AKENTEN APPIAH- MENKA UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT

EFFECTS OF VARYING DOSES OF NEEM SEED OIL ON BODY WEIGHT, HAEMATOLOGY, SERUM BIOCHEMISTRY, AND HISTOLOGY OF LIVER AND KIDNEY IN WISTAR RATS

STEPHEN ADJEI

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BY

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JANURARY, 2025

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Stephen Adjei

Signature: ……. Date: …….

Supervisor's Declaration

I hereby declare that the preparation and presentation of this work was supervised in accordance with the guidelines for supervision of thesis as laid down by the Akenten Appiah- Menka University of Skills Training and Entrepreneurial Development.

Supervisor's Name: Dr. Duodu Addison

Signature: …… Date: …….

ABSTRACT

Neem seed oil, rich in bioactive compounds like azadirachtin, exhibits pharmacological and toxicological effects, with dose-dependent impacts on vital detoxification organs, raising safety concerns for medicinal use. This study evaluated the effects of varying doses of neem seed oil on haematological parameters, liver histology, kidney function, and body weight in Wistar rats. A completely randomized experimental design was employed using 24 female Wistar rats housed under controlled conditions. Neem seeds were collected locally, and oil was extracted via a manual hydraulic press. After a two-week acclimatization, rats were randomized into four groups receiving saline (control) or neem seed oil at low, medium, and high doses via oral gavage for 21 days. Weekly body weights, blood samples, and organ weights were assessed. Hematological, biochemical, and histopathological analyses were performed. Data were analyzed using a two-sample t-test with significance at $p < 0.05$. Low-dose neem oil did not significantly alter hematological parameters, while medium-dose neem oil significantly increased lymphocyte count (LYH) from 2.14 to 5.06×10^9/L ($p = 0.046$), and high-dose neem oil significantly elevated mean platelet volume (MPV) from 6.63 to 7.17 fL ($p = 0.048$). In terms of liver biochemistry, low-dose neem oil significantly increased alanine aminotransferase (ALT) levels from 197.1 to 247.9 U/L ($p = 0.045$), and medium-dose neem oil significantly elevated alkaline phosphatase (ALP) levels from 125.6 to 281.1 U/L ($p = 0.036$). High-dose neem oil had no significant effects on liver biochemical parameters. Neem seed oil showed dose-dependent effects in Wistar rats, with medium doses boosting lymphocyte counts and high doses increasing pro-inflammatory markers. Low and medium doses caused mild liver stress,

while high doses showed minimal impact, suggesting adaptive mechanisms. Kidney function was unaffected, but high doses induced tissue changes, emphasizing dose caution. Recommendations include human studies to confirm findings, further toxicity profiling, exploring weight-reduction mechanisms, and developing innovative approaches for neem oil pharmacological research.

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DEDICATION

I dedicate this study to my brother and special friends for their immense support.

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ABBREVIATIONS

ABBREVIATION		MEANING
PCV	-	Packed Cell Volume
RBC	-	Red Blood Cells
MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
AST	-	Aspartate Aminotransferase
ALT	-	alanine aminotransferase
ALP	-	alkaline phosphatase
TP	-	total protein
GLOB	-	globulin
ALB	-	albumin
LD50	-	Median Lethal Dose
EDTA	-	Ethylenediaminetetraacetic Acid
NaOH	-	Sodium Hydroxide
ANOVA	-	Analysis of Variance
CCl4	-	carbon tetrachloride
CD4 & CD8	-	Glycoprotein
MON	-	monocytes
NEU	-	neutrophils

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Health, as defined by the World Health Organization (WHO), encompasses complete physical, mental, and social well-being and not merely the absence of disease or infirmity (Kalidhasan et al., 2020). This holistic view highlights the importance of maintaining bodily functions and preventing diseases to achieve optimal health. Among traditional remedies, *Azadirachta indica*, commonly known as neem, has gained prominence due to its extensive medicinal properties (Islas et al., 2020). Historically referred to as "Nimba" in Sanskrit, neem has been integral to traditional medicine systems across Asia and Africa (Winterbottom, 2021). Neem's leaves, bark, fruit, flowers, oil, and gum have been utilized to manage conditions such as diabetes, cardiovascular diseases, cancer, and hypertension through mechanisms involving antioxidant activity, cellular maintenance, and immune modulation (Islas et al., 2020; Khadija, 2021). Despite its established therapeutic value, the need to scientifically assess neem's safety and efficacy, particularly neem seed oil, in terms of biochemical and histological parameters remains largely unexplored.

Neem seed oil, extracted from the seeds and fruits of the neem tree, has long been valued in traditional medicine for its broad-spectrum antibacterial, antiviral, antifungal, and anti-inflammatory properties (Wasim et al., 2023; Wylie & Merrell, 2022). Beyond its therapeutic benefits, neem seed oil finds applications in industrial products, including skincare formulations and contraceptive methods, due to its spermicidal properties

(Khanam et al., 2017). Recent studies highlight neem oil's rich bioactive components as the basis for its medicinal properties (Ni Putu Ratna et al., 2024; Nwanekezie et al., 2023). However, concerns about its safety at various dosages have emerged, necessitating research on its potential toxicological effects on vital organs such as the liver and kidneys. The liver, as a detoxifying organ, and the kidneys, responsible for maintaining homeostasis, are especially vulnerable to damage from bioactive compounds (Abou Seif & sciences, 2016; Kieffer et al., 2016). Understanding the dose-dependent effects of neem oil on these organs could inform its safe use as a medicinal supplement.

Herbal medicines are a cornerstone of healthcare in resource-limited settings, where conventional treatments are often inaccessible (Bebell & Muiru, 2014). Traditional herbal remedies are preferred in many African communities due to their cultural acceptance, affordability, and historical usage (Okaiyeto et al., 2021). However, the assumption that herbal remedies are inherently safe has been challenged by reports of adverse effects, including liver and kidney damage, at high doses (Mensah et al., 2019). Neem oil poisoning has been documented to cause serious health complications such as seizures, metabolic acidosis, and renal failure (Martin, 2018). Furthermore, experimental studies on animal models reveal alterations in serum biochemical markers, suggesting hepatotoxicity and nephrotoxicity at certain dosages (Arfat et al., 2014). These findings highlight the importance of determining a safe therapeutic window for neem seed oil use, as overdose and misuse may have deleterious effects on human health.

The increasing global interest in neem as a natural remedy underscores the necessity for comprehensive studies to establish its safety profile (Nwanekezie et al., 2023). While neem's medicinal properties are well-documented, significant gaps remain in understanding its long-term effects and dosage thresholds. Previous research has primarily focused on neem's therapeutic benefits, with limited emphasis on its potential toxicity and histopathological effects on vital organs. This study seeks to address these gaps by systematically evaluating the effects of varying doses of neem seed oil on body weight, haematological parameters, serum biochemistry, and histological changes in the liver and kidneys of Wistar rats. By bridging the knowledge gap, this research aims to contribute to the safe application of neem oil in both traditional and modern healthcare practices.

1.2 Problem Statement

Neem seed oil (NSO) is widely regarded for its therapeutic and industrial applications due to its bioactive compounds (Khanam et al., 2017). It is traditionally used in managing various ailments, including bacterial, viral, and fungal infections (Brai et al., 2024). However, recent studies suggest potential risks associated with excessive consumption or application of NSO, including toxicity to vital organs such as the liver and kidneys (Martin, 2018). Clinical reports have documented adverse effects such as encephalopathy, seizures, and renal dysfunction linked to NSO poisoning (Kumar et al., 2016). Animal studies further indicate alterations in haematological and biochemical markers at high doses, emphasizing its toxic potential. Despite these findings, there is limited data on the safe dosage thresholds for NSO and the mechanisms underlying its effects on physiological systems. The absence of established dosage guidelines and evidence of dose-dependent toxicity raises concerns

about its safety as a medicinal product. This study seeks to address these gaps by evaluating the effects of varying doses of NSO on growth performance, haematology, serum biochemistry, and histological changes in the liver and kidneys of Wistar rats.

1.3 Significance of the Study

This study provides vital insights into the dose-dependent effects of neem seed oil (NSO) on growth, haematology, serum biochemistry, and organ histology, addressing critical gaps in its safety profile. Researchers and healthcare practitioners will benefit from evidence-based data to guide the safe use of NSO in traditional and modern medicine. Policymakers in health and agriculture will gain valuable information to regulate herbal product use and ensure public safety.

Regionally, this study supports the African Union's Agenda 2063 goals of promoting indigenous knowledge and improving healthcare access through safe, affordable remedies. Globally, it aligns with the World Health Organization's (WHO) Traditional Medicine Strategy, which emphasizes evidence-based use of herbal medicines.

The general public, particularly in rural areas reliant on traditional remedies, will benefit from heightened awareness of the potential risks of improper NSO use. Ultimately, the study contributes to safer applications of NSO, promoting health while preventing organ damage and other adverse effects.

1.4 Objectives

1.4.1 Main Objective

The main objective of this study is to evaluate the effects of varying doses of neem seed oil on the body weight, liver and kidney functions, haematological parameters, and organ histology of Wistar rats.

1.4.2 Specific Objectives

1. To assess the effects of varying doses of neem seed oil on the growth performance of Wistar rats.
2. To evaluate the impact of neem seed oil doses on the haematological parameters of Wistar rats.
3. To analyze the effects of neem seed oil doses on the serum biochemistry of Wistar rats.
4. To examine the histological changes in the liver and kidneys of Wistar rats administered different doses of neem seed oil.

1.5 Research Questions

1. What effects do varying doses of neem seed oil have on the growth performance of Wistar rats?
2. How do different doses of neem seed oil affect the haematological parameters of Wistar rats?
3. What are the effects of varying doses of neem seed oil on the serum biochemistry of Wistar rats?

4. What histological changes occur in the liver and kidneys of Wistar rats administered different doses of neem seed oil?

1.6 Research Hypothesis

It is hypothesized that the administration of high doses of neem seed oil (1.5 mg/kg body weight) negatively impacts the growth, haematology, serum biochemistry, and organ histology of Wistar rats (Njoroge, 2012; Patil et al., 2022).

1.7 Justification

In many low-income settings, herbal remedies are often the only accessible form of healthcare (Kim et al., 2020). The neem tree, with its rich history of medicinal use, is a cornerstone of traditional medicine in such communities (Ahmed, 2023). Neem seed oil, a versatile product derived from the tree, is widely used for its therapeutic benefits. However, the lack of standardized dosage guidelines poses a significant public health risk, as overdoses can result in severe toxicological outcomes (Ijinu et al., 2024).

This study is crucial in addressing the knowledge gap regarding the safety and efficacy of neem seed oil at various dosages. By systematically evaluating its effects on growth, haematology, serum biochemistry, and organ histology, this research will provide empirical data to inform dosage recommendations. The findings will contribute to the broader understanding of neem oil pharmacology, ensuring its safe use in traditional medicine and potentially guiding its application in modern therapeutic practices.

1.8 Scope of the Study

This study evaluates the effects of varying doses of neem seed oil (NSO) on the growth performance, haematology, serum biochemistry, and histology of the liver and kidneys in Wistar rats. It investigates dose-dependent impacts using biochemical and histological markers, focusing on safety thresholds and physiological responses. The findings provide insights into NSO's medicinal use and safety, particularly its implications for liver and kidney health.

1.9 Thesis Organization

The study is divided into six main chapters. The first chapter addresses the background of the study, the problem statement, significance of the study, objectives, hypothesis for the study, justification, scope and organization of the study. In the second chapter, relevant literature related to this research topic was thoroughly examined. Chapter three focuses on presenting the study area and the methodology employed to conduct the research. Moving on to chapter four, the study data is presented. Chapter five discussed the findings of the study. Lastly, in chapter six, the summary of the results is presented, along with drawn conclusions based on the main findings and offering recommendations based on the study's outcomes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Geographical Distribution of Azadirachta indica A. Juss.

Azadirachta indica, commonly known as neem, is a versatile, fast-growing, and resilient tropical tree belonging to the Meliaceae family. Often referred to as the "nim tree" or "margosa tree" in English, neem has been widely studied for its economic, ecological, and medicinal significance (Khanwale, 2018). The species is believed to have originated from the Indian subcontinent and regions of Senegal (Chowdhary & Singh, 2009; Kumar & Navaratnam, 2013). It is now naturalized in over 72 countries, spanning continents such as Asia, Africa, Oceania, and the Americas (Koul & Millennium, 2007). This broad distribution accentuates its adaptability to various climatic and soil conditions.

Neem's introduction to Africa, particularly Ghana, dates back to 1915 when it was first cultivated in the country (Nanang, 1996). Since then, its prevalence has grown significantly, particularly in semi-arid regions where its drought-resistant nature allows it to thrive despite low annual rainfall as minimal as 130 mm (Tomar et al., 2008). The tree's ability to adapt to nutrient-deficient and acidic soils makes it particularly valuable in Northern Ghana, where it is extensively used for afforestation and land restoration (Nanang, 1996). This aligns with its recognized role in ecological sustainability and combating desertification globally (Tomar et al., 2008).

Neem's geographic spread is attributed to its exceptional ability to tolerate diverse environmental conditions. The tree grows in regions with annual rainfall ranging from 450 mm to 1,200 mm and can survive in areas receiving as little as 150 mm of precipitation (Chowdhary & Singh, 2009). Its optimal growth occurs at altitudes of up to 1,500 meters and temperatures ranging from 0°C to 49°C (Chowdhary & Singh, 2009). However, it is intolerant of waterlogged soils and regions with poor drainage, as standing water significantly hampers its growth (Council et al., 1992).

Despite neem's extensive distribution and ecological benefits, research on its adaptability to specific microclimates and its role in improving soil health in degraded lands remains limited. For example, while neem's calcium-mining properties to counteract soil acidity are acknowledged (Dare, 2022), empirical studies quantifying its long-term impact on soil restoration in Ghana are scarce. Furthermore, limited attention has been paid to its genetic diversity and potential for breeding programs in African contexts compared to South Asia. Addressing these gaps could inform more effective conservation and utilization strategies for neem in resource-limited settings.

The literature reflects some disparities regarding neem's origin. While (Biswas et al., 2002) emphasize its Indian roots, (Chowdhary & Singh, 2009) highlights its potential African origin, particularly Senegal. This discrepancy suggests the need for genetic studies to trace its evolutionary lineage. Additionally, while neem's drought resistance and adaptability to adverse conditions are widely agreed upon (Muriithi, 2023), there is less consensus on its ability to thrive in high-salinity soils.

Neem's historical introduction to Ghana has positioned it as a critical resource for ecological and agricultural sustainability. For example, its widespread use in afforestation projects in semi-arid regions addresses land degradation challenges (Nanang et al., 1997). Additionally, its cultural and economic significance extends beyond its ecological roles, as it is widely utilized for traditional medicine, pest control, and agroforestry (Biswas et al., 2002). However, comprehensive studies examining the socioeconomic impacts of neem cultivation in Ghana, including its role in livelihoods and biodiversity conservation, are lacking.

2.2 Etymology and Botanical Description of Neem

The Persian word "azadirachta indica" is the source of the Latinized name. Together, the words "free" (Azad) and "tree" (dirakht) and "Indian origin" (i-Hind) form the phrase "the free tree of India." Two species of Azadirachta Indica have been reported viz. *Indica Azadirachta* Native to the Indian subcontinent is A. Juss, whereas the Philippines and Indonesia are the homelands of Azadirachta excels kack. According to (Kuravadi & Gowda, 2019), neem belongs to the mahogany family. The following is the taxonomic taxonomy of neem, as described by De Jussieuin in 1830 (Asnakew, 2024).

Table 2.1: Taxonomic position of Neem

Kingdom	Plantae
Order	Rutales
Suborder	Retinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	Azadirachta
Species	Indica

Figure 2.1: Neem plant adapted from (Andersa et al., 2024)

Figure 2.2 Images of the Neem products

(A) Twigs, (B) Leaves, (c) Fruits (D) Seeds (with endocarp) (E) Seeds (without endocarp)

Table 2.2: Biological Activity and Functions of Neem

Biological Activity	Function	References
Anti-fungal	Work against *Candida, Microsporum, Trichophyton, Geotrichum, Epidermophyton, Trichosporon*	Sitara *et al.*,2008;
Anti-bacterial	It inhibits the activity of bacteria such as *Salmonella typhi*, *Staphylococcus aureus Streptococcus mutants*,*M. tuberculosis, Vibrio Cholerae, Pneumoniae. M. pyogenes and Klebsiell*	Pandey *et al.* 2014; Aslam *et al.*, 2009; and Mohmoud *et al.*, 2011.
Anti-viral	Treatment of fowl pox, smallpox, chicken pox, Vaccinia virus, warts, moderate inhibition hepatitis B virus, Chikungunya, herpes virus, and measles virus.	Faccin *et al.*, of 2012,
Insecticidal	Effective against maggots, horn flies, Headlice, blowflies and biting flies	Tiwiri *etal.*, 2010; Schmutterer *et al.*, 1990
Promotes Oral Health	Neem twigs are used as toothbrushes averting periodontal diseases and gum inflammations	Singh & Purohit 2013
Treatment of arthritis,	Chagas disease Ailments (kissingbugs that transmit the parasites)	Kumar& Navaratnam 2013
Treatment of Malaria,	fever,pain, burning sensations, ringworm, respiratory, disorders eczema, intestinal helminthiasis rheumatism and constipation	Patel *et al.*, 2016
Immunostimulant Activates	Cell-mediated immune pathways toprovoke an enhanced response tosubsequent mitogenic or antigenic encounter.	Upadhyay *et al.*, 1992
Anti-diabetic	Reduces blood sugar level and precludes adrenaline and glucose-inducedhyperglycaemia	Patil *et al.*, 2013
Anti-ulcer	Produces highly potent antiulcer activity	Rair *et al.*, 2013

Anti-fertility	Avoids pregnancy and could be used as a way of contraception	Garg *et al*, 1991
Anti-cancer	Inhibit cell carcinoma in oral mucosaby modulation of glutathione and its metabolizing enzymes	Paul *et al.,* 2011
Anti-oxidant	Eliminates toxins, filter blood, and inhibit damage caused due to free radicals in the body	Nahak *et al.*, 2010

2.3 Bioactive Compounds Present in *Azadirachta indica* Seed Oil

The multifaceted medicinal properties of *Azadirachta indica* (Neem) have long intrigued natural product chemists, with early recognition attributed to the pioneering work of Salimuzzaman Siddiqui (Soukup & Soukup, 2014). In 1942, Siddiqui extracted three key bioactive acids from Neem oil: nimbin, nimbinin, and nimbidin (Sarkar et al., 2021). Using a series of solvents including ethyl acetate, dilute alcohol, petroleum ether, and ether Siddiqui separated these compounds, laying the groundwork for their structural and functional characterization. Among these, nimbidin emerged as the most active antibacterial and bitter component, with significant stability and potency (Siddique et al., 2023). Nimbin, a crystalline, sulfur-free compound with a melting point of 205 °C, and nimbidin, a cream-colored, sulfur-containing substance with a melting point of 90-100 °C, were identified as key bioactive constituents. Notably, these compounds were also found to possess significant insecticidal properties (Siddique et al., 2023).

Subsequent research has expanded on the pharmacological potential of compounds in Neem seed oil, revealing a diverse array of bioactive constituents, particularly triterpenes, which exhibit pronounced medicinal properties. The triterpene nimbin has garnered

attention for its antifungal, antipyretic, antibacterial, and antihistamine activities. Additionally, nimbin demonstrates antioxidant and anti-inflammatory effects, notably through the reduction of reactive oxygen species (ROS) formation (Sudhakaran et al., 2022). These properties underscore its potential to mitigate oxidative and inflammatory damage. Neem oil also contains other bioactive molecules such as protein kinases, phosphodiesterase, flavonoids, and endoperoxides, which have been implicated in anti-inflammatory pathways. For instance, flavonoids present in Neem oil have been shown to inhibit prostaglandin synthesis (Naik et al., 2014; Sarkar et al., 2021).

Extensive phytochemical analyses confirm that Neem seed oil is rich in saponins, flavonoids, and triterpenes, with relatively lower concentrations of catechins and nimbins (Naik et al., 2014). Neem extracts also contain metabolites such as limonoids, tannins, alkaloids, terpenoids, reducing sugars, catechins, and gallic acid, further enhancing its medicinal potential (Atawodi & Atawodi, 2009; Naik et al., 2014). Among these, azadirachtin, a complex tetranortriterpenoid limonoid, has been identified as the principal insecticidal agent. Its toxic effects on insects underscore the dual utility of Neem seed oil in medicinal and agricultural applications.

The comprehensive understanding of Neem seed oil's bioactive compounds not only highlights its therapeutic potential but also underscores its role in sustainable pest management. Future research focusing on the mechanistic pathways and synergistic effects of these compounds could unlock new dimensions of their applicability in pharmaceutical and agricultural domains.

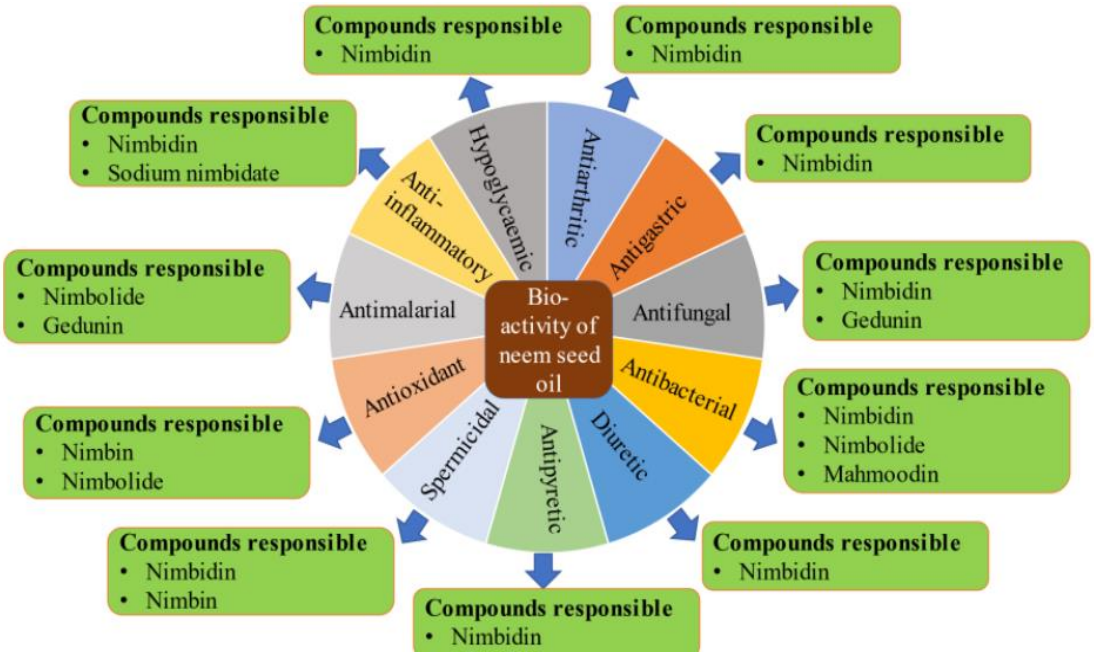


Figure 2.3: Bio-activity of compounds extracted from neem seed oil. (Biswas et al, 1995)

2.4 Mechanism of Action of Active Compounds of Neem

Azadirachta indica's therapeutic potential stems from its rich antioxidant content as well as other advantageous active components such as nimbidin, salannin, quercetin, nimbolinin, Nimbin, and azadirachtin. Still unknown, though, is the exact molecular process by which this prophylaxis works. Numerous parts of the Neem tree have antibacterial qualities; these parts prevent germs from multiplying and breaking down cell walls. A secondary metabolite found in Neem seeds, azadirachtin is an active component of the plant. It is a molecule in the limonoid group of chemicals. Azadirachtin is a tetranortriterpenoid that is extensively oxidised and has a lot of oxygen-bearing functional groups. This crucial ingredient is responsible for the insect-toxic and antifeedant actions (Gupta et al., 2017). Neem is a powerful weapon against free radicals due to its abundance of antioxidants.

Concentration exhibited a relationship between the antiradical scavenging activity and reductive potential of the active components nimbolide and azadirachtin; nimbolide exhibited the highest potency, followed by azadirachtin and ascorbate (Manikandan et al., 2009). Through its effects on cell signalling pathways, the neem component has emerged as a key player in cancer management. In terms of gene activity, neem influences apoptosis (e.g., bcl2, box), angiogenesis (VEFG), and several tumour suppressor genes (e.g., p53, pTEN). Another way neem reduces inflammation is by controlling the production of enzymes that promote inflammation, such as cyclooxygenase (COX) and lipoxygenase (LOX).

2.5 Compounds found in Neem Seed Oil and Their Biological Activities

Neem oil, derived from the neem tree's fruits, seeds, and blossoms, is a viscous liquid with a very foul and awful smell and a colour that varies from golden to dark brown. (Ni Putu Ratna et al., 2024) state that it contains a variety of components, including fatty acids, limonoids, vitamin E, triglycerides, antioxidants, and calcium. Here are some of the chemicals found in neem seed oil:

2.5.1 Nimbidin

The anti-inflammatory effects of nimbidin and sodium nimbidate have been demonstrated in studies involving carrageen-induced acute paw oedema in rats and formalin-induced arthritis (Arora et al., 2022). The action varies with the dose. According to what David described and confirmed in 1969, nimbidin also has antipyretic effects. The oral dose of nimbidin had a significant hypoglycemic effect in rabbits who were fasting (Hassanein et

al., 2021). According to (Sharma & Shukla, 2020; Uddin et al., 2018), nimbidin effectively prevents stomach lesions caused by acetylsalicylic acid, indomethacin, stress, or serotonin, as well as duodenal ulcers caused by histamine or cystamine. Not only can histamine and carbachol enhance gastric acid output, but nimbidin can also suppress basal stomach acid output (Sharma & Shukla, 2020). On top of that, it can help with ulcers and could work as an antihistamine by blocking H2 receptors. Among its antifungal properties, nimbidin reduced the growth of Tinea rubrum (Sharma & Shukla, 2020). Research conducted in 1959 by Sharma and Saksena found that it was bactericidal and could completely inhibit the growth of Mycobacterium TB in vitro. Also, when sodium nimidinate was given to dogs, it had a diuretic effect (Sharma & Shukla, 2020).

2.5.2 Nimbin

Neem leaves were used to extract the triterpenoid nimbin. The antibacterial, anti-inflammatory, antipyretic, fungicidal, and antihistamine properties of neem oil are supposedly due to Nimbin (Norten, 1999). India, Thailand, and China are among the numerous Asian nations that produce the neem tree-derived substance Nimbin. Nimbin is a chemical member of the limonoids and triterpenoids family. In 1942, the first Nimbin extract was made from neem seed, whose Latin name is Azadirachta indica, by Siddiqi *et al*. The molecular formula was found by mass spectrometry alongside salannin, a molecule that is both physically and chemically comparable to Nimbin. Different parts of the neem tree can have their Nimbin content extracted using a solvent or supercritical carbon dioxide (Sidhu et al., 2004). Several studies point to Nimbin's anti-inflammatory, antibacterial,

antiviral, antifungal, and insecticide properties, which contribute to its multi-useful character (Sidhu et al., 2004; Wasim et al., 2023).

Its use helps alleviate symptoms of several skin conditions, including eczema and psoriasis. There is evidence from laboratory research that viruses can be treated, including SARS COV-2 and Dengue Virus (Popoola et al., 2022; Wylie & Merrell, 2022). However, that has only shown promise in human trials conducted in controlled laboratory settings. A Nimbin variation called N2 was utilised in research on the dengue virus and related subjects, including antimicrobials. Despite Nimbin's mildly hydrophobic nature, scientists have discovered a method to increase its hydrophilicity by including an inclusion complex; this discovery may open the door to its potential direct use.

2.5.3 Nimbolide

Nimbolide has several names and chemical formulas, including C H O (Nagini et al., 2024). Also known as (4alpha, 5alpha, 6alpha, 7alpha, 15beta, 17alpha)-7, 1521, 8-dimethyl-1-oxo-18, 24-dinor-11, 12-secochola-2, 13, 20, 22-tetraene-4, and 11-dicarboxylic acid gamma-lactone methyl ester. Gore et al. reported in 1993 that nimbolide has a decalin skeleton (Gore et al., 1993). As described by Singh in 2008, it is categorised as a tetranortriterpenoid belonging to the C-seco Meliacin-family. Nimbolide has a long list of well-known uses, including its ability to kill insects, prevent pests from feeding, and fight bacterial infections caused by S. aureus and S.coagulase (Malakar & Mandal, 2025; Sharma, 2011; Singh et al., 2021). The capacity to scavenge free radicals and demonstrate antioxidant characteristics are additional benefits of nimbolide. (Wang et al., 2016) proved

that nimbolide was a more potent antioxidant than azadirachtin and ascorbic acid (vitamin C). Also, nimbolide is an important part of neem extract, which is used in traditional Indian Ayurvedic medicine to cure infections, wounds, gastric ulcers, and acne (Singh et al., 2021).

(Kashif et al., 2019) identified nimbolide as the limonoids with the highest level of cytotoxicity among the seven compounds studied. Multiple molecular targets were found as a result of a comprehensive analysis of Nimblolide's impact on multiple cancer cell lines. Hence, its pharmacodynamic activities can be explained mechanically. Further evidence of nimbolide's anticancer and cancer prevention capabilities has been shown by several animal research (Bodduluru et al., 2014; Nagini et al., 2021). All of this points to its promising future as a cancer treatment agent. By reducing Plasmodium falciparum cell multiplication, emblemoide has shown antimalarial activity (Nagini et al., 2021). According to a study by Rojanapo *et al*. (1985), nibalide has antibacterial effects against Staphylococcus aureus and Staphylococcus coagulase.

2.5.4 Gedunin

The antifungal and antimalarial effects of the neem seed oil chemical gedunin have been noted in previous studies (Ghosh et al., 2016; Nagini et al., 2024). There is a lot of gedunin in the Azadirachta indica A. Juss. fruit epicarp; the amounts are highest in the immature, green fruits rather than the ripe ones (Nagini et al., 2024).

Gedunin, though present in trace amounts across various parts of the plant, is found in concentrations below 0.1% in the leaves of *Azadirachta indica* (Ponnusamy et al., 2015). Studies have documented its wide-ranging biological activities, including insecticidal, antibacterial, antifungal, antimalarial, antiallergic, anti-inflammatory, anticancer, and neuroprotective effects (Nainu et al., 2021; Namita & Mukesh, 2012). Structurally, limonoids are formed when the side chain of an apotirucallane or apoeuphane skeleton loses four terminal carbons, followed by the cyclization that forms the 17β-furan ring. These compounds, also known as tetranortriterpenoids, are classified based on the oxidation state of one of the four rings (A, B, C, or D) within the triterpene nucleus (Tan & Luo, 2011; Wang et al., 2022). Among the limonoids, gedunin stands out as a prominent member of the ring D-seco class.

According to (Braga et al., 2020), the δ-lactone in ring D of the azadirone class undergoes oxidative ring expansion via a Baeyer-Villiger type reaction, resulting in a steroid skeleton of 4,4,8-trimethyl-17-furanyl. (Ventura-Aguilar et al., 2023) revealed that the malonic acid (MVA) pathway is the sole source of isoprene units for limonoid biosynthesis in neem trees. However, the isoleucine and leucine biosynthetic pathways also contribute to the formation of functional groups in limonoids.

The chemical formula of gedunin was first published in 1960 by Akisanya and colleagues, who derived it from the West African wood *Entandrophragma angolense* (Welw.) C. DC. with a molecular weight of 482.55 g/mol. In 1961, the same researchers detailed certain gedunin reactions, proposing a structure similar to limonin (Akisanya et al., 1961).

Subsequent analyses, including NMR, MS, and X-ray diffraction, provided insights into gedunin's characterization, composition, and stereochemistry using a derivative of dihydrogen-3β-yl iodoacetate (MacKinnon, 1995).

Carvalho et al. (2012) reported that gedunin crystallizes in the orthorhombic space group $P2_12_12_1$, with two distinct molecules, α- and β-Gedunin, in its asymmetric unit. The crystallization process is believed to occur exothermically and spontaneously (Carvalho Jr et al., 2012). Molecular electrostatic potential (MEP) maps show that electronegative atoms are surrounded by negative potential sites, while hydrogen atoms are associated with positive potential sites. The conformer-to-conformer interaction strength is notably high, measuring 13.09 kcal/mol.

Despite its significant biological activities, complete synthesis of gedunin remains elusive. Semi-synthetic derivatives have been created by chemically modifying the gedunin scaffold, but their biological activity has not matched that of the natural compound (Carrasco, 2013; Wang, 2016). It is reported that Pinkerton and research team pioneered the first synthetic route to the BCD ring system of unsaturated ring D-seco limonoids (Youn, 2022). By employing a Robinson annulation reaction, they successfully constructed the complete ABC ring system, incorporating the 7-acetoxy functional group and finalizing ring formation.

2.5.5 Azadirachtin

According to several research, azadirachtin, a highly oxygenated C-secomeliacin derived from neem seed, has antifeedant and antimalarial characteristics (Kumar et al., 2018; Nagini et al., 2024). It is a chemical that, according to Jones *et al.* (1994), prevents malaria parasites from developing (Jones et al., 1994).

2.5.6 Mahmoodin

Some kinds of human pathogenic bacteria have been shown to show modest levels of antibacterial activity against the deoxygedunin called Mahmoodin, which was isolated from seed oil (Pandey et al., 2014).

2.6 Phytochemistry of Azadirachta indica Seed Oil

Phytochemicals are biologically active compounds found in plants that, when consumed in adequate amounts, can benefit human and animal health (Leitzmann, 2016). Over 4,000 phytochemicals have been identified and are broadly categorized into primary and secondary components based on their roles in plant metabolism, protective functions, and chemical properties (Alamgir et al., 2018). Primary components include nucleic acid-derived sugars, chlorophyll, purines, and pyrimidines. Secondary components, such as alkaloids, saponins, flavonoids, and phenolics, provide protective and therapeutic benefits (Mariya et al., 2020).

Plant parts such as seeds, fruits, roots, leaves, and flowers can accumulate phytochemicals at various stages of growth or processing (Tiwari et al., 2015). Common food sources of

phytochemicals include fresh fruits, vegetables, nuts, seeds, legumes, and whole grains (Kimondo, 2020). Among these, phenolic compounds are particularly abundant and diverse (Kimondo, 2020).

2.6.1 Phenolic Compounds

Phenolics are the largest class of phytochemicals, consisting of hydroxyl groups attached to aromatic hydrocarbons (-OH). They include flavonoids, phenolic acids, and polyphenols (Hoda et al., 2019). Flavonoids are the predominant group, while phenolic acids, such as hydroxybenzoic and hydroxycinnamic acids, are noted for their high molecular weight and bioavailability. Phenolics exhibit antioxidant properties that protect against diseases caused by oxidative stress, including cancer, cardiovascular diseases, and inflammation (de la Rosa et al., 2019; Kaurinovic & Vastag, 2019). These compounds are bioavailable through direct consumption or metabolic processes in the liver and kidneys (de la Rosa et al., 2019). Their antioxidant and anti-inflammatory properties are instrumental in combating free radicals, reducing oxidative stress, and preventing degenerative diseases (Hoda et al., 2019).

2.6.2 Flavonoids

Flavonoids are polyphenolic compounds classified as flavones or isoflavones based on their chemical structure. Found in fruits, vegetables, tea, coffee, and other beverages, flavonoids play vital roles in human health. They exhibit antioxidant, anti-inflammatory, antibacterial, and anticancer activities (Garg et al., 2019).

Flavonoids like catechins and luteolin have superior antioxidant properties compared to vitamins C and E, attributed to their unique chemical structures and hydroxyl group arrangements (Panche et al., 2016). These properties make flavonoids essential in reducing oxidative stress and mitigating health risks.

2.6.3 Antioxidants

Antioxidants, whether endogenous (produced by the body) or exogenous (obtained from diet), combat free radicals to prevent cell damage. Common dietary antioxidants include beta-carotene, selenium, and vitamins A, C, and E (Bratovcic, 2020). Free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are unstable molecules that can cause DNA damage, lipid peroxidation, and cellular dysfunction (Engwa et al., 2022). Antioxidants mitigate these effects, reducing the risk of cancer, cardiovascular diseases, and oxidative stress-induced conditions (Engwa et al., 2022).

2.6.4 Alkaloids

Alkaloids are nitrogen-containing organic compounds found in plants, animals, and fungi. Known for their bitter taste and therapeutic properties, alkaloids include nicotine, morphine, and quinine (Alamgir et al., 2018).

Alkaloids exhibit diverse medicinal effects. For instance, quinine is an anti-malarial agent, and vincristine and vinblastine from *Vinca rosea* are used in cancer treatment (Bhambhani et al., 2021). These compounds also demonstrate vasoconstrictive, anti-inflammatory, and

chemotherapeutic properties, making them valuable in pharmaceutical applications (Bhambhani et al., 2021).

2.6.5 Tannins

Tannins, water-soluble polyphenols, are found in many plant-based foods and influence protein digestibility and nutrient absorption (Ribas-Agustí et al., 2018). While high tannin levels in the diet may reduce nutrient bioavailability, they also possess antioxidative properties that protect against oxidative stress, cancer, and mutations (Günal-Köroğlu et al., 2023).

Studies have linked tea consumption, a rich source of tannins, to both carcinogenic and anti-carcinogenic effects, depending on associated components and individual health conditions (Bag et al., 2022; Hayat et al., 2015).

2.6.6 Saponins

Saponins, found in vegetables, legumes, and plants like soapwort and soybeans, are known for their foaming properties and health benefits. They exhibit anticancer, anti-inflammatory, and immune-boosting properties (Dhanalakshmi & Divya, 2024).

Research suggests that saponins inhibit cancer cell growth by interacting with cholesterol-rich cell membranes (Kozińska et al., 2021). Additionally, they contribute to cholesterol regulation, bone health, and oxidative stress reduction, enhancing their therapeutic potential (Kozińska et al., 2021).

In industrial applications, saponins are used in food, cosmetics, and beverages for their emulsifying and foaming properties (Dhanalakshmi & Divya, 2024).

2.7 Effect of Neem Seed Oil on Hematological Parameters

Neem seed oil, derived from *Azadirachta indica*, has been investigated for its potential effects on hematological parameters. Aloke et al. (2021) examined the impact of neem seed extract administered at doses of 200, 400, and 800 mg/kg body weight on rats (Aloke et al., 2021). Their findings revealed a slight but clinically insignificant reduction in hemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts. Despite these minor changes, all values remained within normal physiological ranges, indicating no adverse hematological effects.

The study also found no statistically significant changes in Hb, RBC, or PCV values across the dosage groups at a 0.05 significance level. Furthermore, the total white blood cell (TWBC) counts did not exhibit significant variations at any dose level ($p>0.05$) (Aloke et al., 2021). The red cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were consistent with normal values, even at the highest dosage of 800 mg/kg body weight. This consistency suggests that neem seed extract does not adversely affect red cell size or hemoglobin concentration.

Regarding leukocyte differentials, there were no statistically significant differences ($p>0.05$) between the treated and control groups in the proportions of lymphocytes,

neutrophils, monocytes, and basophils (Aloke et al., 2021). However, eosinophil counts showed a peak at the highest dose (800 mg/kg body weight), following a gradual decline with increasing dosages, which could indicate a dose-dependent effect on this specific leukocyte type.

Although there was a slight increase in leukocyte counts, these changes remained within normal reference ranges and were not statistically significant ($p>0.05$). This suggests that the extract does not significantly alter immune cell profiles.

2.7.1 Potential Effects on Liver and Kidney Functions

The liver and kidneys play vital roles in metabolism, detoxification, and waste excretion, making them susceptible to toxicological impacts (Bischoff et al., 2018; Gupta et al., 2020). Damage to these organs often results in elevated levels of clinical biochemical markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and urea. These markers are indicative of impaired hepatic or renal function when significantly elevated (Bischoff et al., 2018).

In this study, liver enzyme levels (AST, ALT, and ALP) and kidney function markers (creatinine and urea) were assessed. The findings indicated that A. indica seed extract did not induce hepatotoxicity or nephrotoxicity, as there were no significant increases in these biochemical markers at any dosage level ($p>0.05$) (Aloke et al., 2021). Serum total protein (TP) levels also remained stable across treated and control groups.

2.7.2 Supporting Evidence

The absence of significant hematological and biochemical alterations in treated animals aligns with findings from other studies. For instance, Del Serrone et al. (2015) evaluated neem oil (NO) used for lice control in goats and reported no statistically significant differences in hematological parameters (means ± SD) between treated and untreated groups (Del Serrone et al., 2015).

The findings collectively suggest that neem seed oil, even at high doses of 800 mg/kg body weight, does not pose significant risks to hematological or biochemical parameters in experimental animals (Aloke et al., 2021; Del Serrone et al., 2015). Its lack of adverse effects on liver and kidney functions underscores its safety for potential therapeutic applications. However, further studies are warranted to confirm these findings and assess long-term safety.

7.2.3 Research Gap of Studies on Hematological Effects of Neem Seed Oil

Despite the extensive body of research on *Azadirachta indica* (neem) seed extract, certain gaps in knowledge remain unaddressed. One major limitation is the lack of studies investigating the long-term toxicity of neem seed oil. Most existing studies, such as those conducted by Aloke et al. (2021) and Del Serrone et al. (2015), focus primarily on short-term administration and its immediate effects. The potential cumulative impact of prolonged exposure to neem seed oil on hematological parameters and organ function is yet to be thoroughly explored. This leaves an important gap in understanding its safety for long-term use.

Another critical gap lies in the absence of mechanistic insights into the observed effects of neem seed oil. While studies have reported hematological changes, such as an increase in eosinophil counts at higher doses, and stable biochemical markers, the underlying biochemical and immunological pathways have not been investigated. Understanding these mechanisms would provide a clearer picture of how neem seed oil interacts with biological systems and would enhance its application in various fields.

Furthermore, the research is overwhelmingly based on animal models, such as Wistar rats and goats, with limited data on its effects in humans. Although animal studies are essential for preliminary findings, there is a pressing need for human clinical trials to validate the safety and efficacy of neem seed oil. This is particularly important if the oil is to be used for therapeutic or agricultural purposes on a larger scale.

Additionally, the variability in the chemical composition of neem seed oil poses a challenge. Factors such as geographical origin, plant genotype, and extraction methods can influence the active components of the oil. Unfortunately, most studies fail to account for these variations or standardize the neem seed oil used, making it difficult to replicate and generalize the findings across different populations and settings.

Finally, there is a lack of research focusing on vulnerable populations, such as pregnant animals or individuals with pre-existing health conditions. Understanding the safety of neem seed oil in these groups is critical, especially for its potential use in maternal and child health or for individuals with compromised health. Addressing these gaps in future

research will enhance our understanding of the hematological and biochemical effects of neem seed oil and its broader applications in health and agriculture.

2.8 Effect of Neem Seed on Liver Biochemistry

The hepatoprotective potential of neem (*Azadirachta indica*) and its active components has been widely investigated, with numerous studies indicating its ability to mitigate liver damage and improve liver function. Medicinal herbs like neem are generally regarded as safe and effective for liver protection, and their therapeutic applications have been supported by biochemical, histological, and ultrastructural findings.

Bansal et al. (2014) conducted a study on the hepatoprotective function of azadirachtin A, a bioactive compound derived from neem, in rats with carbon tetrachloride (CCl_4)-induced hepatotoxicity (Bansal et al., 2014). The results showed a dose-dependent reduction in hepatocellular necrosis among animals pretreated with azadirachtin A. Furthermore, animals administered higher doses exhibited partial recovery of liver function, as evidenced by improved histological and ultrastructural parameters. The protective effects of azadirachtin A were comparable to those of silymarin, a widely used hepatoprotective standard.

Similarly, another study demonstrated the hepatoprotective effects of neem leaf extracts on paracetamol-induced liver damage in rats. The aqueous leaf extract of *A. indica* significantly reduced serum bilirubin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels compared to

untreated groups (Bharali et al., 2023). The protective effect was also validated by a marked reduction in histological changes indicative of liver damage.

Althaiban (2019) further explored the hepatoprotective properties of neem in rats exposed to carbon tetrachloride. Both ethanolic and aqueous extracts of neem leaves showed moderate efficacy in mitigating liver damage. The extracts were able to restore liver enzyme levels, including AST and ALT, to near-normal ranges and reduce the extent of liver necrosis (Althaiban, 2019). These findings support the conclusion that neem leaf extracts exhibit hepatoprotective effects through multiple mechanisms.

Hameed et al. (2010) also highlighted the potential of neem extracts to protect liver tissues from various toxic insults. Their study demonstrated that both water and methanolic extracts of neem leaves significantly reduced elevated liver enzymes, indicating effective liver protection (Hameed et al., 2021). Similarly, Lim et al. (2014) showed that neem extracts could protect gastric mucosal tissues from ethanol-induced damage, suggesting broader protective effects on the gastrointestinal system (Lim et al., 2014).

In animal models with CCl_4-induced hepatotoxicity, disease control groups exhibited significantly elevated levels of AST, ALT, and ALP, along with reduced total protein levels. However, pretreatment with azadirachtin A effectively mitigated these changes, improving enzyme levels and reducing hepatocellular necrosis (Lim et al., 2014). Histological and ultrastructural studies corroborated these findings, indicating reduced tissue damage in the pretreated groups.

Notably, neem leaf extracts have also been shown to protect against antitubercular drug-induced liver toxicity. In this context, aqueous leaf extracts were more effective than merely discontinuing the drug treatment. They not only reduced elevated liver enzyme levels but also reversed histological evidence of liver damage, highlighting their therapeutic potential (Ugwu & Suru, 2023).

These findings collectively demonstrate the hepatoprotective potential of neem and its active compounds. While neem leaf and seed extracts exhibit promising effects in reducing liver enzyme abnormalities and mitigating tissue damage, further research is necessary to elucidate the precise molecular mechanisms and long-term safety profile. This understanding is essential for optimizing the therapeutic use of neem in liver-related disorders.

2.9 Effect Neem Seed Oil on Kidney Function

The potential nephroprotective effects of neem (*Azadirachta indica*) and its bioactive components have been investigated in various studies, especially in the context of drug-induced kidney damage. The kidneys, being primary organs for filtration and excretion, are highly susceptible to toxicants, making the study of protective agents like neem essential. Seriana et al. (2021) explored the protective effects of neem leaf extract and vitamin E on cisplatin-induced nephrotoxicity in Wistar rats. Cisplatin, a widely used chemotherapeutic agent, is known to cause kidney damage as a side effect. In the study, rats were administered a single intraperitoneal dose of cisplatin (10 mg/kg) to induce nephrotoxicity, followed by oral treatment with neem leaf extract (500 mg/kg/day) for 14 days (Seriana et

al., 2021). The findings revealed that cisplatin-treated animals exhibited significantly elevated serum urea and creatinine levels and heavier kidneys compared to the control group, indicative of renal dysfunction ($p>0.05$).

Interestingly, rats treated with neem leaf extract showed some attenuation of these effects. While metabolic acidosis and hyperkalemia common signs of severe renal impairment were not observed, the renal histology revealed minor tubular dilations, suggesting partial protection (Seriana et al., 2021). The morphological and biochemical data implied that neem leaf extract mitigates some of cisplatin's renal toxic effects. This protective action is attributed to neem's rich phytochemical composition, particularly its bioflavonoids, which are powerful antioxidants.

The researchers further hypothesized that the nephroprotective effects of neem might result from its ability to scavenge oxygen-derived free radicals produced during tissue inflammation (Seriana et al., 2021). Cisplatin-induced nephrotoxicity is often associated with oxidative stress and inflammation, processes that neem extract potentially counteracts through its anti-inflammatory and antioxidative properties. Bioactive compounds such as nimbidin and other neem derivatives are thought to play a critical role in reducing inflammation and neutralizing reactive oxygen species, thereby alleviating renal tissue damage.

In addition to its biochemical effects, neem's influence on renal morphology supports its therapeutic potential (Alzohairy & Medicine, 2016). The absence of severe

histopathological changes like necrosis or extensive tubular damage in neem-treated groups suggests that it helps preserve kidney structure. The study's findings align with previous research indicating neem's protective role against various oxidative and inflammatory insults.

The results of Seriana et al. (2021) stress the potential of neem as an adjunct therapy in mitigating cisplatin-induced nephrotoxicity. While the protective effects were not absolute, the study provides compelling evidence of neem's ability to reduce biochemical and structural markers of kidney damage. This protective mechanism highlights neem's therapeutic promise in managing drug-induced renal injuries.

Despite these promising findings, further research is needed to fully understand the molecular pathways through which neem exerts its nephroprotective effects. Additionally, clinical trials are necessary to establish its efficacy and safety in human populations, paving the way for its integration into renal protective strategies.

2.10 Effect of Neem Seed Oil on Liver and Kidney Histology

Neem seed oil (*Azadirachta indica*) has been studied for its effects on the histology of vital organs such as the liver and kidneys (Gowda et al., 1996; Lisanti et al., 2018). These investigations provide insights into its potential toxicity, therapeutic value, and dose-dependent effects on organ structure and function.

2.10.1 Liver Histology

Histological analysis of the liver in male control mice revealed no signs of toxicity or abnormalities, with liver slices showing intact central veins and normal hepatocyte plates surrounded by portal tracts (Lisanti et al., 2018). However, exposure to neem seed oil at doses of 0.25 and 0.50 mg/kg body weight (bw) over 36 days caused apoptotic changes in hepatocytes, which persisted post-treatment. This damage appeared to be dose-dependent, with higher doses leading to more pronounced and lasting effects (Lisanti et al., 2018). The active components of neem, including terpene glycosides (e.g., actein and cimicifugoside), alkaloids, flavonoids, and tannins, are believed to influence these outcomes. While these compounds possess therapeutic properties, they can also contribute to hepatotoxicity under specific conditions. For example, physical histology from Islas et al. (2020) demonstrated continuous liver damage with prolonged neem seed oil treatment, including disruptions to hepatocyte morphology and central veins (Islas et al., 2020). Evidence of caspase-3 activity, a marker of apoptosis, was observed, highlighting neem's potential to induce programmed cell death (Ogbuewu et al., 2017).

Histological changes in the liver often begin with extracellular fluid leakage into cells, causing cytoplasmic swelling and vacuolization. These changes can become permanent lesions if sustained. Studies indicate that higher doses, such as 0.05 mg/kg bw of neem aqueous seed extract, result in more severe and long-lasting hepatic damage. This damage may also be linked to pyrrolizidine alkaloids and diterpenoids found in neem, which cause hepatocellular damage and hepatocyte death, respectively (Islas et al., 2020; Lisanti et al., 2018).

2.10.2 Kidney Histology

Studies also highlight neem's effects on kidney histology. Kpela et al. (2012) investigated the protective effects of neem leaf extract on cisplatin-induced nephrotoxicity in Wistar rats. Cisplatin-treated rats exhibited significant renal abnormalities, including increased serum urea and creatinine levels and heavier kidneys (Kpela et al., 2012). While neem leaf extract attenuated some of these effects, histological analysis revealed minor tubular dilations, indicating partial nephroprotection. The nephroprotective action of neem is attributed to its bioflavonoids, which scavenge free radicals and reduce oxidative stress.

However, higher doses of neem seed oil can also induce nephrotoxic effects. The histopathological changes observed include tubular damage, vacuolization, and disruptions in renal morphology. These changes underscore the importance of understanding dose thresholds to minimize adverse effects while leveraging neem's therapeutic potential.

2.10.3 Effects on Reproductive Organ Histology

Neem's impact on histological structures is not limited to the liver and kidneys. Shaikh et al. (2009) examined the effect of neem oil on the ovaries of albino female rats. High doses caused significant alterations in ovarian morphology, including atresia of both immature and mature follicles, fluid-filled developing follicles, and disrupted stromal organization (Shaikh et al., 2009). Conversely, low doses showed minimal changes, with primary, secondary, and mature follicles still observable in histological sections. These findings indicate a dose-dependent effect of neem on ovarian histology, highlighting its potential to impact reproductive health.

Neem seed oil has demonstrated dose-dependent effects on the histology of the liver, kidneys, and other organs. While low doses may provide protective benefits, high doses can result in significant organ damage, including hepatotoxicity, nephrotoxicity, and reproductive organ alterations. The findings emphasize the need for careful dose regulation and further research to understand neem's therapeutic and toxicological profiles fully.

2.11 Effect of Neem Seed Oil On Body Weight

The impact of neem seed oil and related neem-derived products on body weight has been the focus of various studies, particularly concerning their potential effects on animal growth and physiology. These studies demonstrate a range of outcomes, with neem's effects varying based on dosage, duration of exposure, and the specific animal model used.

2.11.1 Decrease in Body Weight

Research conducted by Ogbe et al. (2019) investigated the effects of neem seed oil on the skeletal and intramuscular weights of adult male Wistar rats. The findings revealed a statistically significant decrease ($p>0.05$) in the body weights of experimental animals compared to controls (Ogbe et al., 2019). This reduction in body weight was accompanied by decreases in the weights of specific organs, including the testes, liver, kidneys, and brain. These changes suggest that neem seed oil may influence systemic physiology, potentially impacting metabolism or nutrient utilization.

Further support for neem's weight-reducing effects was provided by Heinrich et al. (2012), who studied the effects of neem leaves on bulls. Their results showed a statistically

significant difference ($p>0.01$) in the body weights of bulls fed neem leaves over 28 days compared to those in the control group (Heinrich et al., 2012). This study highlights neem's potential influence on weight regulation in larger animal models, though the mechanisms remain unclear.

2.11.2 Dose-Dependent Effects on Weight

In contrast to studies reporting weight reduction, some research suggests that neem products can promote weight gain under specific conditions. Meshram et al. (2022) evaluated the effects of neem leaf powder on the weight of broiler chicks. They found that the addition of 6 g of neem leaf powder per kilogram of standard feed yielded the best results, with significant increases in body weight (Meshram et al., 2022). However, when the dosage was increased to 8 g per kilogram of feed, a slight decrease in body weight was observed.

This dose-dependent response indicates that neem's effects on body weight are not linear and that optimal dosing is critical. The broiler chicks thrived when fed 6 g of neem leaf powder per kilogram of feed, suggesting that neem can support growth and weight gain at moderate doses while potentially exerting negative effects at higher doses.

2.11.3 Mechanisms Behind Weight Changes

The observed effects of neem on body weight may be attributed to its bioactive compounds, including flavonoids, terpenoids, and alkaloids (Kumar et al., 2018; Sarah et al., 2019). These compounds are known to modulate metabolic processes, such as lipid metabolism

and protein synthesis, which could influence overall weight. Additionally, neem's antimicrobial and anti-inflammatory properties might impact gut health and nutrient absorption, further contributing to its effects on body weight.

The effects of neem seed oil and neem-derived products on body weight vary significantly depending on the species, dosage, and context of administration (Sarah et al., 2019). While neem can promote weight loss in certain animal models, particularly at higher doses, it has also demonstrated growth-promoting effects in broiler chicks at moderate dosages. These findings underscore the need for careful dose optimization to harness neem's benefits while minimizing potential adverse effects on growth and development. Further research is necessary to elucidate the underlying mechanisms and determine safe and effective dosages for various applications.

2.12 Safety, Toxicities, and LD50 Values of Neem

Understanding the safety and toxicological profile of neem is essential for its application in health management. While neem and its components have demonstrated some adverse effects in certain scenarios, numerous animal studies and clinical trials have affirmed its safety when used within specified limits (Murugan et al., 2014). However, reports of toxicity, especially in children, highlight the need for cautious use. Several studies have documented cases of neem oil poisoning in children, emphasizing the importance of dosage and context in its administration (Murugan et al., 2014; Patil et al., 2022).

Animal studies have extensively evaluated the safety of neem. Braga et al. (2021) observed that low doses of neem leaf sap had an antianxiety effect in rats, while higher doses did not produce a significant impact, indicating a dose-dependent response (Braga et al., 2021). Similarly, study in 2004 demonstrated that azadirachtin, a major bioactive compound in neem, was safe in rats at doses up to 5 g/kg body weight (Boeke et al., 2004). Research on rabbits further confirmed the non-toxic nature of neem extract at tested levels, with both test and control animals gaining weight during the study, showing no signs of toxicity (Dimetry, 2020).

The LD50 (median lethal dose) values of neem and its components have been widely studied to assess toxicity levels. Neem oil was found to have an LD50 of 31.95 g/kg in an acute toxicity study (Deng et al., 2013). In chickens, the intraperitoneal LD50 of neem leaf aqueous extract was 4800 mg/kg, with dose-dependent clinical signs observed (Biu et al., 2010). A study reported LD50 values of 489.90 mg/kg for neem stem bark extract and 31.62 mg/kg for neem leaf extract (Alzohairy & Medicine, 2016). Other studies, such as Bakr (2013), identified LD50 values of 9.4 mL/kg for neem seed extract and 6.2 mL/kg for neem leaf extract (Najeebullah et al., 2021; Saleem et al., 2018). In mice, an LD50 of approximately 13 g/kg in an acute oral toxicity test was found (Martin, 2018).

Despite its general safety at recommended doses, neem can have adverse effects if dosages exceed safety thresholds. High doses have been linked to hepatotoxicity, with studies showing liver cellular abnormalities. Neem oil has also been associated with neurotoxicity in children, resulting in toxic encephalopathy in severe cases. Gastrointestinal irritation is

another concern at elevated doses, reflecting the importance of adhering to safe usage guidelines.

Neem has a well-documented safety profile when used within appropriate limits. The LD50 values provide a benchmark for safe usage across different species, ensuring that neem can be applied effectively without significant risk. However, the potential for adverse effects at higher doses underscores the importance of dose optimization and careful monitoring in both therapeutic and non-therapeutic applications.

2.13 Principles of Reagents used for Serum Biochemistry Analysis of this Study

2.13.1 Aspartate amino transferase (AST)

Principle: Ketoglutarate + L-aspartate ─AST─> L-glutamate + oxaloacetate Oxaloacetate + 2,4-dinitrophenyl hydrazine ─NaOH─> 2,4-dinitrophenyl hydrazone

AST present in the sample catalyzes the conversion of L-Aspartate and ketoglutarate to oxaloacetate and glutamate. The oxaloacetate formed reacts with 2,4-dinitrophenyl hydrazine to produce a hydrazine derivative which in alkaline medium (addition of NaOH) produces a colored complex whose intensity is measured.

2.13.2 Alanine Transaminase (ALT)

Principle: Ketoglutarate + L-alanine ─ALT─> Pyruvate + oxaloacetate Pyruvate + 2, 4-dinitrophenyl hydrazine ─NaOH─> 2, 4-dinitrophenyl hydrazone ALT present in the sample catalyzes the conversion of L-Alanine and ketoglutarate to oxaloacetate and pyruvate. The pyruvate formed reacts with 2,4-dinitrophenyl hydrazine to produce a

hydrazine derivative which in an alkaline medium (addition of NaOH) produces a colored complex whose intensity is measured.

2.13.3 Total Protein (TP) Principle

Principle: Protein + Cu++ ─NaOH─> Colored Complex

Total protein determination is based on the principle of biuret reaction (copper salt in an alkaline medium). Protein in plasma forms a blue-colored complex when treated with cupric icons in an alkaline solution. The intensity of the blue colour is proportional to the protein concentration when compared to a solution with a known protein concentration.

2.14 Scholarly Analysis, Identification of Gaps, and Relevance of the Study

Existing literature extensively explores neem seed oil's effects on various physiological and biochemical parameters in animal models, including its impact on body weight, haematological profiles, serum biochemistry, and organ histology (Asghar et al., 2022; Saleem et al., 2018). Studies have demonstrated dose-dependent changes in body weight, with both increases and decreases observed depending on neem dosage (Rahal et al., 2019). Haematological and serum biochemical parameters also show alterations under neem treatment, often reflecting systemic responses to its bioactive components (Rahal et al., 2019). Histological investigations highlight potential toxicities, particularly at higher doses, underscoring the organ-specific impact of neem seed oil.

However, significant gaps remain in understanding the detailed mechanisms underlying these effects, particularly the dose-response relationship and long-term implications for

organ function. Most studies have focused on isolated parameters or used limited dose ranges, leaving room for comprehensive studies that integrate body weight, haematology, biochemistry, and histological outcomes in a single framework. Additionally, inconsistencies in methodologies and findings across studies highlight the need for standardized approaches.

This study addresses these gaps by systematically examining the effects of varying doses of neem seed oil on growth performance, haematological profiles, serum biochemistry, and liver and kidney histology in Wistar rats. By employing a holistic approach, this research provides valuable insights into the dose-dependent effects of neem, contributing to the broader understanding of its safety, therapeutic potential, and toxicological profile. The findings will inform future applications and guidelines for neem seed oil usage in health management.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in the laboratory of the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (AAMUSTED), Mampong Campus, located in the Ashanti Region of Ghana. AAMUSTED has two campuses: the main campus in Kumasi and the Mampong campus, where this research was carried out. Mampong Municipality is one of the 27 districts within the Ashanti Region (Blay & Abunyuwah, 2024). It is situated approximately 57 kilometers from Kumasi, the regional capital (Blay & Abunyuwah, 2024). The municipality is bordered by the Sekyere East District to the north, Afigya-Sekyere District to the east, and Ejura-Sekyeredumasi District to the south and west (Dwumfour-Asare et al., 2018). Geographically, Mampong lies within the coordinates of 6.55°N to 7.30°N latitude and around longitude 0.05°W (Frimpong, 2015). The area experiences an average temperature of 28°C and a relative humidity of approximately 63%, creating a conducive environment for laboratory studies (Frimpong, 2015).

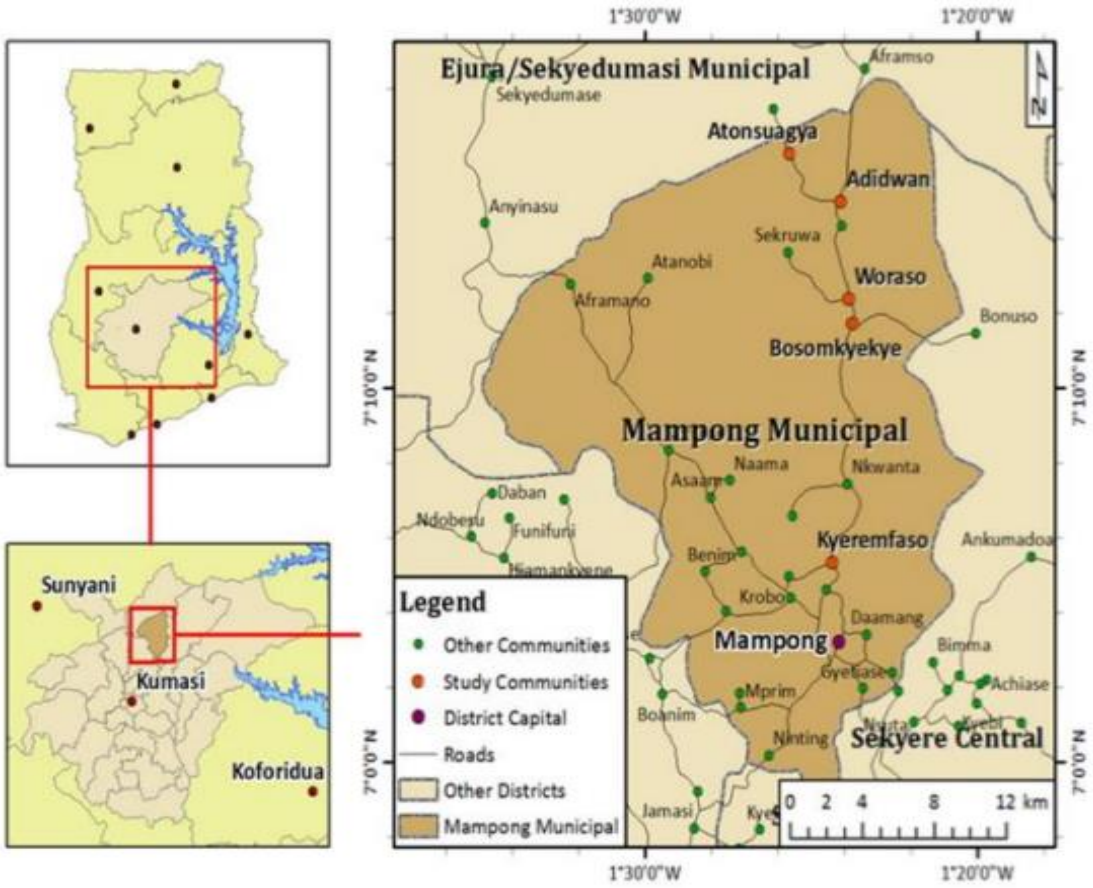


Figure 3.1: Map of Asante Mampong

3.2 Materials

3.2.1 Animals and Housing

The study utilized 24 female Wistar rats of similar age and weight, purchased from a local vendor in Mampong, Ashanti Region, Ghana. The animals were housed in the Animal Science Department of the College of Agriculture Education at the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong Campus. They were kept in a dry, cool environment under a natural light-dark cycle. During the acclimatization period and experiment, the rats were provided with ad libitum access to clean water and a diet of pelletized grower mash.

3.2.2 Neem Seed Sample Collection and Preparation

Fresh ripened fruit samples of *Azadirachta indica* Juss. (neem) were collected from the Mampong Municipality in June 2023. The fruits, along with their seeds, were thoroughly washed with tap water to remove debris and dirt. The washed fruits were packed in carrier bags and transported to the oil extraction site at the Asante Akim Ofoase Palm Oil Extraction Farm.

3.2.3 Equipment and Reagents

- Manual hydraulic pressing unit for oil extraction.
- Electronic balance for weight measurements (5KG/1KG LCD Digital Kitchen Food Scale; sensitivity ± 0.1 kg).
- Haematology auto-analyzer (Rayto RT-7600s).
- Laboratory kits for serum biochemistry (Randox Laboratories, UK).

3.3 Methods

3.3.1 Experimental Design

The study followed a completely randomized design, dividing the 24 rats into four groups of six, with each group receiving different treatments:

- **Group A (Control):** Normal saline (1.0 mL per body weight per day).
- **Group B (Low Dose):** Neem seed oil at 0.5 mL per body weight per day.
- **Group C (Medium Dose):** Neem seed oil at 1.0 mL per body weight per day.
- **Group D (High Dose):** Neem seed oil at 1.5 mL per body weight per day.

The dosage of neem seed oil was selected based on the protocol described by Idu et al. (2017).

3.3.2 Acclimatization

Before the experimental phase, the animals were acclimatized for two weeks to ensure they adapted well to their housing environment and feeding conditions.

3.3.3 Extraction of Neem Seed Oil

The neem seed oil was extracted using the procedure described by Eksteen et al. (2001). The process utilized a manual hydraulic pressing unit (Eksteen et al., 2001). The fresh neem fruits were placed in the container of the pressing unit, and a large screw was used to compress them. Oil extraction began at a pressure of 138 bars and continued until the oil flow ceased at approximately 412 bars. The entire extraction process took approximately four hours. The extracted oil was collected in a large container ("Voltic water" bin) and filtered through filter paper to remove impurities.

3.3.4 Treatment Administration and Monitoring

Neem seed oil was administered orally by gavage daily for 21 days. The body weight of each rat was measured at the start and end of each week to monitor changes.

Plate 3.1: images showing administration of neem seed oil through gavaging

3.3.5 Sample Collection and Processing

3.3.5.1 Blood Collection

At the end of the 21-day period, the rats were fasted overnight and euthanized under chloroform anesthesia. Blood samples were collected via cardiac puncture and transferred into lithium heparinized containers to prevent clotting. The samples were centrifuged, and the plasma was extracted for liver function tests.

Plate 3.2: Blood samples collected through cardiac puncture into EDTA and gel and clot activator tubes

3.3.5.2 Organ Collection

The livers and kidneys were harvested, weighed, and preserved in sterile bottles containing 10% formalin for histopathological analysis.

Plate 3.3: Pictures of animal dissection and organ harvesting

3.3.6 Growth and Body Weight Monitoring

The body weights of all experimental Wistar rats were measured weekly using an electronic balance. The process involved:

- Determining the mass of an empty container.
- Placing the rats inside the container and measuring the combined weight.
- Subtracting the container weight to obtain the individual weights of the rats.

3.3.7 Haematological Analysis

Blood samples were collected through cardiac puncture using sterile syringes and needles. The samples were transferred into:

- EDTA tubes for haematological analysis.
- Gel and clot activator tubes for serum biochemical analysis.

The haematological analysis was performed using an Automatic Haematology Analyzer (Rayto RT-7600s, Guangzhou, China). The following indices were analyzed:

- Red blood cells (RBCs).
- Haemoglobin (Hb).
- Hematocrit (HCT).
- Mean corpuscular volume (MCV).
- Mean corpuscular haemoglobin (MCH).
- Mean corpuscular haemoglobin concentration (MCHC).
- Monocytes (MON), neutrophils (NEU), and lymphocytes (LYM).

The analysis was conducted at the Ashanti Mampong Maternity Medical Center.

3.3.8 Serum Biochemistry Analysis

3.3.8.1 Determination of Liver Biochemical Indices

Blood plasma was used for the assay of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), globulin (GLOB), albumin (ALB) and total protein (TP) level using a standard laboratory kit from Randox laboratories, UK.

3.3.8.2 Aspartate amino transferase (AST)

Table 3.1: AST reagent composition

Contents	Initial concentration of solutions
R1. Buffer Phosphate	100 mmol/l, pH 7.
Phosphate buffer	100 mmol/l
L-aspartate.	2 mmol/
α -oxoglutarate	2 mmol/l
R2. 2,4-dinitrophenylhydrazine 100	

a. Procedure for reagent blank

250µl of reagent 1 was added to 50L of distilled water. The solution was mixed and allowed to stand for 30 minutes at 37℃. Reagent 2 was added and the solution was allowed to stand for 20 minutes at 25℃. 2500 µl of 0.4mol NaOH was added. The solution was mixed and the absorbance was taken at 546nm.

b. Procedure for sample

50 µl of blood plasma was added to 250 µl of reagent 1. The solution was mixed and allowed to stand for 30 mins at 37℃. Reagent 2 was added and the solution was allowed to stand for 20 mins at 25℃. 2500 µl of 0.4mol NaOH was added. The solution was mixed and the absorbance of the sample was read at 546nm against the reagent blank after 5 minutes.

3.3.8.3 Alanine Transaminase (ALT)

Table 3.2: ALT reagent composition

Contents	The initial concentration of solutions
R1. Buffer Phosphate	100 mmol/l, pH 7.
Phosphate buffer	100 mmol/l
L-aspartate.	2 mmol/
α -oxoglutarate	2 mmol/l
R2. 2,4-dinitrophenylhydrazine 100	2 mmol/l

a. Procedure for reagent blank

250 µl of reagent 1 was added to 50 µl of distilled water. The solution was mixed and allowed to stand for 30 minutes at 37℃. Reagent 2 was added and the solution was allowed to stand for 20 mins at 25℃. 2500 µl of 0.4mol NaOH was added. The solution was mixed and the absorbance was taken at 546nm.

3.3.8.4 Total Protein (TP) Principle

Table 3.3 TP reagent composition

Contents	**The initial concentration of solutions**
R1. Biuret reagent	
Sodium hydroxide	100 mmol/l
Na-K-tartrate	16 mmol/
Potassium iodide	15 mmol/l
Cupric sulphate	6 mmol/l
R2. Blank reagent	
Sodium hydroxide	100 mmol/l
Na-K-tartrate	16 mmol/l
CAL. Standard	
Protein	
Sodium Azide	<0.1% w/v

R1 was diluted with 400ml of distilled water. The contents of R2 were diluted with 400ml of distilled water.

a. Procedure for reagent blank

20µl of distilled water was added to 1000 l of R1. The solution was mixed and incubated for 30 minutes in the water bath at 25℃.

b. Procedure for standard

20 µl of standard (CAL) was added to 1000 µl of R1. The solution was mixed and incubated at 25℃.

c. Procedure for sample

20 µl of blood plasma was added to 1000 µl of R1. The solution was mixed and incubated at 25℃. The absorbance of the sample and the standard was measured against the reagent blank at 546 nm.

3.3.9 Histopathological Examination

Histopathological analysis of the livers and kidneys involved embedding the tissues in molten paraffin wax, sectioning, and staining with hematoxylin and eosin. Stained slides were examined under a light microscope, and images of the micrographs were captured using a photomicroscope (Motic, Canada) at 100× magnification.

3.3.10 Organ Harvesting Procedure

Following anaesthesia, a midline abdominal incision was performed on the ventral surface of the rats using sterilized surgical instruments. The rats were positioned supine on a dissection board and securely pinned to minimize movement during the procedure. The organs were carefully harvested and weighed.

3.3.11 Kidney Function Test (Urea and Creatine)

An additional aliquot of blood was collected into sterile vacutainer tubes, which were subsequently subjected to centrifugation at a speed of 13000 revolutions per minute for five minutes. The serum was subsequently aspirated, and the Urea and Creatine parameters in the serum were analyzed using commercially accessible kits and a standard BS-120 Mindray Chemistry Analyzer.

3.3.12 Liver and Kidney Histology

Using the protocols outlined by several studies reported earlier (Effah-Yeboah et al., 2021; Mezban & Hussein, 2015). The liver and kidney of rats were fixed in 10% neutral buffered formalin solution for 24 h, dehydrated in an alcohol series, and cleared in xylol solution followed by embedding on the paraffin block. Tissues embedded in the paraffin block were cut using a rotary microtome into 4 μm thick, mounted on glass slides, and stained with hematoxylin and eosin(H&E) procedure, as described by (Andrés-Manzano et al., 2015)., and examined under a light microscope at a magnification of 10 × 10 and 40 × 40.

3.3.13 Statistical Analyses

The data collected were entered into an Excel spreadsheet for statistical analysis. The study results were analyzed using Minitab statistical software, version 20.0. The mean values were calculated and presented alongside the standard error of the mean (SEM). Comparisons between the groups' parameters were made using a one-way analysis of variance (ANOVA), followed by a post-hoc Tukey-HSD test. All of the data were evaluated using a confidence range of 95%, and the findings were considered to be highly significant when the p-value was less than 0.05.

CHAPTER FOUR

RESULTS

4.0 Introduction

This chapter presents the findings of the study, aligned with the specific objectives. The results are organized under the following subheadings: Effects of Neem Seed Oil on Hematological Parameters, Liver Biochemistry, Kidney Function, and Body Weight.

4.1 Effect of Neem Seed Oil on Hematological Parameters

Tables 4.1A, 4.1B, and 4.1C show that the administration of neem seed oil had varying effects on the hematological parameters of Wistar rats. Low-dose neem oil showed no statistically significant effect on any of the hematological parameters measured (Table 4.1A). Medium-dose neem oil significantly increased the lymphocyte count (LYH) from 2.14 to 5.06 × 10^9/L ($p = 0.046$) (Table 4.1B). High-dose neem oil significantly elevated the mean platelet volume (MPV), increasing from 6.63 to 7.17 fL ($p = 0.048$) (Table 4.1C).

Table 4.1A: Effect of low-dose neem oil on haematological parameters of Wistar rats

Hematological Parameters	Normal Control (mean ± SEM)	LDN/0.5ml (mean ± SEM)	T-Test	P-Value
WBC (10^9/L)	3.38 ± 0.73	4.85 ± 1.69	- 0.80	0.509
LYH (10^9/L)	2.14 ± 0.58	3.05 ± 0.63	-0.73	0.520
MID (10^9/L)	0.45 ± 0.03	0.52 ± 0.15	-0.45	0.697
GRA (10^9/L)	0.79 ± 0.12	1.28 ± 0.48	-0.99	0.428
LYH%	61.30 ± 5.21	60.90 ± 5.58	0.05	0.962
MID%	14.23 ± 2.53	12.33 ± 3.54	0.44	0.692
GRA%	24.47 ± 2.79	26.77 ± 2.34	-0.63	0.572
RBC(10^{12}/L)	6.72 ± 0.38	6.80 ± 0.21	-0.18	0.872
HGB (g/L)	15.27 ± 0.81	14.80 ± 0.20	0.56	0.633
HCT%	40.20 ± 1.65	37.80 ± 1.10	1.21	0.313
MCV (μm^3)	57.53 ± 0.82	55.73 ± 1.67	0.97	0.435
MCH (pg)	21.80 ± 0.36	21.80 ± 1.00	0.00	1.000
MCHC (g/L)	37.93 ± 1.08	39.13 ± 1.57	-0.63	0.574
RDW-SD (fL)	37.80 ± 1.24	36.23 ± 2.54	0.56	0.634
RDW-CV%	13.77 ± 0.20	13.77 ± 0.69	0.00	1.000
PLT (10^9/L)	675.3 ± 46.47	821.7 ± 56.99	-1.99	0.140
MPV (fL)	6.63 ± 0.18	6.50 ± 0.15	0.55	0.618
PDW%	11.13 ± 0.43	10.43 ± 0.59	0.95	0.411
PCT%	0.45 ± 0.04	0.54 ± 0.04	-1.45	0.243
P-LCR%	5.43 ± 0.94	5.53 ± 0.26	-0.10	0.928

Note: *Values are expressed as mean ± SEM; LND; Low dose neem oil. There was no significant difference among the parameters; RBC; Red blood cells, RBC; White blood cells, WBC; haematocrit, HCT; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC; Platelets, PLT; lymphocytes, LYM when compared with the control.*

Table 4.1B: Effect of medium-dose neem oil on haematological parameters of Wistar rats

Hematological Parameters	Normal Control (mean ± SEM)	MDN/0.5ml (mean ± SEM)	T-Test	P-Value
WBC (10^9/L)	3.38 ± 0.73	9.51 ± 3.17	-1.89	0.199
LYH (10^9/L)	2.14 ± 0.58	5.06 ± 0.67	-3.30	**0.046**
MID (10^9/L)	0.45 ± 0.03	1.50 ± 0.90	-1.16	0.367
GRA (10^9/L)	0.79 ± 0.12	2.95 ± 1.66	-1.34	0.312
LYH%	61.30 ± 5.21	59.9 ± 10.06	0.12	0.915
MID%	14.23 ± 2.53	13.20 ± 3.91	0.22	0.838
GRA%	24.47 ± 2.79	26.9 ± 6.30	-0.35	0.761
RBC(10^{12}/L)	6.72 ± 0.38	6.18 ± 1.46	0.36	0.752
HGB (g/L)	15.27 ± 0.81	15.27 ± 0.95	0.00	1.000
HCT%	40.20 ± 1.65	33.4 ± 7.22	0.92	0.455
MCV (μm^3)	57.53 ± 0.82	54.67 ± 1.78	1.46	0.282
MCH (pg)	21.80 ± 0.36	27.8 ± 6.59	-0.91	0.461
MCHC (g/L)	37.93 ± 1.08	50.4 ± 11.10	-1.12	0.380
RDW-SD (fL)	37.80 ± 1.24	40.63 ± 5.23	-0.53	0.650
RDW-CV%	13.77 ± 0.20	16.20 ± 2.21	-1.10	0.387
PLT (10^9/L)	675.3 ± 46.47	740.3 ± 54.45	-0.91	0.430
MPV (fL)	6.63 ± 0.18	6.50 ± 0.15	0.55	0.618
PDW%	11.13 ± 0.43	10.47 ± 0.23	1.35	0.269
PCT%	0.45 ± 0.04	0.48 ± 0.040	-0.58	0.601
P-LCR%	5.43 ± 0.94	3.10 ± 1.56	1.28	0.290

Note: *Values are expressed as mean ± SEM; LND; MDN; Medium Dose Neem oil. There was no significant difference among the parameters; RBC; Red blood cells, RBC; White blood cells, WBC; haematocrit, HCT; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC; Platelets, PLT; lymphocytes, LYM when compared with the control.*

Table 4.1C: Effect of High-dose neem oil on haematological parameters of Wistar rats

Hematological Parameters	Normal Control (mean ± SEM)	HDN/0.5ml (mean ± SEM)	T-Test	P-Value
WBC (10^9/L)	3.38 ± 0.73	4.92 ± 1.38	-0.98	0.398
LYH (10^9/L)	2.14 ± 0.58	3.24 ± 0.90	-1.02	0.381
MID (10^9/L)	0.45 ± 0.03	0.670 ± 0.26	-0.85	0.483
GRA (10^9/L)	0.79 ± 0.12	1.01 ± 0.27	-0.73	0.539
LYH%	61.30 ± 5.21	66.17 ± 2.51	-0.84	0.488
MID%	14.23 ± 2.53	12.80 ± 2.21	0.43	0.698
GRA%	24.47 ± 2.79	21.03 ± 1.16	1.14	0.373
RBC(10^{12}/L)	6.72 ± 0.38	6.16 ± 0.22	1.27	0.295
HGB (g/L)	15.27 ± 0.81	13.90 ± 1.01	1.06	0.369
HCT%	40.20 ± 1.65	35.20 ± 1.92	1.97	0.143
MCV (μm^3)	57.53 ± 0.82	57.10 ± 1.64	0.24	0.836
MCH (pg)	21.80 ± 0.36	22.60 ± 1.50	-0.52	0.657
MCHC (g/L)	37.93 ± 1.08	39.50 ± 1.64	-0.80	0.482
RDW-SD (fL)	37.80 ± 1.24	36.10 ± 1.15	1.01	0.388
RDW-CV%	13.77 ± 0.20	13.40 ± 0.05	1.74	0.224
PLT (10^9/L)	675.3 ± 46.47	401 ± 20.46	1.31	0.321
MPV (fL)	6.63 ± 0.18	7.17 ± 0.12	-2.41	**0.048**
PDW%	11.13 ± 0.43	12.67 ± 0.82	-1.65	0.197
PCT%	0.45 ± 0.04	0.29 ± 0.15	0.98	0.430
P-LCR%	5.43 ± 0.94	7.47 ± 3.09	-0.63	0.594

Note: *Values are expressed as mean ± SEM; HDN, High dose neem oil. There was no significant difference among the parameters; RBC; Red blood cells, RBC; White blood cells, WBC; haematocrit, HCT; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC; Platelets, PLT; lymphocytes, LYM when compared with the control.*

4.2 Effect of Neem Seed Oil On Liver Biochemistry

The administration of neem seed oil had varying effects on liver biochemistry across different dosage levels. Low-dose neem seed oil significantly increased Alanine Aminotransferase (ALT) levels from 197.1 to 247.9 ($p = 0.045$). Similarly, medium-dose neem seed oil significantly elevated Alkaline Phosphatase (ALP) levels from 125.6 to 281.1 ($p = 0.036$). However, high-dose neem seed oil had no significant effect on any of the liver biochemical parameters measured, with no differences observed between the control and treatment groups ($p > 0.05$).

Table 4.2A: Effect of low-dose neem oil on liver Biochemistry of Wistar rats

Hepatological	Normal control (mean ± SEM)	LDN/0.5ml (mean ± SEM)	T-Test	P.Value
AST (U/L)	46.17 ± 1.41	46.07 ± 2.15	0.04	0.971
ALT (U/L)	197.1 ± 13.46	247.9 ± 7.22	-3.32	**0.045**
ALP (U/L)	125.6 ± 27.39	144.1 ± 42.36	-0.37	0.738
GLOB (g/L)	38.07 ± 0.99	39.83 ± 2.38	-0.68	0.564
ALB (g/L)	42.03 ± 3.57	45.97 ± 4.62	-0.67	0.549
TP (g/L)	80.10 ± 3.82	85.80 ± 2.23	-1.29	0.288

Note: *LND; Low dose neem oil; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; GLOB, globulin.*

Table 4.2B: Effect of medium-dose neem oil on liver Biochemistry of Wistar rats

Hepatological	Normal control (mean ± SEM)	MDN/1.0ml (mean ± SEM)	T-Test	P.Value
AST (U/L)	46.17 ±1.41	48.867 ± 0.42	-1.83	0.209
ALT (U/L)	197.1± 13.46	258.6 ±25.43	-2.14	0.122
ALP (U/L)	125.6 ±27.39	281.1± 12.65	-5.16	**0.036**
GLOB (g/L)	38.07 ±0.99	46.07 ± 3.20	-2.39	0.139
ALB (g/L)	42.03± 3.57	52.00 ±5.63	-1.50	0.232
TP (g/L)	80.10 ±3.82	98.00 ±7.05	-2.24	0.111

Note: *MDN; Medium Dose Neem oil; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; GLOB, globulin.*

Table 4.2C: Effect of high-dose neem oil on liver Biochemistry of Wistar rats

Hepatological	Normal control (mean ± SEM)	HDN/1.5ml (mean ± SEM)	T-Test	P.Value
AST (U/L)	197.1±13.46	215.0±10.52	-1.05	0.371
ALT (U/L)	46.17±1.41	50.00 ±9.42	-0.40	0.727
ALP (U/L)	125.6±27.39	182.00±86.70	-0.62	0.598
GLOB (g/L)	38.07 ±0.99	35.33±3.20	0.82	0.500
ALB (g/L)	42.03± 3.57	45.80 ±2.19	- 0.91	0.431
TP (g/L)	80.17± 3.82	81 .17± 4.08	- 0.19	0.861

Note: *HDN, High dose neem oil; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; GLOB, globulin.*

4.3 Effect of Neem Seed Oil On Kidney Functions

The results, summarized in Tables 4.3A, 4.3B, and 4.3C, indicate that neem seed oil, across all dosage levels (low, medium, and high), had no significant effect on kidney function parameters. The levels of urea (mmol/L), creatinine (μmol/L), and the urea/creatinine ratio remained comparable between the control and treatment groups, with no statistically significant differences observed ($p > 0.05$).

Table 4.3A: Effect of low-dose neem oil on Kidney Functions of Wistar rats

Nephrological	Normal control (mean ± SEM)	LDN/0.5ml (mean ± SEM)	T-Test	P.Value
Urea/mmol/L	7.58 ± 1.21	6.760 ± 0.16	0.67	0.574
Creatine/μmol/L	55.33 ± 5.36	54.33 ±2.33	0.17	0.880
Urea / Creatine Ratio	66.0±13.06	58.37±3.09	0.57	0.626

Note: *LND; Low dose neem oil. There was no significant difference among the parameters measured (Urea, Creatine, Urea to Creatine ratio).*

Table 4.3B: Effect of medium-dose neem oil on Kidney Functions of Wistar rats

Nephrological	Normal control (mean ± SEM)	MDN/1.0ml (mean ± SEM)	T-Test	P.Value
Urea/mmol/L	7.58±1.21	6.55±0.25	0.83	0.495
Creatine/μmol/L	55.33±5.36	62.7±14.85	-0.47	0.687
Urea / Creatine Ratio	66.0±13.06	55.3±13.93	0.56	0.615

Note: *MDN; Medium Dose Neem oil. There was no significant difference among the parameters measured (Urea, Creatine, Urea to Creatine ratio).*

Table 4.3C: Effect of high-dose neem oil on Kidney Functions of Wistar rats

Nephrological	Normal control (mean ± SEM)	HDN/1.5ml (mean ± SEM)	T-Test	P.Value
Urea/mmol/L	66.0±1.21	56.6±6.70	0.64	0.587
Creatine/μmol/L	55.33 ±5.36	41.70±9.30	1.28	0.292
Urea / Creatine Ratio	7.58±13.06	4.93±0.93	1.71	0.187

Note: *HDN, High dose neem. There was no significant difference among the parameters measured (Urea, Creatine, Urea to Creatine ratio).*

4.4 Effect of Neem Seed Oil on Body Weight

The administration of neem seed oil at low, medium, and high doses showed no significant effect on the body weight of Wistar rats across the observed time points (Day 1, Day 7, Day 14, and Day 21). As presented in Tables 4.4A, 4.4B, and 4.4C, body weight measurements remained consistent between the control and treatment groups, with no statistically significant differences detected ($p > 0.05$).

Table 4.4A: Effect of low-dose neem oil on Body Weight of Wistar rats

Body Weight	Normal control (mean ± SEM)	LDN/0.5ml (mean ± SEM)	T-Test	P.Value
DAY 1	167.00±6.24	172.4± 7.63	-0.71	0.501
DAY 7	191.6± 24.16	187.20± 7.39	0.23	0.833
DAY 14	184.80±14.10	192.40±7.34	-0.62	0.559
DAY 21	180.40±15.02	188.20±8.32	-0.59	0.579

Note: *LND; Low dose neem oil. There was no significant difference between the parameters when compared with the control.*

Table 4.4B: Effect of medium-dose neem oil on Body Weight of Wistar rats

Body Weight	Normal control (mean ± SEM)	MDN/1.0ml (mean ± SEM)	T-Test	P.Value
DAY 1	167.00±6.24	169.6±13.00	-0.23	0.825
DAY 7	191.60±24.16	187.2±7.39	0.23	0.833
DAY 14	184.8±14.10	175.8±11.44	0.64	0.542
DAY 21	180.40±15.02	173.00±8.43	0.53	0.615

Note: *MDN; Medium Dose Neem oil. There was no significant difference between the parameters when compared with the control.*

Table 4.4C: Effect of high-dose neem oil on Body Weight of Wistar rats

Body Weight	Normal control (mean ± SEM)	HDN/1.5ml (mean ± SEM)	T-Test	P.Value
DAY 1	167.00±6.24	165.0±15.37	0.16	0.882
DAY 7	191.60±24.16	167.00±14.45	1.13	0.302
DAY 14	184.80±14.10	180.0±10.11	0.36	0.731
DAY 21	180.40±15.02	171.6±13.12	0.57	0.586

Note: *HDN, High dose neem oil. There was no significant difference between the parameters when compared with the control.*

4.5 Effects Neem Seed Oil on the Histology of the Liver

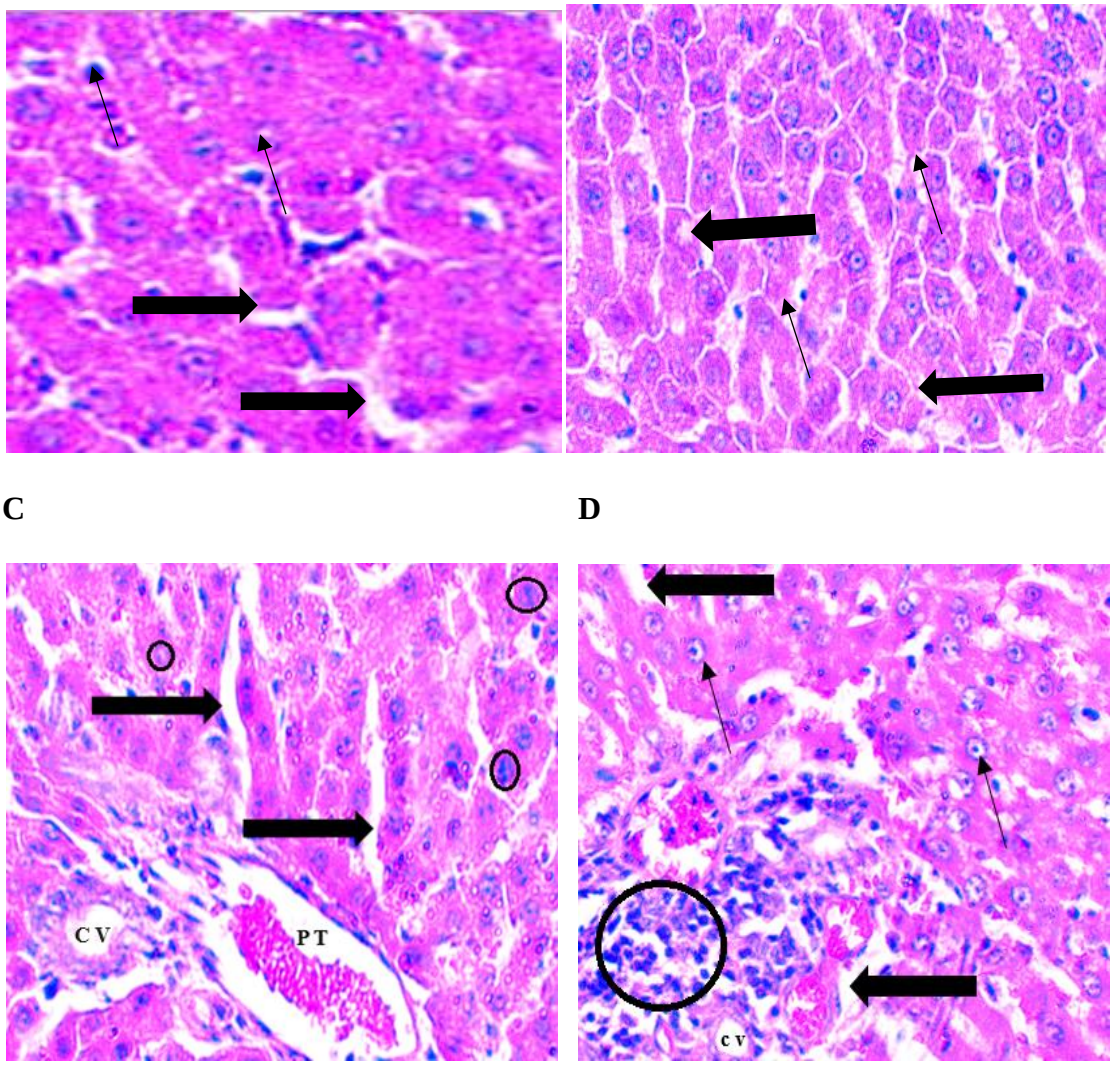
As shown in plate 4.1 below, Plate 4.1A shows normal liver histology indicative of the presence of normal and regularly shaped oval nuclei (thin arrows, →). It has normal sinusoids (pale spaces, thick arrows ➞).

Plate 4.1 B has a normal liver histology and architecture indicative of the polygonal shape and distinct nuclei of hepatocytes. The nuclei are moderately large nuclei (thin arrows, →) and it has a comparably normal sinusoid (thick arrow ➞). Normal liver cells radially arranged around portal tracts and central veins were evident in 0.5 mL/kg.

Plate 4.1C on the other hand has a relatively larger Portal tract (PT), few pale oval vacuolated structures (circle o) and relatively larger sinusoids or pale spaces (thick arrow ➞). The nucleus is well distinct. Moderate to mild fatty liver tissues were observed in 1.0 mL/kg of neem oil–treated animals'plate4.1 G3t3).

Plate 4.1D also displays a moderate liver architecture. The central vein(C V) of the liver in g4t4 (image 4.1) is slightly hypertrophied with distinct regularly oval-shaped nuclei (thin arrows, →). It also has moderately large sinusoids (pale spaces, thick arrow ➞) and an oval pale vacuolated structure. It has a distinct basophilic (blue) coloration which likely suggest proliferated nuclei of cells, such as hepatocytes (liver cells), Kupffer cells (specialized macrophages in the liver), and endothelial cells lining blood vessels.

A B



C D

Plate 4.1: Liver Histology – Indications of Cell Morphology

4.6 Effects Neem Seed Oil on the Histology of Kidney

As shown in plate 4.2 below, plate 4.2A shows a normal kidney histology indicative of the normal renal corpuscles(thin arrows) with glomeruli (GL) that perfectly fit in the Bowman's capsule with a little Bowman's space (thick arrow). Plate 4.2B shows a normal kidney physiology. It depicts a normal Glomeruli (GL) that fits well in the Bowman's capsule with a normal Bowman's space (thick arrow) and well distinct renal corpuscles

(thin arrow). Plate 4.2C on the other hand has relatively larger Bowman's spaces (thick arrows), a well distinct renal corpuscle plate 4.2D also has a relatively larger Bowman's space (thick arrow), it has an oval vacuolated pale structure that resembles an empty Bowman's Capsule (circle o) and a small oval structure (^). The glomeruli were also shrunk in the animal treated with high dose neem seed oil.

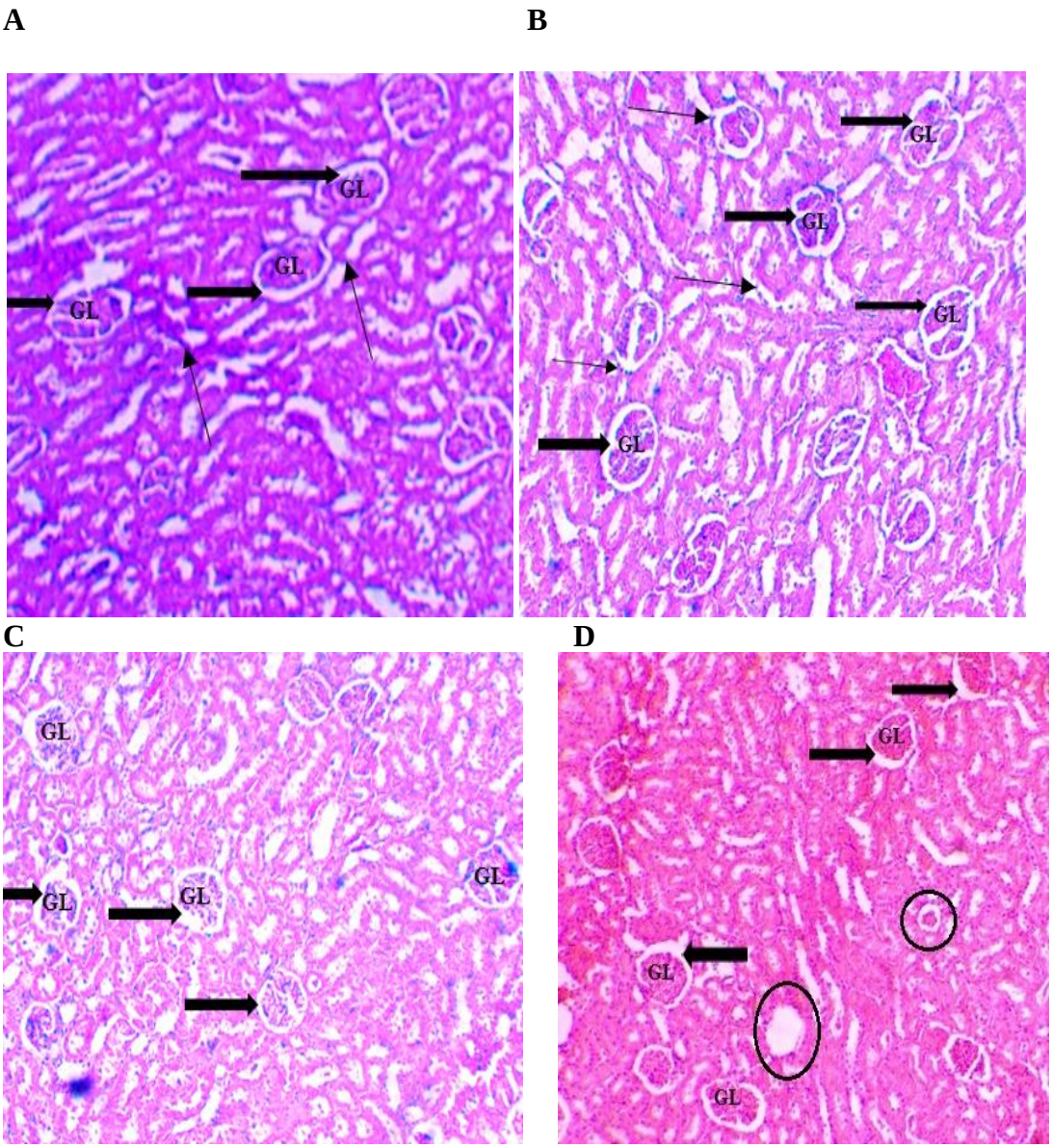


Plate 4.2: Kidney Histology – Indications of Cell Morphology

CHAPTER FIVE

DISCUSSION

5.0 Introduction

This study was conducted to evaluate the effects of varying doses of neem seed oil on haematological parameters, liver histology, kidney function, and body weight in Wistar rats. The specific objectives focused on assessing the growth performance, haematological changes, serum biochemistry, and histological alterations in the liver and kidneys of Wistar rats following neem seed oil administration. The discussion of key findings is presented under the following thematic areas: effects of neem seed oil on haematological parameters, liver biochemistry, kidney function, body weight, and liver and kidney histology. Physiological responses in experimental animals are dose-dependent and vary based on the administered compound's bioactive properties. Neem seed oil is known for its rich bioactive constituents, including azadirachtin, which is reported to exhibit pharmacological and toxicological effects. The liver and kidneys, being vital organs for detoxification and homeostasis, are highly sensitive to such compounds. Assessing haematological indices, biochemical markers, and histological features provides insight into systemic and organ-specific impacts of neem seed oil. Moreover, changes in growth performance may serve as a preliminary indicator of overall health status and metabolic function.

This chapter discusses the study findings in light of existing literature, providing an in-depth interpretation of how neem seed oil doses influence body weight, blood parameters, organ functions, and histological integrity.

5.1 Effect of Neem Seed Oil on Hematological Parameters

This study investigated the dose-dependent effects of neem seed oil on the hematological parameters of Wistar rats, revealing notable findings. Low-dose neem seed oil administration showed no statistically significant changes in any measured parameters, indicating minimal biological impact at lower concentrations. Medium doses, however, significantly increased lymphocyte count (LYH) from 2.14 to 5.06 × 10^9/L, suggesting an immunomodulatory effect that may be attributed to bioactive compounds like azadirachtin and nimbin. Conversely, high-dose neem oil elevated mean platelet volume (MPV) from 6.63 to 7.17 fL, indicating potential platelet activation or production changes, likely due to oxidative stress or inflammation at higher concentrations (Feldman et al., 2000).

These findings align partially with previous studies, reinforcing the immunostimulatory properties of neem oil at medium doses (Biswas et al., 2002) but contrasting with earlier reports suggesting significant effects even at low doses (Girish & Shankara, 2008). The increase in lymphocyte count supports neem oil's established role in promoting immune function, while the elevated MPV aligns with known pro-inflammatory effects of high-dose administration. Differences in preparation methods or experimental conditions might explain the lack of significant effects at low doses compared to prior research. These observations emphasize the complex, dose-dependent biological activities of neem seed oil (Talwar et al., 1997), necessitating further exploration of its mechanisms.

The results carry theoretical, practical, and policy implications. Medium-dose neem oil's ability to enhance lymphocyte counts highlights its therapeutic potential for immune-

compromised conditions, while the pro-inflammatory effects at high doses emphasize the need for careful dose optimization to mitigate risks (Talwar et al., 1997). These findings provide a basis for future studies on neem oil's applications in biomedical and veterinary contexts, offering insights into its safe use. By addressing gaps in knowledge regarding neem seed oil's hematological effects, this study contributes to advancing the understanding of its benefits and risks, reinforcing the importance of evidence-based guidelines for its utilization.

5.2 Effect of Neem Seed Oil On Liver Biochemistry

This current study revealed dose-dependent variations of neem seed oil on liver biochemistry in Wistar rats, with a focus on key liver enzyme parameters such as Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP). Low-dose neem seed oil significantly increased ALT levels from 197.1 to 247.9, suggesting mild hepatocellular stress. Medium-dose neem seed oil elevated ALP levels significantly from 125.6 to 281.1, indicating increased liver activity or mild cholestasis. Surprisingly, high-dose neem seed oil exhibited no significant effect on liver biochemical parameters, with no differences observed between the control and treatment groups. These findings highlight the complex biochemical interactions associated with neem seed oil and its dose-dependent effects on liver function.

The observed increase in ALT at low doses suggests mild hepatocellular injury, as ALT serves as a marker of liver cell damage (Navarro et al., 2008). Elevated ALP at medium doses may reflect increased bile duct activity or hepatobiliary dysfunction, consistent with

studies reporting altered liver enzyme activity following exposure to neem oil's bioactive compounds (Rahman & Sultana, 2006). The lack of significant changes at high doses was unexpected, as higher concentrations of neem oil are often associated with hepatic stress or toxicity (Mukherjee et al., 2010). This anomaly may be due to adaptive metabolic responses or the detoxification of bioactive compounds, a hypothesis warranting further investigation.

These findings align with some studies while contrasting with others. A study by Althaiban (2019) reported significant changes in ALT and ALP levels with moderate doses of neem oil, similar to the medium-dose effects observed in this study (Althaiban, 2019). However, the absence of significant effects at high doses diverges from the findings of Elnakady et al. (2017), who noted hepatotoxicity at elevated doses (Elnakady et al., 2017). This discrepancy might result from differences in experimental design, such as the duration of treatment, preparation of neem seed oil, or the metabolic capacity of the animal model used. Additionally, several studies have shown that bioactive components in neem, such as azadirachtin and nimbin, can induce liver enzyme changes, further supporting the dose-dependent observations in this study (Manukumar et al., 2017; Subramaniam et al., 2021). The observed increases in liver enzymes at low and medium doses highlight the potential risks of liver stress or mild dysfunction when neem seed oil is used therapeutically. The lack of significant effects at high doses raises questions about a possible threshold beyond which the liver's metabolic and detoxification pathways mitigate adverse effects. These findings underscore the need for dose optimization to maximize therapeutic benefits while minimizing risks. Additionally, the study contributes to the growing body of knowledge on

neem oil's effects on liver biochemistry, offering insights for its application in biomedical research and therapeutic interventions.

5.3 Effect of Neem Seed Oil On Kidney Functions

The findings showed no statistically significant impact of neem seed oil (NSO), across low, medium, and high doses, on key renal function indicators, including urea levels (mmol/L), creatinine levels (μmol/L), and the urea/creatinine ratio. These parameters remained consistent between the control and treated groups, with all differences found to be statistically insignificant ($p > 0.05$). These results suggest that NSO, even at higher doses, does not induce nephrotoxicity or disrupt normal kidney function in Wistar rats.

The absence of significant changes in kidney function parameters indicates that NSO is metabolized and excreted efficiently without causing renal stress. Urea and creatinine levels, reliable markers of renal health, staying within normal ranges across all groups further reinforce the non-toxic nature of NSO. Interestingly, even at higher doses, no evidence of renal impairment or physiological burden was observed. This aligns with the hypothesis that NSO, widely used in traditional medicine, would not adversely affect kidney function when administered at controlled doses (Brai et al., 2024; Khanam et al., 2017). However, the complete absence of any measurable effect, even at the highest dose, may point to the efficient renal clearance mechanisms in Wistar rats or the inherent safety of NSO's bioactive components (Khanam et al., 2017).

These findings are consistent with prior research suggesting that neem extracts are generally safe for internal use (Biswas et al., 2002; Puri, 2021). For example, Biswas et al. (2002) and Puri, (2021) highlighted neem's low toxicity profile and potential nephroprotective effects. However, some studies have reported mild toxicity at very high doses, suggesting that NSO's safety profile is dose-dependent (Brandelli et al., 2011). By confirming the safety of NSO within the tested dosage range, this study builds on existing literature and provides additional evidence supporting its therapeutic potential. The lack of nephrotoxic effects in this study suggests that NSO could be safely used in pharmaceutical or nutraceutical formulations if appropriately dosed (Ibrahim et al., 2022).

Theoretically, they highlight the potential of NSO as a natural product with minimal risk of renal toxicity. Practically, the results provide a foundation for further research into NSO's applications in health and medicine, particularly for products requiring long-term use. From a policy perspective, these findings can inform the development of safety guidelines for the use of neem-based products in human and veterinary medicine. By demonstrating the safety of NSO for renal function, this study contributes to the growing body of knowledge on the pharmacological potential of neem and supports its broader application in natural medicine.

5.4 Effect of Neem Seed Oil on Body Weight

The findings of this study reveal that varying doses of neem seed oil did not significantly affect the body weight of Wistar rats across the observation periods (Day 1, Day 7, Day 14, and Day 21). Tables 4.4A, 4.4B, and 4.4C show consistent body weight measurements

between control and treatment groups, with no statistically significant differences observed ($p > 0.05$). This suggests that neem seed oil, at low, medium, and high doses, does not influence body weight, indicating a neutral effect on this specific physiological parameter. The lack of impact on body weight implies that neem seed oil does not alter metabolic processes related to weight regulation, such as appetite, energy expenditure, or fat accumulation (Dare, 2022; Yarmohammadi et al., 2021). These results align with the hypothesis that neem seed oil's effects are dose-dependent, and the concentrations used in this study fall within a safe and non-detrimental range (Yarmohammadi et al., 2021). This finding is consistent with prior research, where neem and its compounds have demonstrated minimal systemic side effects at controlled dosages (Kumar & Navaratnam, 2013).

Interestingly, although higher doses were expected to potentially induce some metabolic effects, the absence of significant weight changes suggests that neem seed oil may lack active components capable of disrupting weight regulation pathways. This contrasts with studies involving other plant oils, which have been associated with weight loss or gain depending on their fatty acid composition (Cousineau, 2024). The consistent body weight findings indicate a favorable safety profile for neem seed oil, supporting its continued use in therapeutic contexts without adverse effects on body weight.

In comparison with existing literature, previous studies have also reported minimal or no weight alterations in animals treated with neem extract, aligning with the current findings (Kumar et al., 2021; Kumar & Navaratnam, 2013). These results contribute to the broader understanding of neem seed oil's safety profile, highlighting its suitability for use in health

formulations. Furthermore, this study addresses a knowledge gap by providing empirical evidence on the neutral metabolic impact of neem seed oil, which is crucial for developing its therapeutic applications in a variety of medical fields.

5.5 Effect of Neem Seed Oil on Liver and Kidney Histology

The biochemical results and histological examinations of liver tissues in both groups are consistent with each other. Both the control group and the animals given low doses of neem seed oil showed normal liver histopathology. The Portal tract, on the other hand, is rather bigger. After administering a high dose of neem seed oil to Wistar rats, researchers found that the rats' portal tracts grew larger. This finding raises the possibility that the rats' livers have become inflamed and fibrotic as a result of the harmful effects of neem oil. A structure known as the portal tract houses the bile duct, hepatic artery branches, and portal vein. It carries serum and bile to and from the organ of liver function. Portal hypertension and impaired liver function can result from neem oil-induced enlargement and scarring of the portal system.

Once again, the liver biochemistry did not show a significant increase; however, the rats given the high dose of neem seed oil had somewhat hypertrophied central veins in plate 4.1D due to the elevation in mean values. According to Lee *et al.* (2020), biliary blockage can be caused by prolonged exposure of the liver to neem seed extract, which is indicated by this hypertrophic reaction (Lee et al., 2020). It is consistent with liver biochemistry that rats exposed to high doses of neem seed oil have dilated and congested blood vessels in the liver, which may explain why these rats grow a bigger sinusoid. Blood flows from the

portal vein and the hepatic artery to the central vein via the sinusoid, a capillary type that passes between the hepatocytes (liver cells). Damage to the liver from neem oil can lead to portal hypertension and impaired liver function due to an enlarged and blood-filled sinusoid. In addition, the Wistar rats that were given a high dose of neem seed oil showed signs of proliferating cell nuclei, such as hepatocytes, Kupffer cells, and endothelial cells. This could indicate that the rats had developed abnormalities in the growth and division of their liver cells as a result of the toxic effects of neem oil when taken in excess. The liver's primary cells responsible for metabolism, detoxification, and synthesis are known as hepatocytes. The liver's indigenous macrophages, known as Kupffer cells, engulf and kill pathogens, damaged cells, and other foreign substances.

The endothelial cells control the permeability and blood flow of blood vessels. As a protective strategy to restore liver function after neem oil injury, these cells may either hypertrophy (grow in size) or hyperplasia (increase in number). However, dysplasia (imperfect cell structure) or neoplasia (uncontrolled cell growth), both of which can develop into cancer (Zhou et al., 2024). The study examined the effects of N-methyl-N'-nitro-N-nitrosoguanidine on gastric cancer development and cell proliferation in Wistar rats. By 24 weeks, it had discovered microscopic cancer, and by 27 weeks, it had discovered macroscopic malignancies (Zhou et al., 2024).

This new study contrasts with an earlier one that investigated how different neem leaf extracts (in water and methanol) affected several haematological indicators in Wistar rats (Ashafa et al., 2012). In contrast to urea, the data demonstrated that the extracts did not

affect AST, ALT, or creatinine concentrations. Additionally, the histological evaluation revealed no unusual alterations in the liver.

A study tested whether neem seed oil could prevent liver damage in Wistar rats caused by carbon tetrachloride (Baligar et al., 2014). A dose-dependent reduction in AST, ALT, and alkaline phosphatase levels was observed in the treated group compared to the control group following neem seed oil administration. According to the histological evaluation, neem seed oil treatment improved liver function and decreased adipose tissue buildup (Sepehrizadeh et al., 2021).

Both plate 4.2A and 4.2B kidney histopathology analyses came back normal. In terms of kidney histology, rats administered a medium dose of neem seed oil plate 4.2C showed an increased Bowman's space compared to the normal and low dose groups plate 4.2B. This finding may indicate that the rats experienced damage and atrophy of the kidney glomeruli as a result of the toxic effects of neem oil at high doses, which is in line with kidney function. Nephron components that encircle the glomerulus a system of capillaries responsible for blood filtration and urine production make up what is known as the Bowman's space. High-dose neem oil causes scarring and shrinkage of the glomerulus in the kidneys, which lowers their filtering ability and widens the Bowman's gap. In plate 4.2D, we also noticed a few enlarged lymphocytes and a few smaller glomeruli. Chronic neem seed extract administration may cause this change in glomeruli shape, which in turn indicates lower renal perfusion (Negi & Mirza, 2020).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Limitations

This study faced several limitations. Firstly, the use of Wistar rats may not fully represent human physiological responses, limiting the generalizability of the findings to humans. Additionally, the investigation focused solely on hematological parameters, potentially overlooking other systemic effects of neem seed oil.

Furthermore, the study's scope was restricted to Wistar rats, which may not accurately reflect human liver responses. The absence of long-term exposure assessments and other liver-related biomarkers restricts a comprehensive understanding of neem seed oil's impact. This narrow focus limits the ability to generalize findings to human liver health.

Lastly, the study's short duration restricted the observation of long-term renal effects and human kidney function. While renal function indicators were prioritized, other related biochemical markers may have been neglected, reducing the overall depth of the analysis. Additionally, the use of Wistar rats limits the extrapolation of findings to human renal health.

6.2 Conclusions

Neem seed oil, at medium doses, significantly increased lymphocyte counts, supporting its potential immunomodulatory benefits. However, high doses raised mean platelet volume

(MPV), indicating pro-inflammatory effects. Further research is necessary to optimize dosage and better understand its full impact.

Low and medium doses of neem seed oil had a noticeable effect on liver biochemistry, resulting in mild hepatocellular stress and cholestasis. In contrast, high doses did not significantly affect liver function, suggesting a dose-dependent relationship and possible adaptive mechanisms at higher concentrations. Neem seed oil did not significantly impact kidney function indicators such as urea and creatinine levels, even at higher doses. This indicates its safety for renal health and minimal risk of nephrotoxicity in Wistar rats.

Additionally, high-dose neem seed oil induced histopathological changes in liver and kidney tissues, such as portal tract enlargement and increased Bowman's space, highlighting the need for careful dose management to ensure safety.

6.3 Recommendations

1. It is recommended that a human model of this study be designed using full blood count and ultrasound monitoring of the internal organs to determine if the same changes seen in the rats would also be seen in humans.
2. It is recommended more work needs to be carried out on determining the toxicity profile (biosafety) of neem seed oil.
3. Further studies should be done to define the pathway involved in these weight reductions.

4. New ideas, methodologies, and markers for neem oil pharmacology study should be assessed.

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