EFFECT OF MULTIVITAMINS ON THE FERTILITY O F NEEM (*Azadirachta indica*) TREATED LABORATORY FEMALE ALBINO RATS (*Rattus norvegicus)*

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A THESIS SUBMITTED TO THE SCHOOL OF SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (ANIMAL PHYSIOLOGY), UNIVERSITY OF ELDORET, KENYA

2025

DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for any award in any other institution. No part of this thesis may be reproduced without prior written permission of the author and/or the University of Eldoret.

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DEDICATION

This thesis is dedicated to my beloved husband Henry and my beloved daughter Samantha for their undying support and prayers that gave me strength to press on.

ACKNOWLEDGEMENT

I sincerely wish to acknowledge the Almighty God for His abundant grace and unending love that He granted unto me throughout this long journey of my studies.

I wish to thank my beloved mother Mama Alice Makungu Aduvaga for her strong spirit of never giving up, no matter the situation. This spirit enabled me to keep pushing.

I also wish to thank Dr. Anthony Sifuna, the Dean of the school of medicine, MMUST for allowing me to carry out my experiments in the animal house at MMUST.

To professor Lilechi Barasa, Head of quality assurance at MMUST, your input in preparation of Neem extracts cannot go unmentioned. I'm so grateful.

Mr. Willy Aganyanya], the Chief technician at the Biotech lab, MMUST, will not go unmentioned for his tireless support throughout the alcoholic extraction of Neem, constitution of Neem and multivitamin doses, to preparation of sera. Thanks a lot.

Additionally, I would really like to thank my dear friend Dr. Jared Ngurwe for introducing me to the Masinde Muliro University staff, whom we worked together with to bring this research to a success.

My special thanks also goes to Mr. Apollonary Ouma of Zoology Lab at the University of Eldoret for tirelessly working to collect the neem twigs, shade drying Neem leaves and grinding them to fine powder.

To the assistant staff in the biotech lab of MMUST: Mr. Oliver Ouma Ojiambo and Arnold Asava who did assist a lot though extraction, dispensing of doses, feeding of animals, treatment of animals until sacrificing and preparation of sera. I will forever remain grateful to you.

To the staff of the main MTRH lab, specifically Mr Chelimo Charles for carrying out the bioassays on my samples, I wish to say thank you so much for your dedicated service.

My special thanks also go to Mr Haruki Boniface (GIS specialist and data analyst) who analyzed my data.

Dr. Chemoiywa Emily of the Biological Science department of the University of Eldoret won't go unmentioned for all the rigorous work of keeping me updated about every requirement to be fulfilled and in good time. Special thanks.

To the staff of the University of Eldoret Library: Ms Petronillah Omete, Ms Sarah Lelei, and Mr. Titus Too for proper guidance on library processes, thank you so much.

Lastly I wish to say this to my destiny helpers Pastor Epiphany Nyirahabimana Ochieng and Eunice Opondo of CITAM Kisumu for fervently praying for me and encouraging me not to stop until the job is done. You are the best!!!!

ABSTRACT

Azadirachta indica (Neem) is a widely used medicinal plant valued for its therapeutic properties, particularly in regions with limited access to conventional healthcare. It has been reported to exert antifertility effects by altering reproductive hormones in animals, yet the potential modulatory effects of multivitamin supplementation remain unclear. 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 Christ Is The Answer Ministries (**CITAM**) Kisumu church compound and the University of Eldoret. Forty female albino rats sourced from Maseno University were acclimatized 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 and without multivitamins, for 28 days. Post-treatment, blood serum samples were analyzed for follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estrogen 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 in the negative control to 3.27±0.21 ng/mL in the highest neem dose (100 mg/kg), which was not significantly different in the positive control group (3.03±0.15 ng/mL). LH declined from 6.17±0.35 ng/mL in the negative control to 2.90±0.20 ng/mL (Neem100), versus 2.57±0.21 ng/mL in the positive control group. Estrogen levels fell from 52.59±2.55 pg/mL in the negative control to 13.53±1.25 pg/mL in Neem100, while controls and positive control had comparable levels (50.97±1.36 pg/mL). Multivitamin supplementation (done at the dosage of one pill per 70kg of body weight per day) partially reversed these suppressive effects of neem, increasing FSH to 5.87±0.15 ng/mL, LH to 5.13±0.15 ng/mL, and estrogen 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 estrogen ($F_{0.05(4,10)}=306.53$, $p<0.0001$). Correlation analysis showed a very strong positive correlation between FSH and LH (r=0.992, $p<0.0001$), while estrogen exhibited weak, non-significant correlations with FSH and LH. In conclusion, neem leaf extract exerts potent contraceptive effects through dose-dependent suppression of reproductive hormones, with multivitamin supplementation partially mitigating these effects. These findings highlight the importance of understanding interactions between traditional herbal remedies and nutritional supplements and warrant further investigation into long-term outcomes and mechanisms.

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LIST OF ACRONYMS AND ABBREVIATIONS

3Rs principle	Replacement, Reduction, and Refinement
ANOVA	Analysis of Variance
CITAM	Christ Is The Answer Ministries
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FSH	Follicle Stimulating Hormone
IACUC	Institutional Animal Care and Use Committee
KVB	Kenya Veterinary Board
LH	Luteinizing hormone
MG/KG.	Milligrams per kilogram
MMUST	Masinde Muliro University of Science and Technology
MTRH	Moi Teaching and Referral Hospital
MG/KG	Milligram per kilogram of body weight
NACOSTI	National Commission for Science, Technology, and Innovation
Neem100	Neem leaf extract at 100 mg/kg
Neem100 + MV	Neem leaf extract at 100 mg/kg combined with multivitamins
Neem50	MV Neem leaf extract at 50 mg/kg combined with multivitamins
Neem50	Neem leaf extract at 50 mg/kg
Neem75	Neem leaf extract at 75 mg/kg
Neem75 + MV	Neem leaf extract at 75 mg/kg combined with multivitamins
ng/mL	Nano grams per milliliter
pg/ml	Pico grams per milliliter

NMR	Nuclear magnetic resonance
ROS	Reactive Oxygen Species
SD	standard deviation
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Thousands of curative plant extracts have served a chief role in old healing systems for a long time and continue to be a vital resource in modern pharmacological research. Worldwide, more than 25,000 higher plant species are documented for their important medicinal value. The World Health Organization (**WHO**, 2022), over 80 percent of the populace in developing countries depends on traditional treatment as their principal healthcare option. The growing demand for plant-based therapies is largely as a result of their seeming safety, affordability, cultural significance, and effectiveness in managing chronic and infectious diseases (Fehér et al., 2020). Moreover, global concerns such as antibiotic resistance, side effects of artificial drugs, and limited healthcare access in rural areas have reignited interest in the use of medicinal plants (Abdallah *et al*., 2023; Izah *et al*., 2023).

Across different parts of the world, plants like *Zingiber officinale* (ginger), *Curcuma longa* (turmeric), *Allium sativum* (garlic), *Ocimum sanctum* (holy basil), and *Aloe vera* are celebrated for their strong anti-inflammatory, antioxidant, antimicrobial, and immune-boosting properties. For instance, turmeric contains curcumin, known for its anticancer and antiarthritic effects, while garlic's allicin has demonstrated effectiveness in combating cardiovascular and microbial conditions (Prajapati et al, 2020). These natural compounds have provided the foundation for several modern pharmaceutical drugs, illustrating the deep connection between traditional ethnobotanical knowledge and modern medicine (Yuan et al., 2016).

In Latin America, traditional healing systems like Curanderismo and the Amazonian pharmacopoeia heavily rely on plants such as *Uncaria tomentosa* (cat's claw) (Bussmann, & Sharon, 2015), *Echinacea purpurea*, and *Bixa orellana* to treat inflammatory, respiratory, and reproductive disorders (Nascimento M Neto *et al*., 2020). Similarly, in Asia, particularly in countries like China and India, centuries-old systems like ancient Chinese Medication and Ayurveda utilize plants like *Withania somnifera* (ashwagandha), *Tinospora cordifolia*, and *Trigonella foenum-graecum* (fenugreek) to manage reproductive and metabolic health (Rajendram, Preedy & Patel, 2023; Tripathy *et al*., 2024).

Africa, especially sub-Saharan Africa, is widely known for its rich and diverse biodiversity, hosting approximately 45,000 plant species, with more than 5,000 recognized for their medicinal properties (Moyo, Aremu & Van Staden, 2015). Indigenous knowledge concerning medicinal plants is widespread across West, Central, and East African communities. In West Africa, plants such as *Vernonia amygdalina* (bitter leaf), *Gongronema latifolium*, and *Tetrapleura tetraptera* are frequently used to manage malaria, digestive disorders, and reproductive conditions (Asuzu, 2018). In East Africa, *Warburgia ugandensis* (East African greenheart) is commonly used to treat respiratory infections, while *Prunus africana* (African cherry), found in the highlands of Kenya, Uganda, and Cameroon, is vital in the treatment of benign prostatic hyperplasia (Karani *et al*., 2017).

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 referred to as neem, is especially notable for its versatility and cultural significance (Wylie & Merrell, 2022; Mursal *et al*., 2024).

Neem is part of the Meliaceae family and is inherent 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 "*Muarobaini*" 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.

Phytochemical studies of neem have identified a variety of bioactive compounds, including azadirachtin, nimbin, quercetin, salannin, and limonoids (Gupta *et al*., 2019). These compounds are the cause of neem's broad therapeutic range, which includes antibacterial, antifungal, antiparasitic, antioxidant, anti-inflammatory, and liver-protective effects (Sarah *et al*., 2019). Neem has been found effective against conditions such as malaria, skin infections, gastrointestinal problems, and diabetes (Gupta *et al*., 2019; Latif, *et al*., 2020).

Importantly, neem has shown potential antifertility effects, sparking interest in its possible use as a natural contraceptive (Patil *et al*., 2021). In males, extracts from neem leaves and seeds have been shown to disrupt sperm production, reduce sperm motility, and cause abnormalities such as headless or tailless sperm (Ali, 2020). In females, neem has been found to suppress the estrous cycle, inhibit ovulation, and prevent embryo implantation, leading to early resorption or miscarriage (Patil *et al*., 2021). These reproductive effects are thought to result from changes in hormone levels, damage to reproductive tissues, and biochemical changes in the uterus.

Experiments with laboratory animals have also revealed that neem can alter levels of reproductive hormones like follicle-stimulating hormone (FSH) and luteinizing hormone (LH), suggesting endocrine disruption. However, these effects appear to vary with dose, method of administration and treatment duration (Njoga *et al*., 2022). Although research on neem plant use in the medicine field is expanding, there is still no agreed-upon safe dosage particularly when it is used for non-medical or preventive purposes across the globe. In contrast, multivitamin supplements are widely taken to support both reproductive and general health which typically contain key nutrients such as vitamins A, C, D, E, and the B-complex group as noted by Wong et al. (2019). This has been linked to improved hormonal balance, sperm quality and overall protection of reproductive tissues against oxidative stress (Walke et al., 2023). Notably, vitamins C and E serve as antioxidants that safeguard sperm and ovarian cells, while vitamin B12 and folate are essential for DNA synthesis and the development of gametes. Despite this, it remains uncertain how multivitamins interact with neem extract. Whether these supplements can offset or perhaps amplify overall neem's antifertility effects has yet to be determined. This presents a significant research gap, especially given the growing trend in many African countries, including Kenya, of using herbal remedies alongside over-the-counter supplements without medical oversight.

Given the traditional importance of neem and the increasing consumption of multivitamins, it is essential to investigate the dose-related effects of neem on fertility and assess whether multivitamins can influence these outcomes. Such research could help guide the safe use of herbal contraceptives or strategies for restoring fertility lead to the development of regulated, evidence-based herbal medicine practices in Kenya as well as in other nations across the globe.

1.2 Statement of the problem

In many parts of the globe, especially in low- and middle-income regions, people continue to rely heavily on herbal medicines to manage their health needs. One such plant is *Azadirachta indica*, popularly regarded as neem, which is widely utilised for its medicinal value across Africa and Asia. In Kenya and other African countries, neem is often used to treat infections, manage diabetes, and even support reproductive health (Tibebu, Haile, & Kebede, 2017; Mbugi et al., 2021). However, recent studies have raised concerns about the plant's potential impact on fertility. Research in animal models shows that neem can interfere with reproductive functions by affecting reproductive hormone levels, impairing sperm production, and disrupting ovulation and embryo implantation (Patil et al., 2021).). On the other hand, there is a continuous increase in use of multivitamin supplements aimed at improving reproductive health. These supplements, which often include vitamins A, C, E, D, B12, and folate, are known to support sperm quality, regulate hormonal balance, and protect reproductive tissues from oxidative stress (Wróblewski, Wróblewska & Sobiesiak, 2024). Despite this, little is known about how these vitamins interact with neem, especially when both are taken together, a practice that is becoming more common in Kenya, where many people turn to self-medication and traditional remedies without professional guidance.

This lack of clarity presents a serious gap in current knowledge. There is limited scientific evidence on whether multivitamins can reduce or worsen the fertility-suppressing effects of neem. Without proper research, people may unknowingly put their reproductive health at risk, particularly those using neem regularly for medicinal purposes or as a form of natural contraception.

1.3 Objectives

1.3.1 Broad objective

To investigate the dose-dependent effects of *Azadirachta indica* (neem) on reproductive function and evaluate the modulatory impact of multivitamin supplementation on these effects in female albino rats

1.3.2 Specific objectives

i) To determine the levels of FSH, LH and Estrogen when female rats are treated with different doses of neem leaf extract

ii)To assess the effect of multivitamin supplementation on albino rats treated with neem leaf extract.

1.3.3 Research null hypotheses

$H0_1$: There is no significant effect of different doses of neem leaf extract on the levels of FSH, LH and Estrogen of female albino rats treated with neem leaf extract

$H0_2$: Multivitamin supplementation has no significant effect on the levels of FSH, LH and Estrogen of female albino rats treated with neem extract.

1.4 Justification

Multivitamins contain a mix of important vitamins that come from both plants and animals, and they are commonly and widely known for being safe and beneficial to our overall health. The easy accessibility of multivitamins and their ability to be well tolerated makes them qualify for being used in research on testing whether they can help protect the body from any side effects caused by other substances. Neem, a plant heavily utilized in ancient medicine, has many health benefits but is also known to affect fertility by changing hormone levels and damaging sperm and egg cells. What is still unclear, though, is whether taking multivitamins can help reduce or reverse these fertility issues caused by neem.

Since neem is so commonly used in many communities, especially in parts of Africa, it is important to understand how we can safely use it without negatively impacting people's reproductive health. Multivitamins, which support the body's natural defenses and hormone balance, might help lessen the harmful effects neem has on the reproductive system. This study aimed to explore how different doses of neem affect fertility and whether multivitamin supplements could offer some protection. By doing so, this research could provide valuable insights for safely combining traditional herbal treatments with modern supplements.

1.5 Significance

For individual women who may use neem to manage health conditions, its impact on the reproductive system may lead to delays in conceiving especially in settings where having children holds deep cultural significance, such delays can cause considerable stress and emotional strain. If multivitamins are found to act and protect against neem's fertility-reducing effects, they could help shorten the time it takes to conceive and support better reproductive health in those who rely on neem plant extracts. The value of this study therefore goes beyond helping individual users. Its findings could provide practical guidance for healthcare professionals, traditional healers as well as community members on how to combine neem with common supplements safely. This is especially relevant in communities more so those in rural settings where herbal remedies are widely used without expert supervision. By offering clearer, evidence-based recommendations, it becomes possible to enjoy neem's therapeutic benefits while minimizing any risk to fertility and ultimately, this research will therefore be of help to people in making informed choices for neem's benefits without compromising their ability to have children.

1.6 Scope of the Study

This research study was designed to explore the effects of Neem (*Azadirachta indica*) extract on reproductive health, with a specific focus on how it influenced fertility in albino rats. The work aimed to observe how different doses of Neem impacted hormonal balance, reproductive organ structure, and fertility outcomes. Alongside this, the study examined whether supplementing with multivitamins could lessen or possibly reverse any negative reproductive effects caused by Neem.

The research study was carried out entirely under controlled laboratory conditions, using albino rats as the test model due to their reliability in reproductive health studies. It concentrated on the oral route of administration for both Neem and multivitamins, reflecting the most common way these substances are consumed in real-life settings.

This research study did not extend to human trials or field studies. It remained limited to short- and medium-term exposure periods that were adequate to observe significant reproductive changes. While human implications were not directly studied, the results were intended to inform future research and offer a scientific basis for understanding how Neem and multivitamins interact in relation to fertility.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The use of curative plants for managing health has developed rapidly recently, especially in developing countries where they provide a more accessible and affordable alternative to conventional pharmaceuticals. Among these, *Azadirachta indica* better regarded as neem has drawn considerable scientific concentration for its varied range of medicinal benefits. Despite its therapeutic potential, research has also raised concerns about its possible impact on reproductive health. In contrast, multivitamin supplements, well known for their antioxidant and protective properties, may help offset such effects. This chapter explores current knowledge on neem's influence on reproductive function, the potential mitigating role of multivitamins, and the reasoning behind using albino rats as a model for experimental investigation.

2.2 Botanical Profile and Phytochemistry of *Azadirachta indica*

2.2.1 Description of *Azadirachta indica*

Azadirachta indica, widely known as neem, *margosa*, or Indian lilac, is a fast-growing, evergreen tree in the mahogany family (Meliaceae). Inherent in the Indian subcontinent as well as sections of Southeast Asia, neem has spread to many tropical and subtropical regions around the globe as a result of its malleability and varied range of uses (Ahmed, 2023; Malakar & Mandal, 2025).

The neem tree typically grows between 15 and 20 meters tall, although some can get to 40 meters. It has a broad, rounded crown that can span over 20 meters in diameter. The leaves are pinnate, about 20–40 cm long, and made up of 20 to 30 serrated leaflets. The terminal leaflet is often missing, which is a feature that helps distinguish neem from similar-looking species (Michael, 2018).

Neem produces small, fragrant, white flowers arranged in drooping panicles up to 25 cm long. Each tree can bear hundreds of these flowers, which are either bisexual or male and typically bloom before the female parts mature, a trait that encourages cross-pollination (Malakar & Mandal, 2025).

The fruits are smooth, olive-shaped drupes that turn yellowish-white when ripe. They usually measure about 1.5–2.5 cm in length and contain a fibrous pulp surrounding one to three seeds. These seeds are pressed to extract neem oil, which contains bioactive compounds like azadirachtin, known for their medicinal and insecticidal properties (Michael, 2018).

Neem is sometimes confused with its close relative, *Melia azedarach* (chinaberry tree), as both have similar leaves and fruit. However, neem has once-pinnate leaves, while *Melia* has leaves that are twice or even three times pinnate, a helpful feature when distinguishing the two (Nicoletti *et al*., 2012).

2.2.2 Taxonomy of *Azadirachta indica*

The scientific identification of *Azadirachta indica*, widely known as neem, was first established in 1830 by the French botanist Adrien Henri de Jussieu. He recognized its distinct characteristics and placed it in a separate genus, *Azadirachta*, based on clear differences from other related species (Winterbottom, 2021). Here, Neem is classified as follows:

Rank	**Taxonomic Category**
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta*
Species	*Azadirachta indica*

The name ‘azedarach’ has an interesting origin. It comes from the French word *azédarac*, which traces back to the Persian term *āzād dirakht*, meaning “free tree” or “noble tree.” In traditional Persian, neem is also called *azad dirakht e hind*, which translates to “the free tree of India.” This likely reflects an old belief in neem’s natural resistance to pests and diseases, which is consistent with its widely known medicinal and insect repellent properties (Chopra *et al*. 1956 cited in Prusty, Nayak2Ψ & Mohanty, 2014).

2.2.3 Distribution of *Azadirachta indica*

Neem is thought to have originated in the Indian subcontinent, with its native range including regions such as Assam in India, as well as Pakistan and Bangladesh. Beyond South Asia, the species is also considered indigenous to parts of mainland Southeast Asia, including Cambodia, Laos, Myanmar, Thailand, and Vietnam (Malakar & Mandal, 2025). Due to its wide range of beneficial properties, including medicinal and ecological uses, neem has been introduced and successfully cultivated in many tropical and subtropical areas around the world. Today, it grows in countries across Africa, the Caribbean, South America, and Southeast Asia, including Indonesia. Its remarkable ability to thrive in arid and semi-arid conditions has made it especially popular in regions facing environmental challenges such as desertification (Ahmed, 2023).

This global spread reflects not just its ecological resilience but also its increasing recognition as a multipurpose tree in both traditional and modern agricultural and health systems.

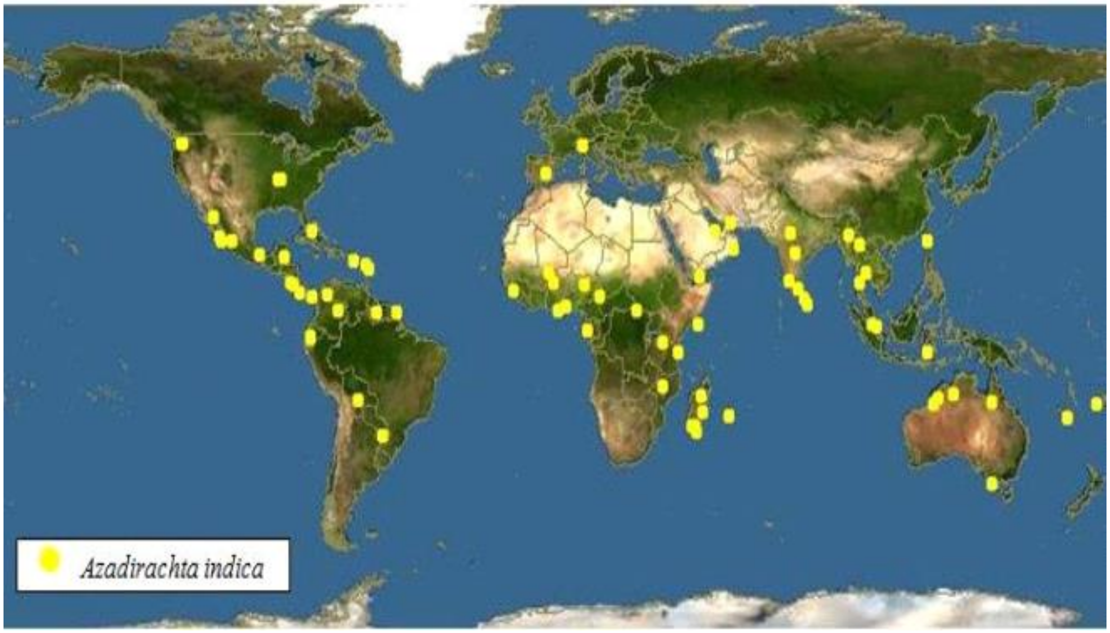

Figure 2.2 Distribution of *A. indica* in the world (Ashwini S et al.,2018)

Azadirachta indica (Neem) is well known for its drought tolerance, thriving mainly in areas with 400 to 1,200 mm of annual rainfall. It can survive in drier regions with less than 400 mm rainfall, relying heavily on groundwater availability The tree grows on various soils but prefers well-drained, deep, sandy soils. It suits tropical and subtropical climates, with average temperatures between 21°C and 32°C, tolerating high heat but not temperatures below 5°C (Malakar & Mandal, 2025).

Neem is valued for providing shade in dry areas, such as parts of India, Pakistan and in major parts of African countries. It requires little water and grows even in poor water quality. Because of this resilience, neem is commonly planted along streets, near public buildings, and in home gardens in tropical countries, also playing a role in land restoration and combating desertification.

2.2.5 Phytochemical Composition

Azadirachta indica contains a wide variety of naturally occurring compounds that contribute to its medicinal and pesticidal properties. These compounds are found across different parts of the plant viz: seeds, leaves, bark, stems, and fruit. One of the most studied and valuable compounds is azadirachtin, primarily found in the seeds. It was first identified in the 1960s and is known for its ability to deter insects from feeding, disrupt their growth, and act as a natural pesticide. Typically, about 5 grams of azadirachtin can be obtained from 2 kilograms of neem seeds (Zheng *et al*., (2018).

Neem seed oil, which is yellowish and has a strong garlic-like smell, also contains beneficial components such as limonoids (about 2%), triterpenoids, polyphenols, nimbolide, glycerides, and beta-sitosterol. These compounds are responsible for many of neem's healing and insect-repelling effects (Subapriya & Nagini, 2005; Ghosh, *et al*., 2016).

The leaves are rich in antioxidants like quercetin, catechins, carotenoids, and vitamin C. This helps support the immune system and may shield the body from oxidative damage (Biswas *et al*., 2002). Additionally, neem's bark and stem contain active substances such as nimbin, nimbidin, and nimbidol, which have demonstrated antibacterial, antifungal, and anti-inflammatory effects. Altogether, these natural compounds explain why neem has been a cornerstone of traditional medicine and continues to gain interest in modern pharmacology and sustainable agriculture.

2.2.6 Medicinal and other uses of *A. indica*

Neem has played a central role in traditional Indian medicine for centuries. Various parts of the tree including its bark, leaves, seeds, and oil have long been used to treat ailments such as skin conditions, fever, inflammation, and rheumatism (Biswas *et al*., 2002; Subapriya & Nagini, 2005).

Despite its widespread use in Ayurveda and folk medicine, modern clinical research has yet to provide strong scientific evidence supporting most of these therapeutic claims. While short-term use of neem products in adults is generally considered safe, especially in small doses, long-term or high-dose consumption may pose health risks. Studies have linked prolonged neem use to potential kidney and liver toxicity (Mishra & Dave, (2013). In children, neem oil can be particularly hazardous even small doses may be toxic and, in rare cases, have led to fatal outcomes (Boeke et al., 2004). Culturally, neem twigs have long been used as natural toothbrushes in Southern India and parts of the Middle East. Rich in antibacterial compounds, these twigs remain a popular, low-cost, and traditional method of maintaining oral hygiene (Hebbar et al., 2004).

The neem plant holds a wide range of practical applications. Neem is also valued as a natural pesticide; seed extracts deter pests, disrupt feeding, and reduce egg-laying when applied regularly. For example, in India, dried leaves are traditionally placed among clothes and stored grains to protect them from insect damage as noted by Biswas et al. (2002). Its young shoots and flowers, though distinctly bitter, are incorporated into various dishes across South and Southeast Asia (Boeke et al., 2004). From an environmental perspective, neem contributes to reforestation efforts worldwide, enriches soil quality, and aids in reducing atmospheric carbon as noted in Kenyan coastal as well as in the rift valley region.

2.3 Neem and Reproductive Health: Evidence from Animal and Human Studies

Neem (*Azadirachta indica*) is a plant that has been widely used in traditional medicine for centuries, known for its diverse therapeutic properties. In recent years, researchers have focused on its effects on reproductive health, especially its potential to regulate fertility. Neem extracts have found applications in contraceptive products, with some modern contraceptives containing neem-derived compounds (Subapriya & Nagini, 2005).

The plant contains several bioactive compounds, including nimbidin, nimbandiol, and azadirachtin, which contribute to its medicinal effects. These compounds have been shown to have antibacterial, antifungal, anti-inflammatory, antidiabetic, and immunomodulatory activities (Nicoletti *et al*., 2012)). Traditional uses of neem also include treatment for skin disorders such as eczema and acne, as well as blood purification and immune system enhancement Azadirachtin, one of the key compounds found mainly in neem seeds, is considered the primary active ingredient responsible for neem's contraceptive and spermicidal properties. It is a highly complex molecule whose detailed structure was elucidated only after advanced analytical techniques like nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography were applied (Patil *et al*., 2021).

Currently, most contraceptive methods are designed for women, with very limited safe and effective options available for men. This gap has driven research into plant-based male contraceptives, with neem being a promising candidate due to its spermicidal effects (Patil *et al*., 2021). Studies indicate that neem extracts influence sperm parameters such as count, motility, and morphology. Both neem leaf extracts and seed oil have demonstrated the ability to inhibit sperm proliferation at very low concentrations, highlighting their potent spermicidal action without causing permanent infertility (Ali, 2020).

Experimental evidence further reveals that sodium nimbidinate, a compound derived from neem, exhibits direct spermicidal effects by disrupting sperm membrane integrity and immobilizing sperm cells (Martin, 2018). Exposure to azadirachtin results in sperm abnormalities such as missing or bent tails, absent or malformed heads, and swelling of the midpiece, collectively impairing sperm function and fertilization capability (Ali, 2020). These findings suggest that multiple neem compounds likely act synergistically to produce these effects.

Research has found that even very small amounts of neem oil (around 10 µg/mL) can impair sperm movement. At these low concentrations, it increases the proportion of sperm that remain immobile while reducing the number capable of moving forward effectively (Patil et al., 2021). Interestingly, neem's spermicidal action seems to be highly selective

Table 2.1: Bioactive Compounds in *Azadirachta Indica*

Azadirachtin (A-G)	Seeds and leaves	Alzohairy MA(2016)
Tetranortriterpenoid	Seeds and leaves	Alzohairy MA(2016)
Limonoids	Seeds and leaves	Alzohairy MA(2016)
Nimbolide – Limonoid	Leaves ,and flowers	Alzohairy MA(2016)
Nimbin	Leaves and bark	Alzohairy MA(2016)
Gedunin – Limonoid	Seeds and bark	Alzohairy MA (2016)
Salannin – Limonoid	Seeds	Islas (2020)
Nimbidol/Nimbiol/Nimbandiol	Leaves and twigs	Alzohairy MA(2016)
Quercetin (Flavonoids)	Leaves	Alzohairy MA(2016)

targeting sperm cells while causing little to no harm to normal body cells. This selectivity is encouraging, as it suggests a relatively favorable safety profile for its use as a contraceptive. Its impact on female fertility has also been documented. In animal studies involving rats and primates, oral administration of neem seed extracts has been shown to prevent pregnancy by disrupting early events after fertilization (Tripathi, Shrivastav & Chaube, 2013). Notably, fertility returned once treatment was stopped, indicating that the effect is reversible. Similarly, vaginal application of neem oil before mating has been reported to prevent conception in both animals and humans (Suryawanshi, 2011).

Specific compounds found in neem oil such as azadirachtin, nimbin, and salannin have been studied individually in rodents and linked to antifertility effects (Chaube et al., 2006). Neem oil can also suppress the development of ovarian follicles in female rats, potentially lowering the number of eggs available for fertilization (Ghosh et al., 2016). Moreover, neem leaf extracts have been shown to trigger apoptosis in mammalian oocytes, as indicated by visible structural changes and the activation of caspase-3, a key enzyme in programmed cell death (Tripathi, Shrivastav & Chaube, 2013). These findings help explain how neem can directly influence the ovaries to reduce fertility.

However, toxicological studies on neem's reproductive effects have produced mixed results, highlighting the need for further research to clarify its safety and efficacy. High doses of neem oil administered throughout pregnancy caused fetal malformations and reproductive toxicity in Wistar rats (Braga *et al*., (2021). However, administration of pure azadirachtin at lower doses did not elicit such teratogenic effects, potentially due to protective effects of fatty acids like linoleic acid present in neem oil. This suggests that some components of neem may counteract the toxicity of others, highlighting the importance of considering whole extract composition in safety assessments.

2.4 Determining Optimal Dosage: Approaches and Findings in Animal Models

Finding the right dose of neem extract is essential to make sure it works effectively without causing harm, especially when looking at its effects on reproduction. Researchers often rely on a mix of toxicity tests and biological markers like hormone levels, organ sizes, and tissue health to figure out what amounts are safe to use. (Ahmet A et al.,)

One common starting point is the LD_{50}, which tells us the dose that would be lethal to half the test animals, helping set the boundaries for safe testing. But more importantly, scientists check how lower doses affect reproductive hormones like luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and estrogen since these play key roles in fertility (Oduwole, Huhtaniemi & Misrahi, 2021).

Studies clearly show that the effects of neem on hormones and reproductive organs depend a lot on the dose. For example, Yarmohammadi *et al*. (2021), gave rats neem leaf extract at 200 mg/kg and 800 mg/kg over 30 days. The lower dose caused only minor and reversible changes, but the higher dose led to big drops in LH and FSH and visible damage in testes and ovaries, suggesting a risk to fertility. More recent research has taken a closer look at what is happening at the cellular level.

It is also important to note that some effects of neem can be reversed if the dose is low or the exposure is short. Mishra and Singh (2015) found that once neem treatment stopped, reproductive functions bounced back in many cases. But if the dose is very high or exposure lasts longer the damage might become permanent with tissue scarring and loss of reproductive cells. This highlights why carefully balancing dose and treatment length is crucial.

How neem is administered also matters. Taking neem orally affects the whole body and can cause more widespread hormone changes. On the other hand, applying neem oil locally for example intravaginal can prevent systemic side effects while still working as a contraceptive (Upadhyay, Kaushic & Talwar, 1990). Researchers are even looking into new formulations like Nano emulsions to make such local treatments safer and more effective.

2.5 Role of Multivitamin Supplementation in Modulating Reproductive Toxicity

Vitamins such as A, C, E, and the B-complex group, along with minerals like zinc and selenium, are essential for maintaining reproductive health. Their key role lies in their antioxidant properties, which help neutralize Reactive Oxygen Species (**ROS)** harmful molecules that can injure reproductive tissues. When higher doses neem extract trigger oxidative stress, these nutrients can help shield tissues from harm and support recovery (Agarwal & Majzoub, 2017).

Studies indicate that neem-induced oxidative stress can damage reproductive organs, but antioxidant supplementation can help counteract these effects. For instance, research has shown that rats receiving vitamin E alongside neem extract had healthier testicular tissue and maintained normal testosterone levels compared to those that did not receive the supplement. Zinc is another important nutrient it is essential for making healthy sperm and keeping their shape and movement normal. In animals exposed to toxins, zinc helped improve sperm quality (Fallah, Mohammad-Hasani & Colagar, 2018). Selenium also plays a protective role by boosting antioxidant enzymes that help detoxify harmful molecules in the testes.

Vitamin C, known for being a strong water-soluble antioxidant, has been found to lower damage to sperm DNA and lipids caused by oxidative stress (Ibrahim *et al*., 2025). Plus, B-complex vitamins support the energy production and hormone balance needed for healthy reproductive function (Yang *et al*., 2021).

While these individual vitamins and minerals show promise, there is still a lack of detailed research on how taking a combination of multivitamins together with neem might work. Especially important is understanding whether different doses make a difference, and if these nutrients can fully reverse or prevent the reproductive harm neem might cause. So far, most studies have looked at single nutrients, leaving many questions about how multivitamin supplements interact with neem unanswered.

Since neem is widely used in traditional medicine and is being considered for natural contraceptive methods, figuring out how nutrition can influence its effects is important. That is why the current study aimed to explore how multivitamin supplements could help reduce the reproductive toxicity caused by neem and support recovery after exposure.

2.6 Experimental Studies Using Albino Rats as Models for Reproductive Research

Albino rats are commonly chosen for reproductive toxicology studies because they are easy to handle, reproduce quickly, and have well-understood reproductive systems. Researchers often use them to test how different substances affect fertility by giving the rats various doses of those substances through different methods like oral feeding or injections, over periods ranging from a few days to several weeks or months.

When it comes to neem, many studies have focused on giving albino rats extracts from neem leaves, seed oil, or purified compounds such as azadirachtin (Upadhayay & Vigyan, 2014). Usually, these are given daily at doses from 200 to 800 mg per kilogram of body weight for about a month. These experiments consistently show that neem affects important reproductive hormones like testosterone, LH, and FSH. The higher the dose, the more noticeable the drop in hormone levels (Seriana, Akmal & Wahyuni, 2019).

In addition to hormone changes, scientists carefully look at the structure of reproductive organs under the microscope. In the testes, for example, neem tends to disrupt the organization of the tubules where sperm develop, lowers sperm count, and causes damage to the cells that create sperm (Adewole & Attah, 2020). Similar effects have been seen in the ovaries, where neem exposure leads to fewer developing follicles and more cell death, which helps explain lower fertility in these animals.

Another interesting finding is that neem specifically harms sperm cells. Using special staining techniques, researchers have shown that neem damages the membranes of sperm, making them unable to move properly, while leaving other nearby cells unharmed. This targeted effect suggests neem could work as a natural contraceptive without causing widespread toxicity (Seriana, Akmal & Wahyuni, 2019).

2.7 Role of Albino Rats in Reproductive Research.

Albino rats are typically and commonly used in reproductive studies because their reproductive physiology particularly the hormonal regulation through the hypothalamic–pituitary gonadal axis closely mirrors that of humans according to several studies (Acevedo-Rodriguez et al., 2018; Odetayo et al., 2024). This similarity makes them a reliable model for examining how natural products, such as neem as well as other natural extracts, may affect reproductive function. Their relatively uniform genetic makeup further minimizes variability in the end, helping to ensure consistent results across treatment groups (Aitman et al., 2008; Krubaa & Yogitha, 2024). In addition, they provide a practical and ethically acceptable option for testing fertility-regulating agents prior to progressing to studies involving larger animals or human subjects as noted by Brogi et al. (2019).

2.7 Research gaps and why this study matters

Many researchers have looked into neem's ability to affect fertility, showing its potential as a natural contraceptive (Suryawanshi, 2011). However, most of these studies do not provide detailed information on the exact doses needed to get effective results without causing lasting harm to reproductive health. Also, they often focus on short-term effects, leaving questions about what happens in the long run unanswered. Another important piece that is missing in current research is how multivitamins might protect against the reproductive damage neem can cause. We know that oxidative stress serves a huge purpose in this damage, and antioxidants like vitamins A, C, and E, as well as minerals like zinc and selenium, help defend reproductive tissues from harm (Wróblewski, Wróblewska & Sobiesiak, 2024). But few studies have looked at how giving multivitamins alongside neem might reduce those harmful effects.

2.8 Summary of Literature Review

The literature demonstrates neem's dose-dependent reproductive effects and suggests potential modulation by multivitamin supplementation. However, inconsistencies in dosages, extract types, and animal responses necessitate further controlled studies. This study, therefore, builds on previous findings to evaluate both the toxicity threshold and protective effects of multivitamins in a controlled albino rat model.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

This investigation utilized an experimental design to examine how Neem (*Azadirachta indica*) leaf extract affects reproductive hormones, specifically Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and Estrogen in female albino rats. It also explored whether supplementing with multivitamins could counteract potential antifertility effects. This design allowed close regulation of all variables, providing a robust foundation for reliable and reproducible findings.

3.2 Study Area

The research was undertaken in the Biotechnology and Microbiology Laboratories in the School of Natural and Applied Sciences at the Masinde Muliro University of Science and Technology(**MMUST**), situated along the Kakamega-Webuye road in Kenya's western region. The area 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.

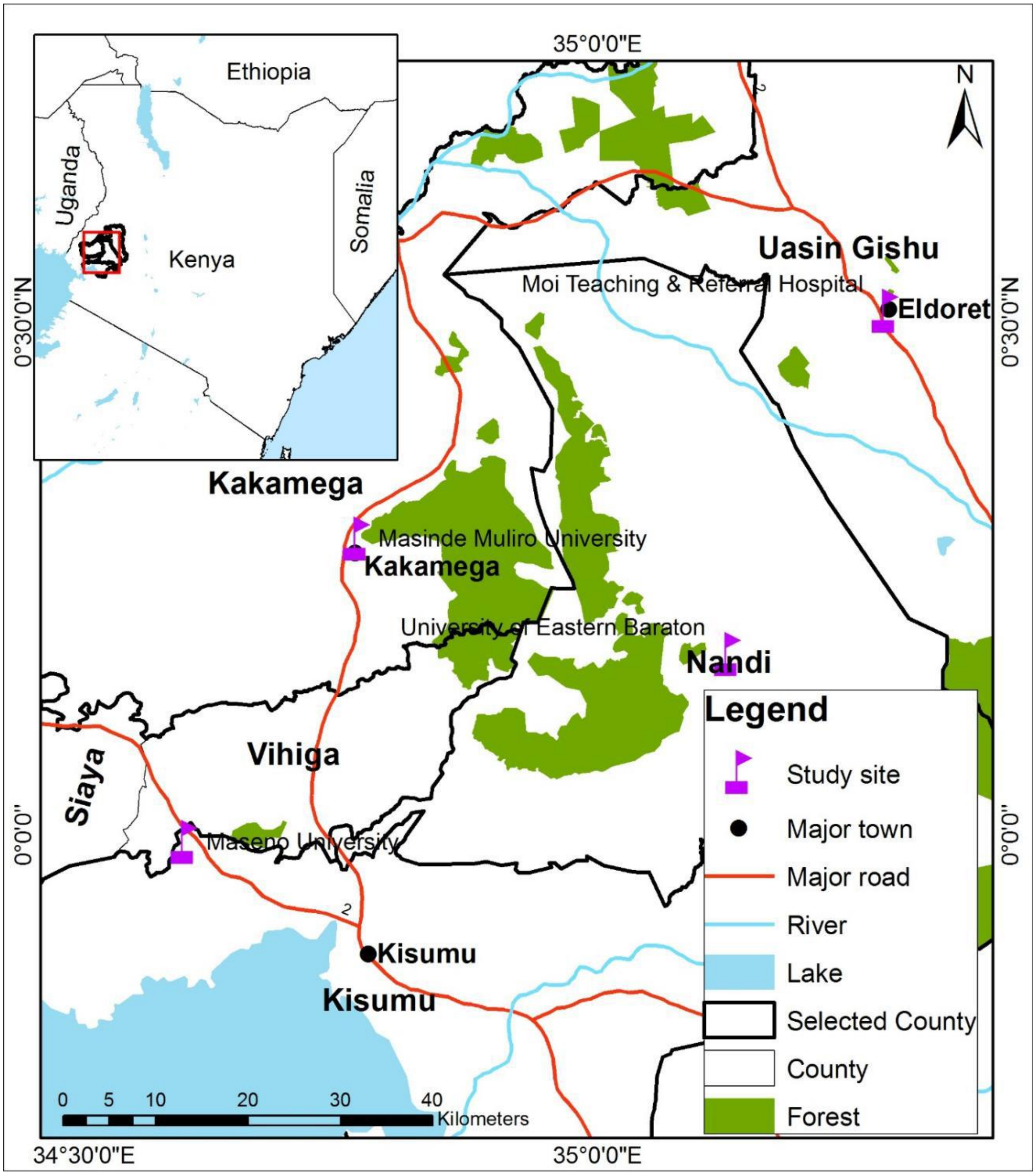

Figure 3.1: Map showing the location of the Universities and MTRH associated with the study in Kenya (Haruki, 2025).

3.3 Study Population

The experimental animals consisted of 40 female albino rats (*Rattus norvegicus*), aged between 8 to 10 weeks and weighing between 180–250 grams. These animals were initially sourced from the School of Physical and Biological Science at Maseno University, a licensed provider of laboratory animals. After being procured, the rats were carefully transported to Masinde Muliro University of Science and Technology (**MMUST**) under humane handling conditions. Once there, they were given 30 days to acclimatize, with daily care, feeding, and health monitoring to ensure they were in good condition for the study. The housing facilities at MMUST were well-suited for this purpose, offering temperature-controlled rooms, standard rodent feed (mice pencils from Unga Limited), and unlimited access to clean drinking water. The university's location was also advantageous, allowing the lead researcher to closely oversee the animals' acclimatization and general well-being. From the initial pool of 40 rats, 24 healthy, sexually mature females showing regular estrous cycles were selected for the experiments. This selection was based on thorough health checks and reproductive assessments in the animal house at the MMUST School of Medicine. (Plate 3.1). Health checks were done by observation of feeding, drinking water, movement and general body appearance. At the completion of 28 days, the animals were humanely euthanized in line with the School of Medicine's animal care guidelines. Blood samples were gathered immediately through cardiac puncture and kept under cold chain conditions while being transported to the Biochemistry Laboratory at Moi Teaching and Referral Hospital (MTRH) in Eldoret for further analysis. All procedures involving serum extraction and subsequent bioassays, including hormonal and biochemical analyses, were carried out at MTRH using standard laboratory protocols.

3.4 Experimental design and treatment protocols

A total of 24 rats selected as a sample was randomly allocated into eight groups of three, representing biological triplicates to ensure statistical robustness. The groups were structured as follows:

Group I (Negative Control): Received only distilled water.

Group IIa: Given neem leaf extract at a dose of 50 mg/kg of body weight.

Group IIb: Given neem leaf extract at a dose of 75 mg/kg of body weight.

Group IIc: Given neem leaf extract at a dose of 100 mg/kg of body weight.

Group IIIa: Received neem leaf extract at 50 mg/kg of body weight together with multivitamins.

Group IIIb: Received neem leaf extract at 75 mg/kg of body weight together with multivitamins.

Group IIIc: Received neem leaf extract at 100 mg/kg of body weight together with multivitamins.

Group IV (Positive Control): Received a standard contraceptive treatment at one pill per day per 70kg body weight.

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

3.5 Inclusion and Exclusion Criteria

The study included only healthy, non-pregnant female rats that showed regular estrous cycles. Any animals displaying signs of illness, abnormal behavior, or irregular reproductive cycles during the acclimatization period were excluded from participation.

3.6 Housing and Feeding Conditions

Each group of rats was housed separately in well labelled transparent polycarbonate cages fitted with stainless steel mesh lids, with dimensions of 45 cm × 30 cm × 20 cm. The cages were located in a temperature-controlled room set at 22 ± 2°C with 55–65% humidity and a 12-hour light/dark cycle.

The rats were fed standard mice pencils (Unga Farm Care, Kenya), formulated to meet all nutritional requirements. Each rat received 15–20 grams of feed daily. Clean tap water was available at all times. Water bottles were washed daily with mild detergent and hot water, and air-dried. Cage bedding made of sterile wood shavings was changed twice weekly, and full cage cleaning was conducted every four days.

3.7 Preparation and Administration of Treatments

Fresh Neem leaves were harvested from the University of Eldoret Arboretum and additional samples of leaves, flowers, and fruits were collected from CITAM (Christ Is The Answer Ministries) Church compound in Kisumu in January 2021 (Plate 3.2). Even though only the leaves were needed for the experiment, the other neem plant parts were collected for a thorough identification of neem plant. Taxonomic identification and verification of the plant materials were carried out with the assistance of the late Dr. B. K. Wanjohi, a renowned botanist from the University of Eldoret.

Fresh Neem leaves were shade-dried for a period of one month, finely ground in a food processor, and subjected to ethanol extraction using 96% Ethanol and Soxhlet apparatus. (LabTech, South Korea). The extract was concentrated using a rotary evaporator (Biobase, China), (Plate 3.4). After evaporation the pure neem concentrate was weighed and 12 grams of the concentrate were obtained. Treatments were administered orally using calibrated stainless steel gavage needles (Kent Scientific, USA) once daily over a 28-day period. All administrations were performed in the morning at 0900HRS to avoid diurnal hormonal variations

A. **Preparation of neem leaf extract doses**.

The following doses of pure neem leaf extract were prepared basing on an average weight of each albino rat at 250 grams:

DOSE 1

The 50mg/kg group

Calculations:

50mg * 250grams/1000grams

=12.5mg

So, this group received a dose of 12.5mg per rat per day.

DOSE 2

The 75mg/kg group

Calculations:

75mg * 250grams/1000grams

=18.75mg

This group received a dose of 18.75mg per rat per day.

DOSE 3

The 100mg/kg group

Calculations:

100 *250grams/1000grams

=25mg

This group received a dose of 25mg per rat per day.

These doses were dissolved in warm distilled water and stored singly in 1ml vials at 4°C.

B. Preparation of multivitamins doses

Multivitamins (Wellwoman®, UK) doses were prepared following the manufacturer's instruction of one pill per day for an average adult human weight of 70kg. This brand of multivitamins contains the following ingredients: Starflower oil, evening primrose oil, vitamins D3, E, C, B1, B2, B3, B6, Folic Acid, Biotin, Vitamin K, natural mixed carotenoids, Iron, zinc, Manganese, Copper, Selenium, Chromium.

The following procedure was followed:

The dosage of one pill per day per adult of average weight 70kg was followed.

Multivitamin pill:1g

Dosage: 1 pill per day

Average weight of human:70kg

Average weight of rat: 250g

The calculations:

Km(human) =37

Km (rat) =6

Conversion factor 37/6

=6.17

Human dose is 1 pill per 70kg

=0.01429pills per kg

Rat dose (tablet /kg) will be:

0.01429 *6.17=0.08881tablets per kg

Therefore, a 250g rat dose will be:

0.08881 * (250/1000)

=0.022025 tablets.

Converting the above into milligrams

1 pill used was one gram by weight (1000 mg)

So, a rat receives 0.022025 * 1000g

=22.025mg

For 28days,

22.025 * 28

5,550mg

5.5g

Which is approximately 6grams hence 6 pills.

Six tablets were crushed to make a stock solution to provide an ml of preparation per rat (252 ml).

1ml * 9rats * 28=252 ml

The stock solution was then dispensed into 252 1ml vials and refrigerated at 4 degrees Celsius. Therefore, from the above calculations, a rat received 23.8 mg of multivitamins per day.

C. Preparation of contraceptive doses (Femiplan)

The human dosage of Femiplan is one pill per person per day. The average weight of an adult human is assumed to be 70 kg.

Human dose per kg =1pill/70kg

Converting to RED (Rat Equivalent Dose) using BSA factor,

Human Km=37

Rat Km=6

RED=Human dose * 37/6

But human dose is 0.0143 pills per day

Hence, RED will be:

0.0143 * (37/6)

=0.0143 * 6.1667

=0.0882 pills per kg of a rat's body weight.

Since the average weight of rats was 250g, the dosage per rat will be:

0.0882 * (250g/1000g)

=0.022pills

The number of rats receiving this dose were 3 and the number of treatment days were 28. Therefore, the number of pills needed for this duration was going to be:

0.022pills * 3 *28 days

=1.848pills

This was estimated to be 2 pills due to the very small margin that remained to reach 2 pills. These 2 pills were to be crushed and dissolved in 42mls of warm distilled water and mixed by a vortex mixer into a homogenous solution to be dispensed into 0.5ml vials making a total of 84 vials to complete the whole treatment period. However, one pill was crushed and dissolved in 21mls of warm distilled water using a vortex mixer. Three 0.5 ml vials were used to dispense the homogenous contraceptive solution. The rest of the mixture was discarded to avoid administering hormones that were already degenerated.

The above procedure was repeated daily until the treatment period elapsed.

3.8 Data Collection Procedures

At the end of the treatment period of 28 days, the rats were subjected to an overnight fast. On day 29 at 8.00 a.m., the rats were humanely sacrificed under deep anesthesia by intramuscular administration of xylazine at the dosage of 16mg per kg of body weight and ketamine administration at 60mg per kg of body weight before sample collection Parasuraman et al (2010). An average of 9ml of blood was drawn from each albino rat via cardiac puncture. Serum was obtained through centrifugation (Bio Base BKC-TL4BII) at 3000rpm for 10 minutes. Bioassay was done at the Biochemistry laboratory at the Moi Teaching and Referral Hospital (MTRH) for hormone analysis using standard laboratory protocols. Hormone concentrations specifically follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estrogen were measured using enzyme-linked immunosorbent assay (ELISA) kits from reputable international suppliers: (Abcam (UK) according to N. Gharaghdaghi et al. (2019), Elabscience (China) according to P.Li et al (2024), and

BioAssay Systems (USA) Y Choi et al (2021). To enhance accuracy and minimize variability, each hormone measurement was carried out in triplicate.

3.9 Ethical Approval and Regulatory Compliance

Ethical qualification for this research was obtained from the Ethics Review Committee of the University of Eastern Africa, Barton. A research permit was also issued by the National Commission for Science, Technology, and Innovation (NACOSTI). All animal handling and experimental procedures complied on the ethical threshold agreed upon by the Institutional Animal Care and Use Committee (IACUC) and the guidelines of the Kenya Veterinary Board (KVB). The study partially followed the 3Rs principle Replacement, Reduction, and Refinement and all activities were carried out under the supervision of certified personnel.

3.10 Data analysis

Data was analyzed using Statgraphic Centurion software v16 (StatPoint Technologies Inc., USA). Hormone concentrations were expressed as mean + or - standard deviation. Differences in means amongst groups were evaluated by the use of one-way Analysis of Variance (ANOVA), and when substantial differences were detected, Tukey's post hoc test was employed to identify where the variations occurred. To determine the optimal dose of the neem extract–multivitamin combination, simple linear regression analysis and dose–response modeling were performed. The best-fitting dosage was estimated from pharmacodynamics regression curves, with model performance evaluated using the coefficient of determination (R^2) and other goodness-of-fit measures. All statistical tests were conducted at a 95% confidence level, and outcomes were regarded as significant at $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Introduction to the chapter

This chapter explored the results of an experiment that assessed how different doses of *Azadirachta indica* (commonly known as neem) affect reproductive hormones in female albino rats. It also examined whether adding multivitamins could help counter these effects. The discussion is structured in two main parts: the first part focuses on how varying neem dosages influenced the levels of three important reproductive hormones (FSH, LH, and estrogen). The second part delves into how multivitamin supplementation may have helped reduce or reverse the hormonal changes caused by neem exposure.

4.2 Effects of Neem Dosage on Reproductive Hormones

The levels of reproductive hormones assessed which included; FSH, LH and estrogen varied significantly across the treatment groups. The negative control group consistently recorded the highest concentrations, whereas the group receiving the highest neem dose (100 mg/kg) showed the lowest values for all hormones assessed. (Table 4.1)

4.2.1 Follicle-Stimulating Hormone (FSH)

FSH concentrations varied notably across the treatment groups. The control group recorded the highest mean FSH level (8.27±0.35 ng/mL), followed by a stepwise decline in the Neem50 (5.70±0.36 ng/mL), Neem75 (4.23±0.25 ng/mL), Neem100 (3.27 ± 0.21 ng/mL), and contraceptive (3.03±0.15 ng/mL) groups. One-way ANOVA revealed a highly significant effect of treatment on FSH levels ($F_{0.05\ (4,\ 10)} = 181.67$, $p < 0.0001$). There were statistically significant differences between the control and Neem50 groups, Neem50 and

Neem75 as well as Neem75 and Neem100. No significant difference was observed between Neem100 and the contraceptive group (Table 4.1).

4.2.2 Luteinizing Hormone (LH)

For LH, the negative control group showed the highest mean concentration (6.17±0.35 ng/mL), followed by Neem50 (4.70±0.30 ng/mL), Neem75 (3.83±0.25 ng/mL), Neem100 (2.90±0.20 ng/mL), and the positive control group (2.57±0.21 ng/mL) with a statistically significant difference among the treatment groups ($F_{0.05\,(4,\,10)} = 88.24$, $p < 0.0001$). Post hoc tests showed significant differences between each successive group: negative control versus Neem50, Neem50 versus Neem75, and Neem75 versus Neem100. No substantial difference was recorded between Neem100 and the positive control group as summarized in Table 4.1.

4.2.3 Estrogen

Estrogen levels exhibited a distinct response pattern. The highest concentrations were observed in the negative control group (52.59±2.55 pg/mL) and the positive (50.97±1.36 pg/mL) groups, which did not significantly differ. Substantially lower estrogen levels were recorded in the neem-treated groups in a dose-dependent manner: Neem50 (30.17±1.65 pg/mL), Neem75 (21.20±1.55 pg/mL), and Neem100 (13.53±1.25 pg/mL) with a significant difference ($F_{0.05\,(4,\,10)} = 306.53$, $p < 0.0001$). Post hoc analysis uncovered a substantial discrepancy between the negative control and Neem50, Neem50 and Neem75, Neem75 and Neem100. No substantial difference was found between the negative control and positive control groups as summarized in Table 4.1.

Table 4.1: Mean ± Standard Deviation (SD) of Reproductive Hormones (FSH, LH, and Estrogen) Across Treatment Groups with Post Hoc Grouping, ANOVA F-Ratios, and P-Values

Hormone	Treatment	Mean ± SD	F-Ratio	P-Value
FSH (ng/mL)	Negative 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}		
	Positive control	3.03 ± 0.15^{a}		
LH (ng/mL)	Negative 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}		
	Positive control	2.57 ± 0.21^{a}		
Oestrogen (pg/mL)	Negative 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}		
	Positive control	50.97 ± 1.36^{d}		

N.B The superscripts a, b, c, d show the differences in significance between treatment groups.

4.2.4 Correlation Analysis of Reproductive Hormones

Across all treatment groups, a clear pattern emerged showing that as FSH levels increased or decreased, LH levels followed a nearly identical trend, reflected in a very strong and statistically significant positive correlation ($r = 0.9916$, $p = 0.0009$). This suggests that the two hormones tended to move together across the various neem dosages and control groups. In contrast, the relationship between FSH and estrogen showed only a moderate positive correlation (r = 0.4619), which was not statistically significant (p = 0.4336), implying that their levels did not consistently rise or fall in tandem. A similar weak and non-significant association was found between LH and estrogen (r = 0.3720, p = 0.5375), suggesting that estrogen levels varied somewhat independently from the other two hormones across the treatments.

Table 4.2: Correlations Analysis of Reproductive Hormones

	FSH	LH	Estrogen
FSH		0.9916 (0.0009)*	0.4619 (0.4336)
LH			0.3720 (0.5375)
Estrogen			

Correlation (P-Value)

* Significantly different at 95% confidence interval

4.3 Evaluation of Multivitamin Supplementation Effects

4.3.1 Effects of Multivitamin Supplementation on Follicle-Stimulating Hormone (FSH)

The highest mean FSH level was observed in the negative control group (8.27±0.35), followed by Neem50 + MV (6.33±0.15), Neem75 + MV (6.00±0.20), Neem100 + MV (5.87±0.15) and the lowest was in the positive control group (3.03±0.15). The differences between these groups were statistically significant ($F_{0.05}$ (4, 10) = 225.29, $p < 0.0001$). Post hoc analysis revealed distinct groupings where the positive control group differed significantly from all others, and the negative control group had significantly higher FSH levels compared to neem treatments as portrayed in Table 4.3.

4.3.2 Effects of Multivitamin Supplementation on Luteinizing Hormone (LH)

LH showed the highest mean in the negative control group (6.17 ± 0.35[c]), followed by Neem50+MV (5.17±0.21), Neem100 + MV (5.13±0.15), Neem75+MV (5.03±0.15), and the lowest in the positive control group (2.57±0.21). These discrepancies were statistically substantial ($F_{0.05\ (4,\ 10)} = 104.53$, $p < 0.0001$). Post hoc comparisons indicated that the positive control group was significantly different from all others, while the neem-treated groups formed a similar cluster as illustrated in Table 4.3.

4.3.3 Effects of Multivitamin Supplementation on Estrogen

The highest mean estrogen level was recorded in the Neem100+MV group (54.33±1.17), followed by the negative control group (52.59±2.55), positive control group (50.97±1.36), Neem75+MV (48.83±1.14), and the lowest in Neem50+MV (40.17±1.38). These differences were significant ($F_{0.05\ (4,\ 10)} = 35.58$, $p < 0.0001$). Post hoc analysis showed multiple distinct groupings, with Neem50+MV significantly lower than all others, Neem100+MV significantly higher, and intermediate groups showing varying degrees of overlap as shown in Table 4.3.

Table 4.3: Effects of Multivitamin Supplementation on FSH, LH and Oestrogen Hormone Levels across Treatment Groups

Hormone	Treatment	Count	Mean ± SD	F-Ratio	P-Value
FSH (ng/mL)	Negative control	3	8.27±0.35[d]	225.29	0.0000
	Neem50+MV	3	6.33±0.15[c]		
	Neem75+ MV	3	6.00±0.20[bc]		
	Neem100+MV	3	5.87±0.15[b]		
	Positive control	3	3.03±0.15[a]		
LH (ng/mL)	Negative 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]		
	Positive control	3	2.57±0.21[a]		
Oestrogen (pg/mL)	Negative control	3	52.59±2.55[cd]	35.58	0.0000
	Neem50+MV	3	40.17±1.38bc		
	Neem75+MV	3	48.83±1.14[b]		
	Neem100+MV	3	54.33±1.17[d]		
	Positive control	3	50.97±1.36[bc]		

4.3.4 Correlation Analysis of FSH, LH and Oestrogen Hormones

FSH revealed a moderate positive correlation with estrogen ($r = 0.504$, $p = 0.0280$), while LH and estrogen were similarly related ($r = 0.483$, $p = 0.0360$) as illustrated in Table (4.4).

There was a strong and statistically substantial correlation between FSH and LH levels ($r = 0.981$, $p < 0.0001$).

Table 4.4: **Pearson Correlation Coefficients Among FSH, LH, and Estrogen Levels Following Multivitamin Supplementation**

Variables	FSH	LH	Estrogen
FSH		0.981 (0.0001)*	0.504 (0.0280)*
LH			0.483 (0.0360)*
Estrogen			

Correlation (P-Value)

* Significantly different at 95% confidence interval

4.4 Chapter summary

In summary**,** this chapter highlights how higher doses of neem led to a noticeable drop in key reproductive hormones (FSH, LH, and estrogen) in albino rats. Interestingly, the highest neem dose had effects similar to those of a standard contraceptive. When multivitamins were added to the treatment, they appeared to ease some of neem's suppressive effects, especially by helping to restore estrogen levels. These results suggest that while neem can interfere with hormonal balance in a dose-dependent way, multivitamin supplementation might help alleviate that impact.

CHAPTER FIVE

DISCUSSION

5.1 Introduction to the chapter

This chapter explores how different doses of *Azadirachta indica* (neem) affect reproductive hormones in female albino rats, and examines whether multivitamin supplementation can reduce these effects. The results are discussed in relation to hormonal interactions and compared with existing studies, including those on synthetic contraceptives.

5.2 Effects of Neem Dosage on Reproductive Hormones

5.2.1 Effects of neem dosages on Follicle-Stimulating Hormone (FSH)

In this study, the control group was recorded to have the highest FSH levels which indicated normal ovarian activity and healthy follicular stimulation. Such hormone levels are consistent with a stable reproductive cycle characterized by regular follicle recruitment and maturation as noted by Roop, Dhaliwal, & Guraya, (2005) and Chou & Chen (2018) studies. Increasing doses of neem extract produced a clear dose-dependent decline in FSH, suggesting progressive disruption of gonadotropin-releasing hormone (GnRH) signaling. This aligns with earlier findings by Kulkarni (2020) and Kiplagat (2020), who in their studies reported that phytochemicals in neem extracts may either suppress hypothalamic GnRH secretion or act directly on pituitary gonadotropes. They also added that compounds such as nimbolide and azadirachtin are thought to be key mediators of this neuroendocrine effect in animal reproduction.

Notably in this research, the group treated with synthetic contraceptives displayed FSH levels compared to those in the high-dose neem group. This pattern suggests that higher concentrations of neem may mimic the gonadotropic suppression achieved by hormonal contraceptives, which prevent ovulation by lowering FSH and LH levels with support from studies by Ravichandran et al. (2009) and Patel, Jacob & Thomas (2024). These findings not only reinforce neem's hormonal action mediated by nimbolide, azadirachtin, and other bioactive constituents but also highlight its capabilities as a natural fertility-regulating agent with mechanisms resembling those of conventional pharmaceutical interventions commonly used.

5.2.2Effects neem dosages on Luteinizing Hormone (LH)

Luteinizing hormone (LH) in this study exhibited a trend similar to that of Follicle-Stimulating Hormone (FSH). The control group recorded the highest LH concentrations, whereas both the high-dose neem group and the contraceptive group revealed general marked reductions. The stepwise decline in LH levels among the neem-treated groups suggests a progressive disruption of the hormonal signals required for ovulation. Since the LH surge is critical for the breakage of the mature follicle and the launch of the ovum, its suppression in this case directly compromises fertility as supported by Dozortsev & Diamond (2020). The nearly identical degree of LH suppression in the high-dose neem and contraceptive groups highlights neem extract's ability to downregulate specifically hypothalamic and pituitary functions. These findings align with the observations of Moore (2012), who reported that neem extract can interfere with neurotransmitter pathways and hormonal feedback loops that regulate pulsatile GnRH secretion, ultimately diminishing the LH surge in the body. Taken together, the results suggest that neem extract particularly at higher doses may act as a plant-based agent capable of modulating reproductive cycles by targeting upstream endocrine pathways in a manner comparable to synthetic contraceptives available.

5.2.3 Effects of neem dosages on Oestrogen

In this research study, the estrogen levels showed a more intricate pattern than the gonadotropins. As expected, the control group maintained elevated estrogen levels concentrations, reflecting active ovarian steroid production and healthy follicular development in normal adult albino rats. Conversely, in the neem extracts-treated groups, oestrogen levels declined in a dose-responsive fashion indicating that neem's action extends beyond central hormonal regulation to include peripheral disruption of ovarian function. Phytochemicals in neem extracts may inhibit aromatase, the enzyme that converts androgens to oestrogens or directly damage granulosa cells responsible for oestrogen synthesis as reported by Patil *et al*. (2021). Evidence from study by Al-awadhi *et al*. (2024), suggests that high concentrations of neem extracts lead to follicular atresia, degeneration of the corpus luteum and disrupted steroidogenic enzyme activity, all of which culminate in reduced oestrogen output.

In contrast, the relatively high oestrogen levels observed in the contraceptive group are likely attributable to exogenous oestrogens present in the formulation, which are included to stabilize the endometrium and prevent breakthrough bleeding (Verma, Cwiak & Kaunitz, 2021). Unlike synthetic contraceptives, which maintain peripheral oestrogen levels, neem appears to shut down both the upstream (GnRH and gonadotropins) and downstream (ovarian) hormonal synthesis pathways.

5.2.4 Hormonal Interrelationships

The strong positive link between FSH and LH across all groups was not surprising, since both are regulated by the same hormone GnRH from the hypothalamus (Blake & Norman, 2020). When neem disrupts GnRH signaling, it triggers a simultaneous drop in both FSH and LH in the body. The contraceptive group displayed a similar trend, reinforcing the idea that neem extracts and synthetic contraceptives locally available both act at the brain level to suppress reproductive hormones. However, estrogen did not mirror this close relationship in this research. In neem-treated rats, estrogen levels showed only a weak correlation with FSH and LH, suggesting that neem may also act directly on the ovaries rather than relying solely on central hormonal suppression as reported by Tran and Hinds (2013).

In contrast, the relatively steady estrogen levels observed in this study in the contraceptive group were likely maintained by the external hormones they received rather than by natural ovarian production. (ESHRE Capri Workshop Group 2001). This divergence between estrogen and pituitary hormone patterns in the neem-treated groups points to a more complex mechanism that happens in the body. While synthetic contraceptives primarily exert their effects through central regulation, neem appears to influence both the brain's hormonal control and ovarian function directly.

5.3 Evaluation of Multivitamin Supplementation Effects

5.3.1 Follicle-Stimulating Hormone (FSH)

In this investigation, animals in the control group had the highest FSH levels, which points to healthy ovarian activity and normal stimulation of follicle development. This likely reflects an intact hypothalamic-pituitary-ovarian (HPO) axis functioning without interference. In contrast, the groups that received neem extract alongside multivitamin supplementation showed moderately reduced FSH levels, and the group on synthetic contraceptives had the lowest. This suggests that neem may suppress FSH production, but the inclusion of multivitamins may help cushion that effect to some extent.

This protective influence might be explained by the role of vitamins in supporting hormonal balance through antioxidant and anti-inflammatory pathways. Some vitamins, such as Vitamin E and Vitamin B-complex, are known to stabilize endocrine function and reduce oxidative stress in reproductive tissues. Findings by Nagy *et al*. (2024) support this view, showing that natural supplements combined with micronutrients help counteract reproductive suppression. Likewise, Njoga *et al*. (2022) observed improved hormonal profiles in animals treated with phytochemicals when vitamin supplementation was introduced.

5.3.2 Luteinizing Hormone (LH)

Similarly, LH levels were highest in the control group and declined progressively across the neem-multivitamin groups, with the lowest levels seen in the contraceptive group. While neem appears to interfere with LH production potentially disrupting ovulation the addition of multivitamins seems to have offered some hormonal support.

Multivitamins may help regulate LH by preserving hypothalamic signals or maintaining pituitary sensitivity. This is in alignment with a study by Diaz-Thomas *et al*. (2023), who found that vitamin D supplementation improved LH levels and overall hormonal regulation in individuals with fertility issues. Another study by Gullo *et al*. (2025), noted that women taking multivitamins showed more stable LH levels, especially under physiological stress.

5.3.3 Estrogen Levels

Interestingly, estrogen levels were highest in the group that got the highest neem dose with multivitamins (Neem100+MV), even surpassing the control group. On the other hand, the group receiving the lowest neem dose with vitamins (Neem50+MV) had the lowest estrogen levels. This pattern suggests that the relationship between neem, vitamins, and estrogen production may not be linear. It is possible that higher doses of neem, when buffered by vitamins, stimulate a kind of hormonal rebound or even enhance aromatase activity, leading to elevated estrogen production.

This observation fits with earlier findings that neem contains compounds with mild estrogenic properties, which could interact with vitamin-based metabolic support to stimulate hormone synthesis. Beck *et al*. (2003) discussed how certain plant extracts might enhance or inhibit estrogen depending on the dose and combination with other nutrients. Similarly, Nagy *et al*. (2024) reported that vitamin-treated rats showed diverse estrogenic responses depending on the botanical extract administered.

5.3.4 Hormonal Correlations

There existed a clear and strong positive association between FSH and LH, while both hormones also showed moderate positive links with estrogen. This implies that the treatments, despite altering hormone levels, did not fully disrupt the functional relationships within the reproductive hormone system. In other words, the hormones still seemed to rise and fall together in expected patterns, indicating that the HPO axis remained responsive.

Such interlinked hormone activity is typical in healthy reproductive function. It confirms that the body still tried to maintain hormonal feedback loops even under neem exposure.

Rahman *et al*. (2023) observed similar trends, where natural treatments did not prevent hormonal cross-talk even when hormone levels themselves were altered.

5.4 Summary of the chapter

Neem extract showed a dose-dependent suppression of FSH, LH, and estrogen, indicating interference with both brain and ovarian hormonal regulation. At high doses, its effects resembled those of synthetic contraceptives. Multivitamin supplementation helped moderate some of these hormonal changes, suggesting a protective role. These results support neem's potential as a natural contraceptive and highlight the influence of nutritional support on reproductive hormones.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This research set out to investigate how *Azadirachta indica* (neem) affects key reproductive hormones namely FSH, LH, and estrogen in female albino rats, while also exploring whether multivitamin supplementation could help mitigate any adverse effects.

The findings in this research study revealed a clear dose-response pattern: as neem intake increased, levels of both Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) fell sharply, with the highest dose of 100 mg/kg producing the strongest suppression. Estrogen levels also dropped in neem-treated groups, but remained higher in the synthetic contraceptive treated group, hinting that neem and pharmaceutical contraceptives may disrupt reproduction through different pathways.

Encouragingly, multivitamin supplementation appeared to ameliorate these hormonal disturbances in this research study. Estrogen levels, in particular, improved when multivitamins were given alongside neem and in the highest supplemented group, estrogen even exceeded levels seen in untreated controls.

6.2 Recommendations

1.Given the significant hormonal effects observed, caution is advised when using neem, particularly in contexts where fertility is a concern. While its ability to suppress reproductive hormones could be harnessed for contraceptive purposes, such applications should be approached with rigorous safety testing and ethical consideration.

2.The promising results from the multivitamin-supplemented groups highlight the need for further research into how nutritional interventions might counteract or reverse the reproductive side effects of herbal or synthetic agents. Future studies should aim to identify which specific vitamins or minerals are most beneficial for hormonal recovery.

3.Lastly, future research should extend beyond short-term hormonal assessments. Larger sample sizes, longer treatment durations, and additional parameters such as sperm quality, ovarian health, and tissue histology are essential for building a complete picture of neem’s impact on reproductive health and the potential of multivitamins to offer protection or recovery.

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APPENDICES

Appendix I: Plates showing photos taken during data collection

Plate 2.1: ***Azadirachta indica*** **showing its leaves and immature fruits (Author, 2025)**

Plate 2.4: ***Rattus norvegicus*** **(Albino rat)** ***(Author, 2025)***

Plate 3.1: Albino rats being received at the school of medicine animal house at MMUST.

Plate 3.2: Fresh Neem tree at CITAM (Christ Is The Answer Ministries) Church compound in Kisumu in January 2021

Plate 3.3: Sieving finely ground neem plant leaves

Plate 3.4: Ethanol extraction of neem plant extracts

Plate 3.5: Dispensing neem doses into vials

Appendix II: NACOSTI Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **868997**

Date of Issue: **26/October/2023**

RESEARCH LICENSE

This is to Certify that Ms.. Zainab Minage of University of Eldoret, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nandi on the topic: EFFECTS OF MULTI-VITAMIN ON NEEM TREATED LABARATORY RATS for the period ending : 26/October/2024.

License No: **NACOSTI/P/23/28914**

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Applicant Identification Number

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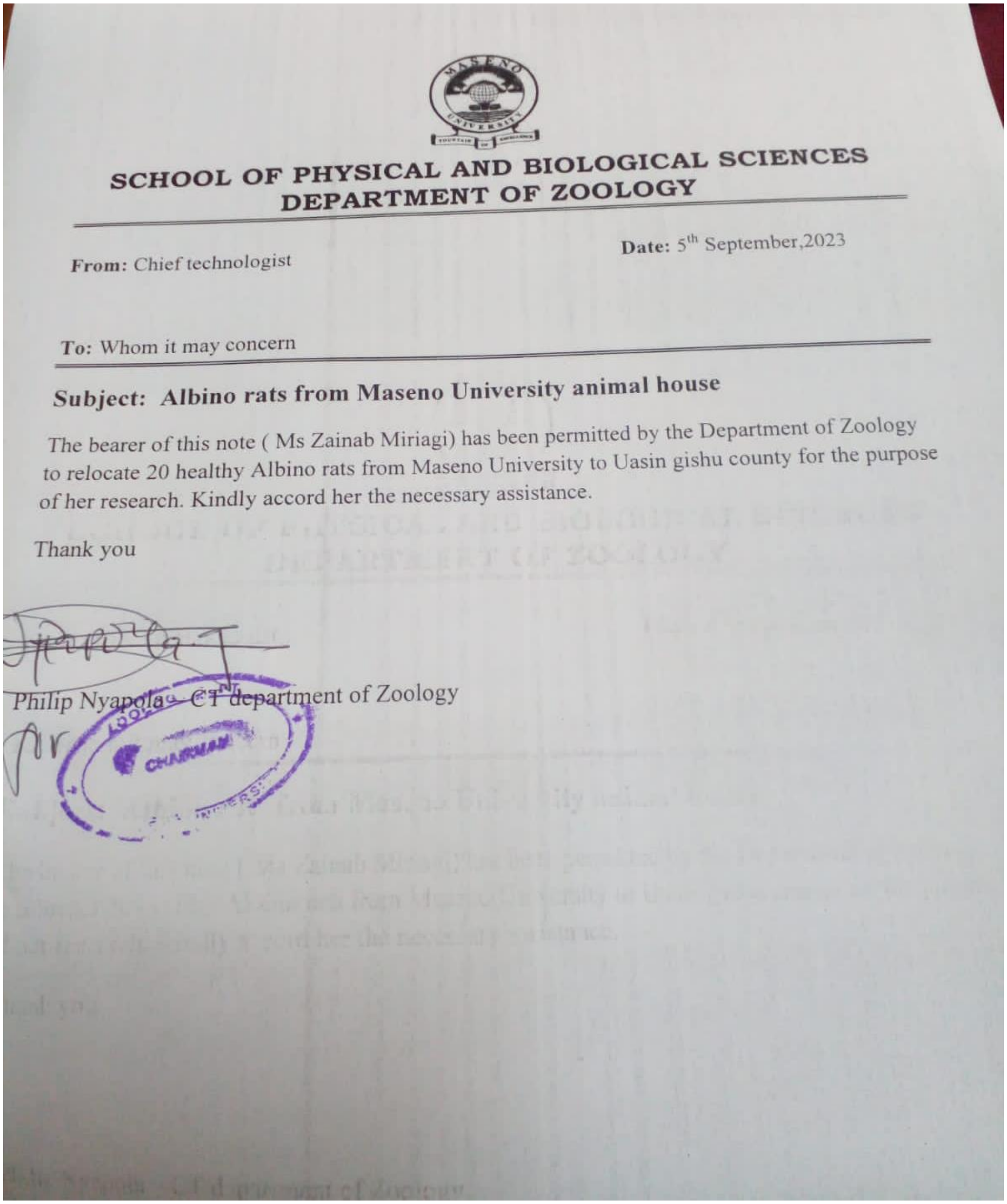
See overleaf for conditions

Appendix III: Authorization letter for albino rats acquisition from Maseno university



SCHOOL OF PHYSICAL AND BIOLOGICAL SCIENCES
DEPARTMENT OF ZOOLOGY

From: Chief technologist

Date: 5th September,2023

To: Whom it may concern

Subject: Albino rats from Maseno University animal house

The bearer of this note (Ms Zainab Miriagi) has been permitted by the Department of Zoology to relocate 20 healthy Albino rats from Maseno University to Uasin gishu county for the purpose of her research. Kindly accord her the necessary assistance.

Thank you

Philip Nyapola – CT department of Zoology

Appendix IV: Authorization letter for albino rat rearing at MMUST

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Technology for Development

INTERNAL MEMO

From: Deputy Vice Chancellor **Date:** 26th June 2024

To: Dean, School of Medicine **Ref:**MMU/COR:311136 (25)

RE: REQUEST TO USE ANIMAL HOUSE BY UNIVERSITY OF ELDORET PHD STUDENT

Reference is made to your memo dated 28th May 2024 forwarding the request of Ms. Zainab Minage to use the University's animal house for her PhD studies.

The animal house being a shared facility among the three schools in the health sciences field, approval has been given for the student to co-share the facility with our internal staff and students.

Kindly inform the student to liaise with the Technician in charge of the animal house to schedule her accordingly.

Prof. J. Kuria Thuo
DEPUTY VICE CHANCELLOR (A&F)

Noted
Student to be informed.
28/06/2024

Appendix V: Similarity Report

University of Eldoret

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Certificate of Plagiarism Check for Thesis

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Similarity	6%
Paper ID	4609351
Total Pages	86
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Signature of Guide

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