy where they acclimatized in temperature-controlled rooms with standard feed, clean water and daily health checks. From this group, 24 healthy, sexually mature females with regular 4-5day estrous cycles, confirmed via daily vaginal cytology/smear analysis, were selected. Following treatment, the rats were humanely euthanized according to institutional animal care guidelines. Blood was immediately drawn via cardiac puncture, stored under a cold chain and taken to the Biochemistry Laboratory at Moi Teaching and Referral Hospital (MTRH), Eldoret, for serum extraction and subsequent hormonal and biochemical analyses using standard protocols.

Sample Size and Grouping

The resulting 24 rats were randomly allocated into eight groups of three, representing biological triplicates to ensure statistical robustness. The groups were structured as follows:

Group I (Negative Control): This group received distilled water only.

Group IIa: This was given neem extract at a dose of 50 mg/kg.

Group IIb: This group received given neem extract at a dose of 75 mg/kg.

Group IIc: This group was given neem extract at a dose of 100 mg/kg.

Group IIIa: This group received neem extract at 50 mg/kg together with a multivitamin supplement at a standardized dose (to be expressed in mg/kg body weight based on manufacturer formulation), administered orally once daily.

Group IIIb: The group received neem extract at 75 mg/kg together with multivitamins.

Group IIIc: It received neem extract at 100 mg/kg together with multivitamins.

Group IV (Positive Control): It received a standard contraceptive treatment containing ethinylestradiol (0.03 mg/kg) and levonorgestrel (0.15 mg/kg),

Each group was housed in separate, well-labeled polycarbonate cages maintained under identical room conditions to ensure consistency in environmental exposure.

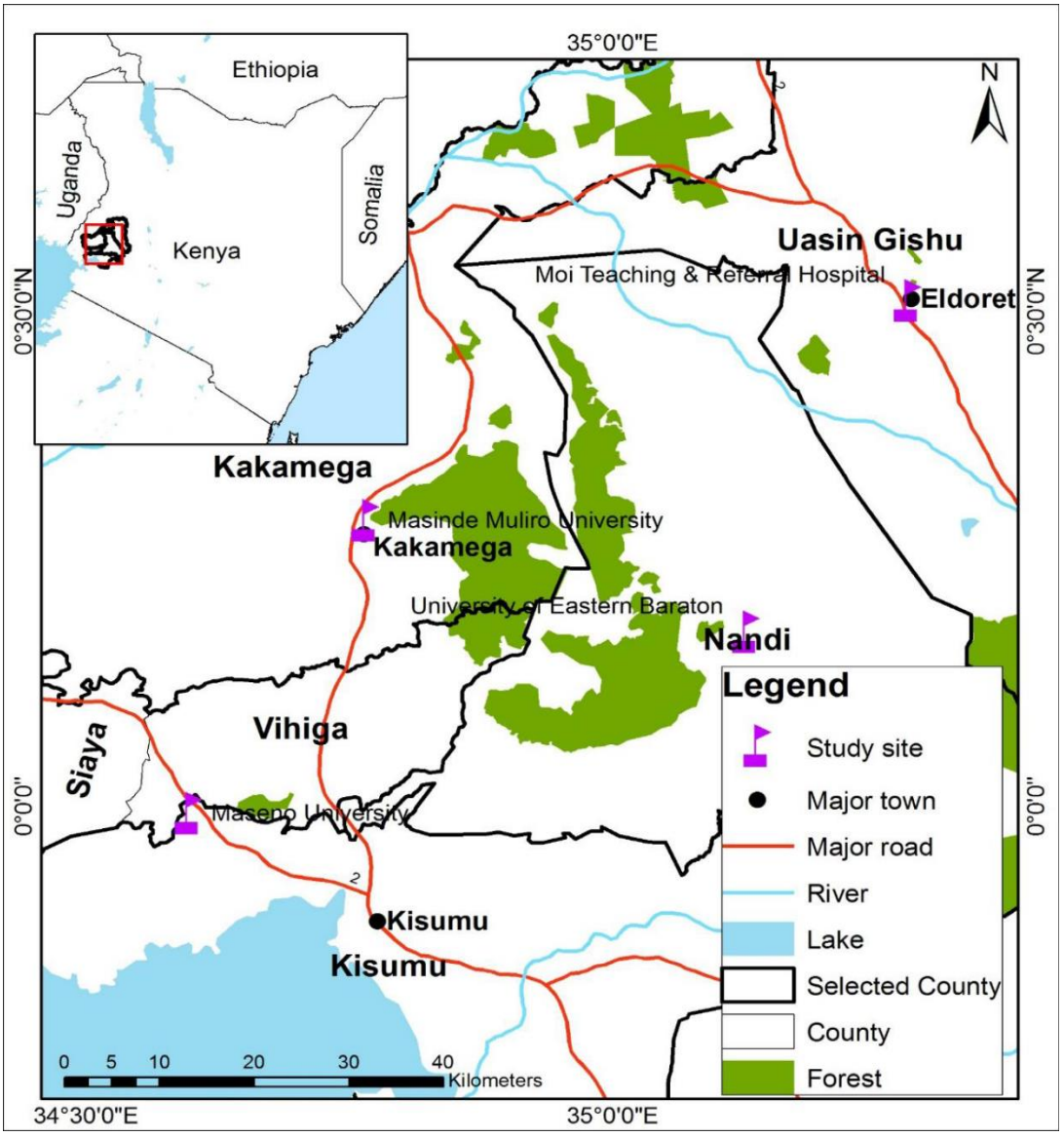

Figure 1: Map showing the locations of the Universities and MTRH associated with the study in Kenya

Housing and Feeding Conditions

Rats in each group were kept in separate, clearly labeled polycarbonate cages (45×30×20 cm) with mesh lids. The room environment was maintained at 22±2°C, 55–65% humidity, and a 12-hour light/dark cycle. They were fed standard rodent pellets sourced from Unga Farm Care, Kenya at 15–20 g per rat per day, with clean tap water available at all times. Feeders and water bottles were washed daily, bedding of sterile wood shavings was replaced twice a week and cages thoroughly cleaned every 3–4 days.

Preparation and Administration of Treatments

Fresh neem leaves were collected in January 2021 from the University of Eldoret Arboretum, with extra leaves sourced from the CITAM Church compound in Kisumu. The late Dr. B. K. Wanjohi, a botanist at the University of Eldoret, confirmed the plant's identity. Leaves were shade-dried, ground, and ethanol-extracted using a Soxhlet apparatus, then concentrated with a rotary evaporator and stored in amber bottles at 4 °C. Multivitamins (Wellwoman®, UK) were prepared in distilled water as per instructions. Both treatments were given orally via stainless steel gavage needles once daily for 28 days, always in the morning to minimize hormonal variation.

Blood Collection, Hormone Analysis and Statistical Methods

Only healthy, non-pregnant female rats with regular estrous cycles were enrolled; any animal showing illness, abnormal behaviour, or irregular cycles during acclimatization was excluded. On day 29 animals were humanely euthanized in line with approved ethical procedures and blood was collected immediately by cardiac puncture to preserve serum quality. samples were allowed to clot at room temperature, centrifuged at 3,000 rpm for 10 minutes, and the serum was aliquoted and stored at −20°C until analysis at Moi Teaching and Referral Hospital. Serum concentrations of FSH, LH and estrogen were measured in triplicate to ensure assay reliability. Primary measurements were performed on the Roche Cobas 6000 automated platform (cobas e601 module) using an electrochemiluminescence immunoassay (ECLIA) that applies a competitive antigen–antibody principle. A subset of samples was cross-checked using commercial ELISA kits (Abcam, UK; Elabscience, China; BioAssay Systems, USA) following the manufacturers' protocols. Statistical analyses were carried out in Statigraphic Centurion v16. Group differences were tested with one-way ANOVA followed by Tukey's post hoc comparisons. Optimal neem–multivitamin dosing was explored using linear regression and dose–response modelling. Results were considered statistically significant at $p < 0.05$. The study was approved by NACOSTI and a research permit was given. It also followed IACUC and Kenya Veterinary Board guidelines, keenly observing refinement and reduction.

Results

Effects of Neem Dosage on Reproductive Hormones

FSH, LH, and Oestrogen levels differed significantly across treatment groups. FSH was highest in the control (8.27±0.35 ng/mL) and declined stepwise in Neem50 (5.70±0.36), Neem75 (4.23±0.25), Neem100 (3.27±0.21), and contraceptive (3.03±0.15) groups ($F_{0.05\ (4,10)} = 181.67$, $p < 0.0001$), with significant differences between most groups except Neem100 and contraceptive. LH followed a similar pattern: control (6.17±0.35 ng/mL) > Neem50 (4.70±0.30 ng/mL) > Neem75 (3.83±0.25) > Neem100

(2.90±0.20) > contraceptive (2.57±0.21) ($F_{0.05\ (4,10)}$ = 88.24, p < 0.0001), with successive groups significantly different except Neem100 and contraceptive. Oestrogen peaked in control (52.59±2.55 pg/mL) and contraceptive (50.97±1.36), which were similar, but decreased sharply with increasing neem dose—Neem50 (30.17±1.65), Neem75 (21.20±1.55 pg/mL), Neem100 (13.53±1.25 pg/mL) ($F_{0.05\ (4,10)}$ = 306.53, p < 0.0001), with all neem-treated groups significantly differing from each other as summarized in Table 1.

Table 1: Mean±Standard Deviation (SD) of Reproductive Hormones (FSH, LH, and Oestrogen) Across Treatment Groups with Post Hoc Grouping, ANOVA F-Ratios, and P-Values

Hormone	Treatment	Mean± SD	F-Ratio	P-Value
FSH (ng/mL)	Control	8.27±0.35^{d}	181.67	0.0000
	Neem50	5.70±0.36^{c}		
	Neem75	4.23±0.25^{b}		
	Neem100	3.27±0.21^{a}		
	Contraceptive	3.03±0.15^{a}		
LH (ng/mL)	Control	6.17±0.35^{d}	88.24	0.0000
	Neem50	4.70±0.30^{c}		
	Neem75	3.83±0.25^{b}		
	Neem100	2.90±0.20^{a}		
	Contraceptive	2.57±0.21^{a}		
OOestrogen (pg/mL)	Control	52.59±2.55^{d}	306.53	0.0000
	Neem50	30.17±1.65^{c}		
	Neem75	21.20±1.55^{b}		
	Neem100	13.53±1.25^{a}		
	Contraceptive	50.97±1.36^{d}		

Means followed by different superscript letter are significantly different for a treatment in a specific hormone in a column

In all treatment groups, FSH and LH levels moved closely together, showing a strong, significant positive correlation (r = 0.9916, p = 0.0009), meaning changes in one were mirrored by the other across neem doses and controls. In contrast, FSH and Oestrogen showed only a moderate, non-significant link (r = 0.4619, p = 0.4336), while LH and Oestrogen had a similarly weak, non-significant correlation (r = 0.3720, p = 0.5375), suggesting Oestrogen levels varied more independently.

Evaluation of Multivitamin Supplementation Effects

FSH was highest in the control group (8.27±0.35) and lowest in the contraceptive group (3.03±0.15), with all differences significant ($F_{0.05\ (4,\ 10)}$ = 225.29, p < 0.0001). LH followed a similar trend, peaking in controls (6.17±0.35) and lowest in the contraceptive group (2.57±0.21), also with significant differences ($F_{0.05\ (4,\ 10)}$ = 104.53, p < 0.0001). Oestrogen was highest in Neem100+MV (54.33±1.17) and lowest in Neem50+MV (40.17±1.38), with significant variation across groups ($F_{0.05\ (4,\ 10)}$ = 35.58, p < 0.0001). Post hoc tests showed the contraceptive group differed from all others, while neem treatments clustered closely for FSH and LH but varied more for Oestrogen as shown in Table 2. FSH showed a moderate positive connection with Oestrogen (r = 0.504, p = 0.0280), while LH and Oestrogen were

similarly related (r = 0.483, p = 0.0360) as illustrated in Table (2). There was a strong and statistically significant link between FSH and LH levels (r = 0.981, p < 0.0001).

Table 2: Effects of Multivitamin Supplementation on Hormone Levels across Treatment Groups

Hormone	Treatment	Count	Mean ± SD	F-Ratio	P-Value
FSH (ng/mL)	Control	3	8.27 ± 0.35^{d}	225.29	0.0000
	Neem50+MV	3	6.33 ± 0.15^{c}		
	Neem75+	3	6.00 ± 0.20^{bc}		
	Neem100+MV	3	5.87 ± 0.15^{b}		
	Contraceptive	3	3.03 ± 0.15^{a}		
LH (ng/mL)	Control	3	6.17 ± 0.35^{c}	104.53	0.0000
	Neem50+MV	3	5.17 ± 0.21^{b}		
	Neem75+MV	3	5.13 ± 0.15^{b}		
	Neem100+MV	3	5.03 ± 0.15^{b}		
	Contraceptive	3	2.57 ± 0.21^{a}		
OOestrogen (pg/mL)	Control	3	52.59 ± 2.55^{cd}	35.58	0.0000
	Neem50+MV	3	40.17 ± 1.38^{a}		
	Neem75+MV	3	48.83 ± 1.14^{b}		
	Neem100+MV	3	54.33 ± 1.17^{d}		
	Contraceptive	3	50.97 ± 1.36^{bc}		

Discussion

In this study, the control group exhibited the highest Follicle-Stimulating Hormone (FSH) levels, reflecting normal ovarian function and healthy follicular growth. Such levels are characteristic of a stable reproductive cycle, consistent with findings by Roop, Dhaliwal, and Guraya (2005) and Chou and Chen (2018). In contrast, increasing doses of neem extract caused a clear, dose-dependent decline in FSH, indicating progressive interference with gonadotropin-releasing hormone (GnRH) signaling. These results align with earlier reports by Kulkarni (2020) who attributed such disruptions to phytochemicals like nimbolide and azadirachtin, which can suppress hypothalamic GnRH release or act directly on pituitary gonadotropes.

Interestingly, the high-dose neem group (Neem100) showed FSH levels comparable to those observed in the synthetic contraceptive group, suggesting that high concentrations of neem may mimic the gonadotropic suppression achieved by hormonal contraceptives. This similarity reinforces neem's potential to act as a natural fertility-regulating agent, as described by Ravichandran et al. (2009) and Patel, Jacob, and Thomas (2024). However, when multivitamins were co-administered with the high neem dose (Neem100+MV), FSH levels rose modestly, indicating that MV supplementation partially reversed the suppression induced by neem. This partial recovery suggests a protective role of vitamins, possibly linked to their antioxidant properties and ability to support pituitary and ovarian function under oxidative stress.

Luteinizing Hormone (LH) followed a similar trend. The control group recorded the highest LH levels, whereas the high-dose neem and contraceptive groups showed marked reductions. Since the LH surge triggers ovulation, its suppression implies impaired follicular

rupture and reduced fertility potential (Dozortsev & Diamond, 2020). The nearly identical LH reductions in the high-dose neem and contraceptive groups suggest that neem exerts a comparable central inhibitory effect on hypothalamic–pituitary signaling. Moore (2012) similarly reported that neem extracts can disrupt neurotransmitter feedback mechanisms controlling pulsatile GnRH release, further supporting this mechanism.

Oestrogen patterns revealed a more complex picture. As expected, the control group maintained high oestrogen concentrations, signifying active ovarian steroidogenesis and normal follicular development. In contrast, oestrogen levels declined steadily in the neem-treated groups, confirming that neem acts not only through central suppression of gonadotropins but also by directly disrupting ovarian function. Phytochemicals in neem may inhibit aromatase, the enzyme responsible for converting androgens into oestrogen or damage granulosa cells, as described by Patil et al. (2021). Al-Awadhi et al. (2024) further demonstrated that high doses of neem can induce follicular atresia, corpus luteum degeneration, and reduced steroidogenic enzyme activity, all of which contribute to decreased oestrogen production.

The relatively high oestrogen levels observed in the contraceptive group are noteworthy, as they arise from exogenous oestrogens within the contraceptive formulation. This distinction is important because it highlights mechanistic differences between neem and synthetic contraceptives: while neem primarily acts as an oestrogen synthesis inhibitor, suppressing both central and peripheral hormone pathways, the contraceptive acts centrally while supplying exogenous oestrogen to stabilize the endometrium and prevent breakthrough bleeding (Verma, Cwiak & Kaunitz, 2021).

A particularly striking observation was that the Neem100+MV group recorded higher oestrogen levels than even the control group (54.33 pg/mL vs. 52.59 pg/mL). This finding is highly significant, suggesting a possible rebound effect or compensatory overproduction of oestrogen once multivitamin supplementation mitigated neem's suppressive impact. The antioxidants and micronutrients in multivitamins such as vitamins A, C, and E, as well as zinc and selenium are known to protect cytochrome P450 aromatase enzymes and ovarian tissues from oxidative stress (Wróblewski, Wróblewska & Sobiesiak, 2024). By shielding these enzymes, multivitamins may enhance aromatase activity and restore oestrogen synthesis once central suppression is partially relieved, resulting in a temporary "overshoot" in hormone production.

Correlation analysis among the hormones supports these interpretations. A strong positive correlation between FSH and LH was observed across all groups, consistent with their shared regulation by GnRH. However, in the neem-only groups, oestrogen showed a weak correlation with FSH and LH, indicating a breakdown in the normal hypothalamic–pituitary–ovarian (HPO) feedback loop likely due to neem's dual central and peripheral effects. Conversely, in the Neem+MV groups, the correlation between oestrogen and gonadotropins strengthened to moderate levels, suggesting that multivitamin supplementation helped restore the hormonal feedback mechanism. This recovery implies that the vitamins supported not only antioxidant defense but also the physiological communication between pituitary hormones and ovarian oestrogen synthesis.

Hence, these findings demonstrate that neem exerts its antifertility effects through both endocrine disruption and oxidative damage, while multivitamin

supplementation can partially reverse these effects. The observed rebound in oestrogen production under Neem100+MV further underscores the protective role of micronutrients in maintaining enzymatic and hormonal balance. Such outcomes highlight the importance of understanding interactions between traditional herbal agents like neem and nutritional supplements, especially in reproductive health contexts. This study confirms that ethanol-extracted Azadirachta indica (neem) leaf extract exerts potent antifertility effects via a dose-dependent hormonal disruption in female rats. The highest dose (100 mg/kg) effectively mimicked the central contraceptive action by causing sharp reductions in FSH and LH, reinforcing neem's potential as a natural central suppressive agent. The results point to a dual mechanism of action: central suppression of FSH/LH and distinct peripheral inhibition of ovarian E2 synthesis, a mechanism differing from the synthetic contraceptive control. The introduction of multivitamin (MV) supplementation significantly compromised neem's antifertility effect. MV co-treatment substantially mitigated hormonal suppression, best demonstrated by an approximately 79% reversal of FSH suppression in the Neem100+MV group. Furthermore, MV supplementation caused Estrogen levels to overshoot, recording values higher than the control group (54.33 pg/mL), suggesting a potent protective and possibly hyper-stimulatory effect on ovarian function, likely via antioxidant support that restores HPG axis responsiveness. Future research is key to isolate the specific nutrients (e.g., Vitamin E or Zinc) responsible for this reversal, investigate the mechanisms behind the E2 overshoot, and conduct long-term studies to assess actual pregnancy outcomes, fetal viability and chronic effects on ovarian morphology.

Conclusion and Recommendation

In conclusion, neem leaf extract exerts potent contraceptive effects through dose-dependent suppression of key reproductive hormones, while multivitamin supplementation partially counteracts these effects. These findings emphasize the need for cautious use of neem-based products among women of reproductive age and highlight the importance of understanding interactions between herbal remedies and nutritional supplements. Further research is warranted to clarify the underlying mechanisms and long-term implications of such interactions.

References

Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R., & Martins, C. H. G. (2023). Back to nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. *Plants*, *12*(17), 3077. https://doi.org/10.3390/plants12173077

Adhikari, A. S. (2022). *Phytochemicals and Antioxidant Activities of Neem (Azadirachta indica) Leaves* (Doctoral dissertation, Department of Nutrition & Dietetics Central Campus of Technology Institute of Science and Technology Tribhuvan University, Nepal. 2021).

Al-awadhi, R. M., Abdelrazek, H. M. A., Abouelhassan, E. M., Kamel, M. S., Attia, N. A., & Awad, A. A. (2024). Neem Leaves Extract Reduces Sex Steroids and Gonadal Function in Female Wistar Albino Rats. *Biomedical and Pharmacology Journal*, *17*(3), 1471-1481. https://biomedpharmajournal.org/vol17no3/neem-leaves-extract-reduces-sex-steroids-and-gonadal-function-in-female-wistar-albino-rats/

Chebii, W. K., Muthee, J. K., & Kiemo, K. (2020). The governance of traditional medicine and herbal remedies in the selected local markets of Western Kenya. *Journal of ethnobiology and ethnomedicine*, *16*, 1-24. https://doi.org/10.1186/s13002-020-00389-x

Chou, C. H., & Chen, M. J. (2018). The effect of steroid hormones on ovarian follicle development. *Vitamins and hormones*, *107*, 155-175.

https://doi.org/10.1016/bs.vh.2018.01.013

Dozortsev, D. I., & Diamond, M. P. (2020). Luteinizing hormone–independent rise of progesterone as the physiological trigger of the ovulatory gonadotropins surge in the human. *Fertility and sterility*, *114*(2), 191-199. https://doi.org/10.1016/j.fertnstert.2020.06.016

Fehér, A., Gazdecki, M., Véha, M., Szakály, M., & Szakály, Z. (2020). A Comprehensive Review of the Benefits of and the Barriers to the Switch to a Plant-Based Diet. Sustainability, 12(10). https://www.mdpi.com/2071-1050/12/10/4136

Gupta, A., Ansari, S., Gupta, S., Narwani, M., Gupta, M., & Singh, M. (2019). Therapeutics role of neem and its bioactive constituents in disease prevention and treatment. *Journal of Pharmacognosy and Phytochemistry*, *8*(3), 680-691.

Izah, S. C., Joshua, M. T., Torru, K. E., Ngun, C. T., Ogwu, M. C., & Hait, M. (2023). Antimicrobial resistance and the role of herbal medicine: challenges, opportunities, and future prospects. *Herbal Medicine Phytochemistry: Applications and Trends*, 1-26.

Khanpara, P., & Jadeja, Y. (2022). A complete review on medicinal plant: Margosa Tree. *Journal of Medicinal Plants*, *10*(5), 131-140. https://www.plantsjournal.com/archives/2022/vol10issue5/PartB/10-5-21-910.pdf

Kulkarni, D. S. (2020). Gonadal histoarchitecture and reproductive hormones studies of female albino rats treated with azadirachtin. *Editorial Board*, *9*(11), 199. http://ijmer.s3.amazonaws.com/pdf/volume9/volume9-issue11(2)-2020.pdf#page=207

Moore, A. (2012). *Investigating steroid hormone feedback in a mouse model of polycystic ovarian syndrome* (Doctoral dissertation, University of Otago, New Zealand. https://ourarchive.otago.ac.nz/esploro/outputs/graduate/Investigating-steroid-hormone-feedback-in-a/9926479184801891

Moyo, M., Aremu, A. O., & Van Staden, J. (2015). Medicinal plants: An invaluable, dwindling resource in sub-Saharan Africa. *Journal of Ethnopharmacology*, *174*, 595-606. https://doi.org/10.1016/j.jep.2015.04.034

Njoga, U. J., Jaja, I. F., Onwuka, O. S., Ilo, S. U., Eke, I. G., Abah, K. O., ... & Ochiogu, I. S. (2022). Reproductive effects of medicinal plant (Azadirachta indica) used as forage and for ethnoveterinary practices: New insights from animal models. *Challenges*, *13*(2), 40. https://www.mdpi.com/2078-1547/13/2/40

Orisakwe, O. E., Orish, C. N., & Nwanaforo, E. O. (2020). Coronavirus disease (COVID-19) and Africa: acclaimed home remedies. *Scientific African*, *10*, e00620. https://doi.org/10.1016/j.sciaf.2020.e00620

Patil, S. M., Shirahatti, P. S., VB, C. K., Ramu, R., & Prasad, N. (2021). Azadirachta indica A. Juss (neem) as a contraceptive: An evidence-based review on its pharmacological efficiency. *Phytomedicine*, *88*, 153596. https://doi.org/10.1016/j.phymed.2021.153596

Ravichandran, V., Arunachalam, G., Subramanian, N., & Suresh, B. (2009). Contraception and its significance in the traditional system of medicines. *Int J Pharm Sci*, *1*, 1-21..

Roop, J. K., Dhaliwal, P. K., & Guraya, S. S. (2005). Extracts of Azadirachta indica and Melia azedarach seeds inhibit folliculogenesis in albino rats. *Brazilian journal of medical and biological research*, *38*, 943-947. https://www.scielo.br/j/bjmbr/a/GLLjQChMzbh9wjSyNrnjdKz/?format=html&lang=en

Sarah, R., Tabassum, B., Idrees, N., & Hussain, M. K. (2019). Bio-active compounds isolated from neem tree and their applications. *Natural Bio-active Compounds: Volume 1: Production and Applications*, 509-528. https://link.springer.com/chapter/10.1007/978-981-13-7154-7_17

Suryawanshi, J. A. S. (2011). Neem-natural contraceptive for male and female-an overview. *International Journal of Biomolecules and Biomedicine*, *1*(2), 1-6. https://innspub.net/wp-content/uploads/2023/03/IJBB-V1-No2-p1-6.pdf

Verma, N., Cwiak, C., & Kaunitz, A. M. (2021). Hormonal contraception: systemic Oestrogen and progestin preparations. *Clinical obstetrics and gynecology*, *64*(4), 721-738. https://journals.lww.com/clinicalobgyn/a

bstract/2021/12000/hormonal_contraception_systemic_estrogen_and.3.aspx

Winterbottom, A. (2021). Becoming "Traditional" a transnational history of neem and biopiracy discourse. *Osiris*, *36*(1), 262-283. https://www.journals.uchicago.edu/doi/abs/10.1086/713897

Wróblewski, M., Wróblewska, W., & Sobiesiak, M. (2024). The Role of Selected Elements in Oxidative Stress Protection: Key to Healthy Fertility and Reproduction. *International Journal of Molecular Sciences*, *25*(17), 9409. https://www.mdpi.com/1422-0067/25/17/9409

Wylie, M. R., & Merrell, D. S. (2022). The antimicrobial potential of the neem tree Azadirachta indica. *Frontiers in pharmacology*, *13*, 891535. https://doi.org/10.3389/fphar.2022.891535