THE EFFECT OF MIRACLE SEED ULTIMA® ON KIDNEY FUNCTION PARAMETERS, HEMATOLOGICAL PARAMETERS, AND GLUCOSE LEVELS IN MALE *WISTAR RATS*

BY

EKRAKE ESTHER EWOMAOGHENE

(BMS2001909)

DEPARTMENT OF MEDICAL BIOCHEMISTRY
BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN,
BENIN CITY, EDO.

NOVEMBER, 2025

THE EFFECT OF MIRACLE SEED ULTIMA® ON KIDNEY FUNCTION PARAMETERS, HEMATOLOGICAL PARAMETERS, AND GLUCOSE LEVELS IN MALE *WISTAR RATS*

BY

EKRAKE ESTHER EWOMAOGHENE

(BMS2001909)

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY IN SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC) DEGREE IN MEDICAL BIOCHEMISTRY, UNIVERSITY OF BENIN

NOVEMBER, 2025

CERTIFICATION

I hereby certify that this project work was written and presented by **EKRAKE ESTHER EWOMAOGHENE** with matriculation number **BMS2001909** of the department of Medical Biochemistry, University of Benin, Benin city, and was supervised by **DR. J. C. ANIONYE.**

.

DR. J. C. ANIONYE	**DATE**
(Project Supervisor)	
DR. N.B. AGUEBOR-OGIE	**DATE**
(A.g. Head Of Department)	
EXTERNAL EXAMINER	**DATE**

DEDICATION

This project work is dedicated to God almighty for his unconditional love, infinite mercies, divine grace and protection.

ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to those who have supported me throughout the journey of completing this project work entitled “Sub-Acute toxicity study of Miracle Seed Ultima® on kidney function, Hematological parameters, and Glucose levels in male *wistar rats*” First and foremost, I wish to extend my deepest appreciation to my supervisor, Dr. J. C. Anionye, for his invaluable guidance, unwavering support, and constructive feedback throughout the research process. His expertise and insights not only facilitated the progression of this dissertation but also inspired me to strive for excellence. I sincerely acknowledge Mrs Ada Ukwuonwo-Ediale, my course adviser, for her guidance and advice through out my academic journey. I’m also grateful the head of department, Dr. N. B**.** Aguebor-Ogie and to the lecturers who taught and guided me, including Dr. Mrs. Ikponwosa-Eweka, Dr. Eluehike, and Dr. Omoruwa, among others and the entire faculty and staff of the Medical biochemistry at University of Benin for creating a stimulating academic environment and providing the resources necessary to pursue my research. Their dedication to fostering a spirit of inquiry has significantly enriched my educational experience.

On a personal note, I offer my deepest thanks to my family and friends for their unwavering support and encouragement. To my family, your belief in me and your constant motivation have been my anchor throughout this academic journey. I am especially thankful to My Dad and Mum, MR and MRS EKRAKE, for their love and understanding, which have given me the strength to persevere. To my Siblings and friends, thank you for your companionship and for being a source of joy and laughter during the long hours of study. In conclusion, I am profoundly grateful to everyone who has played a role in this journey. Your contributions, whether small or significant, have made an indelible impact on both my professional and personal growth. Thank you all for being part of my academic adventure.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Herbal medicines are increasingly used globally, yet comprehensive safety data for many traditional preparations remain limited. This study evaluated the sub-acute toxicity of Miracle Seed Ultima® (MSU), a commercially available herbal product, on kidney function, hematological parameters, and glucose metabolism in male *Wistar rats*. Twenty male *Wistar rats* weighing 120-170g were randomly divided into four groups (n=5): Group 1 (control) received distilled water, while Groups 2, 3, and 4 received MSU at 100 mg/kg, 300 mg/kg, and 1000

mg/kg body weight respectively via oral gavage daily for 28 days. Blood samples were collected at the end of the study for assessment of renal function markers (urea, creatinine, electrolytes), complete blood counts, red cell indices, and fasting blood glucose levels. Key findings revealed significant reductions in plasma urea concentrations in groups receiving 300 mg/kg (74.85±6.3 mg/dL) and 1000 mg/kg (68.62±2.9 mg/dL) compared to controls (102.72±5.7 mg/dL), with $p < 0.05$. Plasma creatinine showed significant differences in the 100 mg/kg group (2.12±0.1 mg/dL) compared to controls (2.79±0.1 mg/dL). However, all electrolyte parameters (sodium, potassium, chloride) remained within normal physiological ranges across all groups. Non-statistically significant differences were observed in all hematological parameters ($p > 0.05$). Fasting blood glucose levels remained normal across all treatment groups. The findings indicate a relatively favorable safety profile for MSU at the tested doses, with no evidence of overt nephrotoxicity, hematotoxicity, or metabolic disturbances. The observed reductions in plasma urea and creatinine may reflect enhanced renal clearance rather than toxicity. These results support the short-term safety of MSU at therapeutic doses.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

The search for novel therapeutic agents from natural sources continues to have intensified due to limitations and adverse effects of synthetic drugs. Medicinal plants provide a rich repository of bioactive compounds with diverse pharmacological properties, making them an important source for drug discovery (Atanasov *et al*., 2021). In recent years, renewed interest in traditional medicine systems has prompted scientific investigations into the safety and efficacy of herbal remedies used empirically for generations (Ekor, 2014).

Miracle Seed Ultima® (MSU) has gained attention as a dietary supplement and traditional remedy. While various plant-based products have been historically been used in the management of metabolic disorders, inflammatory conditions, and infectious diseases (Kumar *et al*., 2020), preliminary reports suggest that MSU seed extracts may possess anti-diabetic, antioxidant, hepatoprotective, and immune-modulatory properties (Eddouks *et al*., 2023). However, the safety toxicological implications of the phytochemicals in combination remain largely unexplored, highlighting a significant knowledge gap.

The kidneys play a pivotal role in maintaining homeostasis through the elimination of metabolic waste products, regulation of electrolyte balance, and control of blood pressure (Perazella, 2019). These organs are particularly vulnerable to xenobiotic-induced toxicity due to their high blood flow and extensive biotransformation activity. Similarly, hepatotoxicity remains a major concern in preclinical studies, as exemplified by acetaminophen-induced liver injury (Yoon *et al*., 2023). The assessment of renal function parameters, including serum creatinine, blood urea nitrogen, and electrolyte levels, constitutes a fundamental component of preclinical toxicity studies.

Hematological parameters are sensitive indicators of physiological and pathological changes, reflecting status of bone marrow, immune system, and overall health (Adeyemi and Akanji, 2022).

Glucose homeostasis represents another critical physiological parameter, particularly for plants with claimed anti-diabetic activity. Medicinal plants may influence blood glucose levels through mechanisms such as enhancing insulin secretion, improving insulin sensitivity, or inhibiting carbohydrate-digesting enzymes (Galicia-Garcia *et al*., 2020; Eddouks *et al*., 2023). While these effects may be therapeutically beneficial in diabetic conditions, they could pose risks in normoglycemic individuals, necessitating careful dose-response evaluation.

Sub-acute toxicity studies conducted over 28 days provide essential data bringing acute and chronic toxicity evaluations, identifying target organs for toxicity, establishing dose-response for future studies (OECD, 2008; Parasuraman, 2021). These studies provide essential information regarding the potential adverse effects of repeated exposure to test substances, identify target organs for toxicity, establish dose-response relationships, and determine appropriate dose levels for subsequent chronic studies (Sellers *et al*., 2019). The use of male *Wistar rats* as the experimental model offers several advantages, including their well-characterized physiology, genetic homogeneity, ease of handling, and extensive historical control data (Sengupta, 2022).

Preliminary phytochemical analyses of MSU formulations indicate the presence of bioactive constituents such as alkaloids, flavonoids, saponins, and phenolic compounds. These compounds are associated with antioxidant activity, immunomodulatory effects, and metabolic regulation, suggesting potential therapeutic benefits. However, the exact toxicological implications of these phytochemicals in combination remain to be fully established.

1.2 Statement of the Problem

Miracle Seed Ultima® (MSU) is widely used in traditional medicine and is increasingly available as a commercial product. However, there is limited information on its safety when taken repeatedly. The kidneys, which are the main organs for removing waste and toxins, may be especially at risk from bioactive compounds in the seeds. While no detailed studies have been conducted on MSU itself, research on similar herbal products suggests that kidney function could be affected (Nwankwo *et al*., 2021). In addition, changes in hematological parameters that could affect the immune system or oxygen transport have not been thoroughly examined for MSU. Studies on other plant preparations indicate that such effects are possible and should be considered (oguntibeju, 2019).

Finally, although MSU may have benefits for blood sugar regulation, these effects need careful study to avoid problems such as low blood sugar in people with normal glucose levels. This lack of comprehensive scientific highlights the need for preclinical investigations to evaluate the safety of MSU properly.

1.3 Significance of the Study

This research holds considerable importance for multiple stakeholders within the healthcare and scientific communities. Primarily, it will provide critical safety data on miracle Seed Ultima® (MSU), enabling evidence-based decisions regarding its therapeutic application (Ekor, 2014). The findings will help establish safe dosage ranges, identify potential adverse effects, and outline appropriate precautions for clinical use, thereby protecting public health and informing regulatory policies.

For healthcare practitioners and herbalists who recommend or prescribe MSU, the study will provide essential information for patient counseling and monitoring protocols (Heinrich *et al*., 2020). Understanding the toxicological profile will enable clinicians to make informed risk-benefit assessments, particularly for vulnerable populations such as individuals with pre-existing kidney conditions, hematological disorders, or metabolic conditions.

From a regulatory perspective, this investigation will generate data to support quality control standards, labeling requirements, and safety warnings for herbal products containing MSU (WHO, 2019). Regulatory authorities increasingly require rigorous preclinical safety data for herbal medicines, and this study will contribute towards meeting those standards. Additionally, the research may serve as a model for toxicological evaluation of other understudied medicinal plants, promoting standardization of safety assessment protocols.

The study's significance extends to the pharmaceutical industry and researchers engaged in natural product development. By elucidating the safety profile and identifying potential toxic constituents, the research may guide the development of safer formulations, standardized extracts, or isolation of specific bioactive compounds with improved therapeutic indices (Newman and Cragg, 2020). Moerover, the findings may reveal novel mechanisms of toxicity, enhancing broader understanding of plant-derived xenobiotic metabolism.

1.4 Justification of the Study

The justification for undertaking this sub-acute toxicity study rests on several compelling factors. First, the increasing global trend toward complementary and alternative medicine has led to widespread use of herbal products, often without adequate safety evaluation (Ekor, 2014; Frass

et al., 2022). Miracle Seed Ultima® (MSU), despite its traditional use, lacks the rigorous toxicological scrutiny required for safe therapeutic application in contemporary medical practice.

Second, the documented nephrotoxic potential of various herbal medicines underscores the necessity of specific renal function assessment (Posadzki *et al*., 2023). The kidneys' critical role in maintaining homeostasis and their vulnerability to toxic insult make them priority organs for safety evaluation. Given that many consumers of herbal products may have undiagnosed renal impairment or concurrent use of nephrotoxic medications, understanding the renal effects of MSU is paramount.

Third, the purported anti-diabetic properties of the plant require careful characterisation of its effects on glucose homeostasis (Eddouks *et al*., 2023). Uncontrolled hypoglycemia represents a potentially life-threatening adverse effect, particularly in non-diabetic individuals or those taking concurrent hypoglycemic agents. Understanding the dose-response relationship for glucose modulation is essential for safe use of MSU.

Finally, the selection of a 28-day exposure period aligns with internationally accepted guidelines for sub-acute toxicity testing (OECD, 2008) and provides sufficient duration to detect cumulative effects while remaining practical for preclinical investigation. The use of male *Wistar rats* offers a validated experimental model with extensive background data, facilitating interpretation of results and comparison with other studies (Sengupta, 2022).

1.5 Aim of the Study

The aim of this study is to evaluate the sub-acute toxicity of Miracle Seed Ultima® (MSU) on kidney function, hematological parameters, and glucose levels in male *Wistar rats*

1.6 Objectives of the Study

The specific objectives of this research are:

1. To determine the effects of different doses of Miracle Seed Ultima® (MSU) on renal function parameters in male *Wistar rats*.
2. To assess the impact of MSU administration on hematological parameters.
3. To evaluate the influence of MSU on fasting blood glucose levels and glucose tolerance in male *Wistar rats*.
4. To compare the safety profile of different doses of MSU with established control groups to determine the potential toxic thresholds.

1.7 Research Questions

This investigation seeks to address the following research questions:

1. Does repeated administration of Miracle Seed Ultima® (MSU) for 28 days cause significant alterations in kidney function markers in male *Wistar rats*?
2. What are the effects of different doses of MSU on hematological parameters?
3. How does sub-acute exposure to MSU influence fasting blood glucose levels and glucose homeostasis in normoglycemic rats?
4. Is there a correlation between dose levels of MSU and the severity or incidence of adverse effects on the measured parameters?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Medicinal Plants and Their Role in Healthcare

Medicinal plants have played a central role in the treatment of various diseases centuries, serving as valuable sources of bioactive phytochemicals (Atanasov *et al*., 2021). The therapeutic potential of these plants has been uncovered through careful exploration of nature and the identification of active constituents in different plant parts, including barks, flowers, fruits, seeds, and stems (Ekor, 2014; Thomford *et al*., 2023). According to the World Health Organization (WHO), traditional medicine encompasses the knowledge and practices used for the diagnosis,

prevention, and treatment of physical, mental, or social imbalances, whether scientifically explained or not (WHO, 2019).

In Africa, traditional healers and plant-based remedies continue to support the healthcare of millions of people, with WHO recognising traditional medicine as one of the most effective ways to achieve comprehensive healthcare coverage for the global population. With the global increase in the use of complementary and alternative medicine, Frass *et al*., 2022 report that herbal therapies and other non-coventional treatments are widely relied upon across different populations, reflecting their growing importance in modern healthcare systems. The affordability of herbal remedies, combined with their perceived lower risk of adverse effects compared to conventional drugs, contributes to their use in developing countries (Ekor, 2014). Medicinal plants are often classified by their physiological and pharmacological effects, including central nervous system activity, anti-inflammatory, anti-allergic, anti-diabetic plants, cytoprotective , antioxidants, and antimicrobial, with many plants exhibiting multiple effects (Newman and Cragg, 2020). Plants with long histories of ethno-medicinal use contain phytochemicals that offer therapeutic benefits against ailments (Heinrich *et al*., 2020).

Despite their natural origin, herbal medicines are not automatically safe and require careful toxicological evaluation before therapeutic application (Yoon *et al*., 2023). Phytochemicals from plants have also become valuable in the pharmaceutical and biotechnology industries, serving as raw materials for drugs, cosmetics, and fine chemicals (Oguntibeju, 2019). Thus, medicinal plants remain indispensable in the drug discovery and development, providing hope for safe and effective future therapies (Posadzki *et al*., 2023; Adebayo and ishola, 2021).

2.2 Overview of Ultima species and Miracle Seed Ultima® (MSU)

The ultima species is a botanical plant that has attracted attention in ethno-pharmacological research due to its reported medicinal properties (Ibrahim *et al.,* 2023). Different part of the plant, including seeds, have traditionally been used across various cultures to manage a range of ailments such as metabolic disorders, inflammatory conditions, infections, and gastrointestinal disturbances (Heinrich *et al*., 2020). These ethno-medicinal uses are largely attributed to the presence of bioactive phytochemicals, which exhibit antioxidant, anti-inflammatory, and anti-diabetic properties (Eddouks *et al*., 2023; Kumar *et al*., 2020).

Miracle Seed Ultima® (MSU), is a commercial formulation derived from the seeds of the ultima species. Although MSU is widely used and promoted for its purported anti-diabetic, antioxidant, hepatoprotective, and immunodeficiency effects, there is limited scientific evidence specifically validating these claims. Existing literature primarily supports the general therapeutic potential and their phytochemical constituents rather than this particular formulation (Ibrahim *et al*., 2023; Eddouks *et al*., 2023).

Therefore, systematic preclinical evaluation of MSU is necessary to establish its safety profile and potential efficacy. Such investigators will provide empirical data to support its therapeutic use and inform safe dosage ranges, contributing to evidence-based application in modern healthcare.

2.2.1 Botanical Description and Distribution

Comprehensive botanical documentation of the specific miracle Seed Ultima® (MSU) is limited in current literature; however, closely related species documented in traditional medicine systems have been well characterised (Adebayo and Ishola, 2021). The plant is believed to be indigenous to tropical regions, where it has been cultivated and utilized for generations in traditional healing practices. Understanding the botanical characteristics, including its botanical characteristics, including, morphological features, growth patterns, and phytochemical composition, is essential for proper identification and standardization of medicinal preparations (Heinrich *et al*., 2020).

Figure 2.2: Commercial MSU- Miracle Seed Ultima® liquid extract products (500ml bottles) (Nigerianherbals.com, 2025).

2.2.2 Traditional Uses and Ethnopharmacological Significance

Traditional medicine systems have employed Miracle Seed preparations for diverse therapeutic purposes (Mohammed *et al*., 2022). Indigenous communities have traditionally used the seeds to manage diabetes mellitus, hypertension, inflammatory conditions, and various infectious diseases. Depending on the intended therapeutic application, the seeds are prepared in different forms, including decoctions, infusions, or powdered formulations (Kumar *et al*., 2020). The empirical knowledge regarding these traditional uses has been transmitted through generations, forming the basis for contemporary scientific investigations into the plant's pharmacological properties (Thomford *et al*., 2023).

2.2.3 Phytochemical Constituents

Preliminary phytochemical analyses of Miracle Seed Ultima® (MSU) and related ultima species indicates the presence of several bioactive constituents, including alkaloids, flavonoids, saponins, tannins, phenolic compounds, and glycosides (Ibrahim *et al*., 2023). These classes of compounds are widely recognized for their contributions to the therapeutic properties of many medicinal plants. Flavonoids are particularly valued for their potent antioxidant activity, which plays a key role in protecting cells from oxidative stress and free radical induced damage (Atanasov *et al*., 2021). Alkaloids present in the seed extract have been associated with various physiological processes, including modulation of neurotransmission and metabolic processes (Newman and Cragg, 2020).

Figure 2.3: *Utima species* flowers displaying natural color polymorphism, with both pure yellow and purple-yellow bicolor forms demonstrating the phenotypic variation within the species (Plantphotography.com, 2025).

Saponins present in the seeds exhibit immunomodulatory, hypocholesterolemic, and anti-inflammatory actions (Kumar *et al*., 2020), while Phenolic compounds contribute significantly to the overall antioxidant capacity of the extract (Ibrahim *et al*., 2023). Tannins, with their characteristic astringent properties, may also play a role in the antimicrobial and anti-inflammatory effects traditionally attributed to the plant (Eddouks *et al*., 2023).

The presence of these diverse phytochemical constituents suggests potential synergistic effects that may enhance the overall therapeutic efficacy of miracle Seed preparations (Heinrich *et al*., 2020). However, the exact composition and concentration of these bioactive compounds can vary significantly depending on factors such as geographical location, cultivation practices, harvesting time, and processing methods, necessitating standardization for consistent therapeutic applications (Thomford *et al*., 2023).

2.3 Kidney Function and Nephrotoxicity

2.3.1 Renal Physiology and Homeostasis

The kidneys play a pivotal role in maintaining homeostasis through multiple essential functions, including the elimination of metabolic waste products, regulation of electrolyte balance, control of blood pressure, maintenance of acid-base balance, and production of hormones such as erythropoietin and renin (Webster *et al*., 2022). These paired organs receive approximately 20-25% of cardiac output, processing about 180 liters of blood daily to produce approximately 1-2 liters of urine (Perazella, 2019). The nephron, as the functional unit of the kidney, consists of the glomerulus, proximal tubule, loop of Henle, distal tubule, and collecting duct, each segment performing specialized functions in urine formation and composition (Webster *et al*., 2022).

The glomerular filtration barrier selectively allows the passage of water, small solutes, and waste products while retaining larger molecules such as proteins and blood cells (Perazella, 2019). The proximal tubule reabsorbs approximately 65-70% of filtered sodium, chloride, and water, along with virtually all filtered glucose and amino acids. The loop of Henle establishes the osmotic gradient necessary for urine concentration, while the distal tubule and collecting duct fine-tune electrolyte balance and water reabsorption under hormonal regulation (Webster *et al*., 2022).

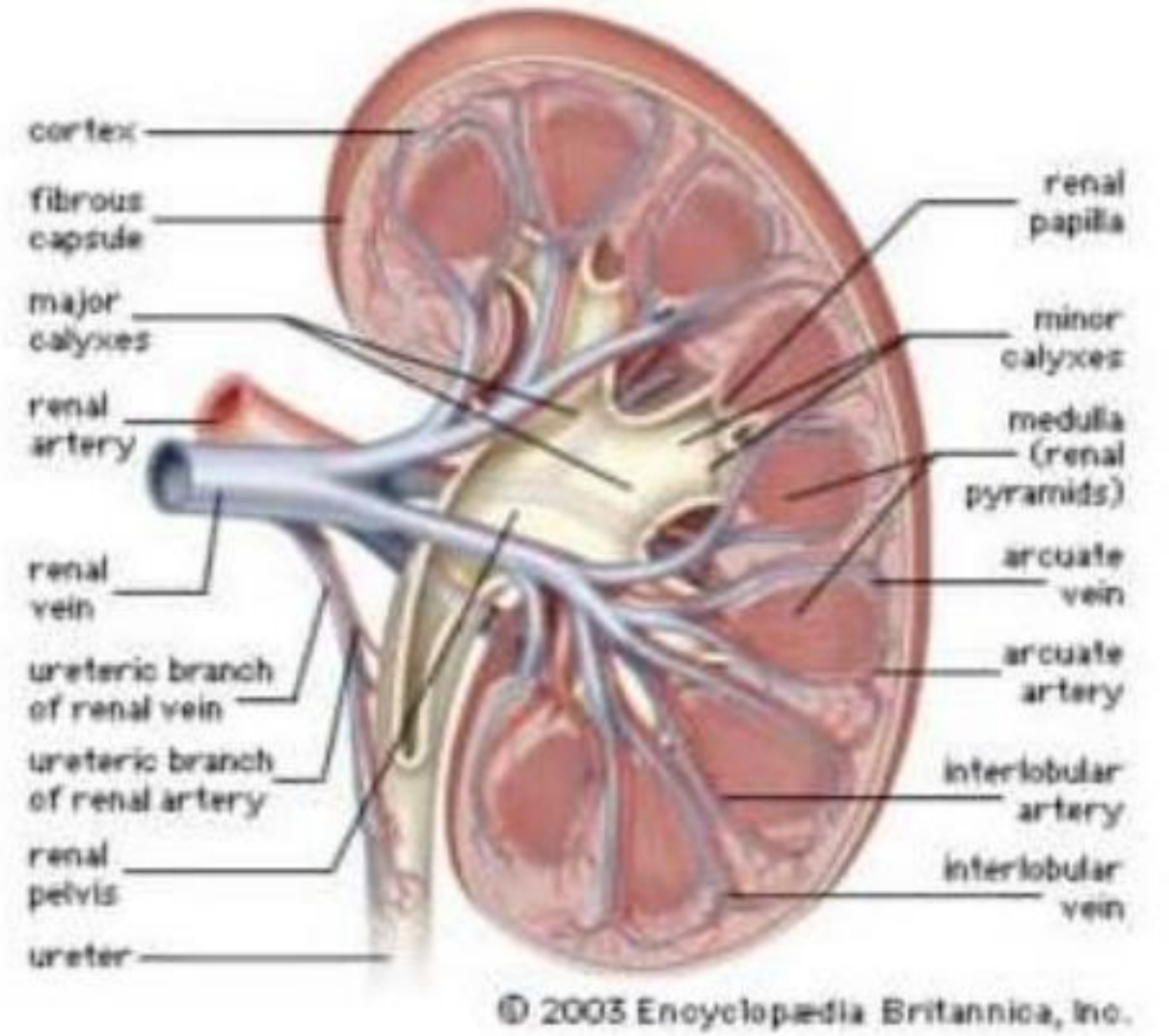

Figure 2.4: Diagram of the kidney (Britannica, 2025).

2.3.2 Renal Vulnerability to Xenobiotics

The kidneys are particularly vulnerable to xenobiotic-induced toxicity due to several anatomical and physiological factors (Perazella, 2019). First, the high blood flow to the kidneys results in disproportionate exposure to circulating toxins and drugs. Second, the concentrating mechanisms within the nephron can significantly increase the local concentration of potentially toxic substances within tubular fluid and cells (Yoon *et al*., 2023). Third, the kidneys possess

extensive biotransformation capacity through various metabolic enzymes, which can generate reactive metabolites that induce cellular injury (Nwankwo *et al*., 2021).

The proximal tubular cells are especially susceptible to toxic injury due to their high metabolic activity, extensive brush border membrane surface area for toxin uptake, and rich complement of transport proteins that facilitate cellular accumulation of xenobiotics (Perazella, 2019). Additionally, the medullary region of the kidney experiences relative hypoxia due to the countercurrent exchange mechanism, rendering cells in this region more vulnerable to oxidative stress and ischemic injury (Webster *et al*., 2022).

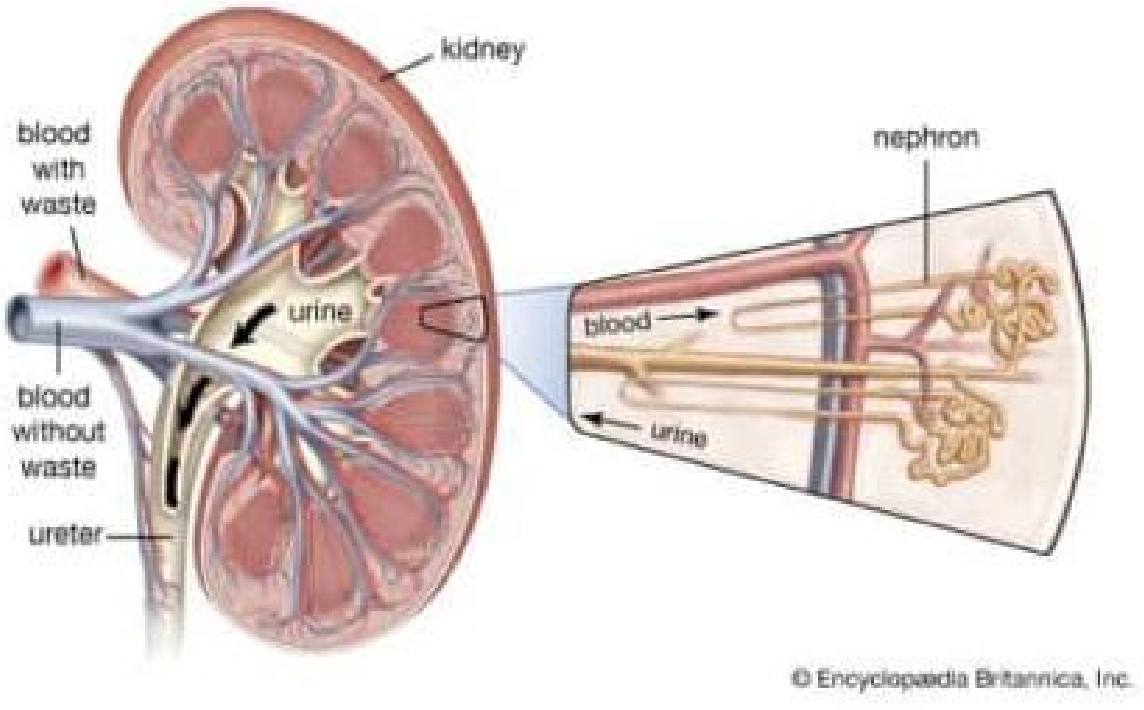

Figure 2.5: Kidney blood supply (Britannica, 2025).

2.3.3 Herbal Medicine-Induced Nephrotoxicity

Despite their perceived safety, herbal medicines have been increasingly recognized as potential causes of kidney injury, with various mechanisms implicated in herbal nephrotoxicity (Yoon *et al*., 2023). Herbal medicine-induced kidney injury can manifest through multiple pathological patterns, including acute tubular necrosis, acute interstitial nephritis, chronic interstitial nephritis, glomerulonephritis, and nephrolithiasis (Nwankwo *et al*., 2021). Several factors contribute to herbal nephrotoxicity, including the inherent chemical composition of plant materials, contamination with heavy metals or pesticides, adulteration with synthetic drugs, improper identification of plant species, and individual patient susceptibility factors (Posadzki *et al*., 2023).

Specific phytochemicals that have been associated with nephrotoxicity include aristolochic acids found in Aristolochia species, which cause progressive tubulointerstitial fibrosis; pyrrolizidine alkaloids present in various plant families, which induce sinusoidal obstruction and hepatorenal syndrome; and oxalates from plants like Averrhoa carambola, which form crystals causing tubular obstruction (Yoon *et al*., 2023). The nephrotoxic potential of many traditional medicinal plants remains inadequately characterized, highlighting the critical need for comprehensive toxicological evaluation before widespread therapeutic application (Nwankwo *et al*., 2021).

2.3.4 Biomarkers of Renal Function

Assessment of kidney function relies on various biomarkers that reflect different aspects of renal physiology and pathology (Webster *et al*., 2022). Serum creatinine represents the most commonly used marker of glomerular filtration rate (GFR), with elevations indicating reduced renal clearance capacity. However, serum creatinine has limitations, including dependence on muscle mass, delayed elevation following acute injury, and insensitivity to early-stage kidney disease (Perazella, 2019).

Blood urea nitrogen (BUN) reflects the balance between urea production and renal excretion, with elevations occurring in conditions affecting GFR as well as in states of increased protein catabolism, dehydration, or gastrointestinal bleeding (Webster *et al*., 2022). The BUN/creatinine ratio can help differentiate prerenal azotemia from intrinsic kidney disease. Serum uric acid levels provide information regarding purine metabolism and renal handling of urate, with elevations associated with gout, kidney disease, and metabolic syndrome (Perazella, 2019).

Electrolyte measurements, including sodium, potassium, chloride, bicarbonate, calcium, and phosphate, reflect the kidney's regulatory functions and can reveal specific tubular dysfunction patterns (Webster *et al*., 2022). Novel biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and cystatin C, offer improved sensitivity for detecting early kidney injury before changes in traditional markers become apparent (Perazella, 2019). The combined assessment of multiple biomarkers provides a comprehensive evaluation of renal function and facilitates early detection of kidney injury in toxicological studies (Sellers *et al*., 2019).

2.4 Hematological Parameters and Toxicity Assessment

2.4.1 Overview of Hematopoiesis

Hematopoiesis is the complex, highly regulated process of blood cell formation occurring primarily in the bone marrow of adult mammals (Adeyemi and Akanji, 2022). This process generates all cellular components of blood, including erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets), from multipotent hematopoietic stem cells through sequential differentiation and maturation stages (Sharma *et al*., 2021). The bone marrow microenvironment, consisting of stromal cells, extracellular matrix, and various growth factors

and cytokines, provides essential signals that regulate stem cell self-renewal, proliferation, differentiation, and apoptosis (Olson *et al*., 2020).

Erythropoiesis, the production of red blood cells, is primarily regulated by erythropoietin, a hormone produced by the kidneys in response to tissue hypoxia (Sharma *et al*., 2021). Mature erythrocytes are anucleate, biconcave discs containing hemoglobin for oxygen transport, with a circulating lifespan of approximately 120 days in humans and 60 days in rats (Adeyemi and Akanji, 2022). Leukopoiesis encompasses the production of various white blood cell lineages, including granulocytes (neutrophils, eosinophils, basophils), lymphocytes (T cells, B cells, natural killer cells), and monocytes, each serving distinct roles in immune defense and inflammatory responses (Sharma *et al*., 2021).

Thrombopoiesis involves the production of platelets from megakaryocytes, with platelets playing crucial roles in hemostasis and wound healing (Olson *et al*., 2020). Thrombopoietin serves as the primary regulator of megakaryocyte development and platelet production. The balanced production and destruction of blood cells maintain stable circulating counts, with various feedback mechanisms ensuring appropriate responses to physiological demands or pathological challenges (Adeyemi and Akanji, 2022).

2.4.2 Hematological Parameters as Toxicity Indicators

Hematological parameters serve as sensitive indicators of physiological and pathological changes in the body, reflecting the functional status of bone marrow, immune system, and overall health (Adeyemi and Akanji, 2022). Routine hematological assessment includes measurement of red blood cell (RBC) count, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration

(MCHC), white blood cell (WBC) count with differential, and platelet count (Sharma *et al*., 2021).

Alterations in erythrocyte parameters can indicate various pathological conditions or toxic effects (Olson *et al*., 2020). Anemia, characterized by reduced hemoglobin concentration, can result from decreased production (bone marrow suppression, nutritional deficiencies), increased destruction (hemolysis), or blood loss. The morphological characteristics of erythrocytes and associated indices help classify anemia and identify potential etiologies (Sharma *et al*., 2021). Conversely, erythrocytosis or polycythemia indicates increased red blood cell mass, which may be primary (bone marrow disorder) or secondary (compensatory response to hypoxia or inappropriate erythropoietin production) (Adeyemi and Akanji, 2022).

Leukocyte alterations provide valuable information regarding immune function, inflammatory responses, and bone marrow status (Sharma *et al*., 2021). Leukocytosis, an elevation in total white blood cell count, can result from infection, inflammation, stress, or hematological malignancies, with the specific pattern of elevation (neutrophilia, lymphocytosis, eosinophilia, monocytosis) suggesting different etiologies (Olson *et al*., 2020). Leukopenia, a reduction in white blood cell count, may indicate bone marrow suppression, overwhelming infection, or autoimmune disorders, potentially compromising immune defense mechanisms (Adeyemi and Akanji, 2022).

Thrombocyte abnormalities reflect alterations in hemostatic capacity (Sharma *et al*., 2021). Thrombocytopenia (low platelet count) increases bleeding risk and can result from decreased production, increased destruction, or splenic sequestration. Thrombocytosis (elevated platelet count) may be reactive (secondary to inflammation, infection, or malignancy) or primary

(myeloproliferative disorder), with potential implications for thrombotic risk (Olson *et al*., 2020). The assessment of platelet function, in addition to platelet count, provides comprehensive evaluation of hemostatic capacity (Adeyemi and Akanji, 2022).

2.4.3 Xenobiotic Effects on Hematopoiesis

Various xenobiotics, including pharmaceutical agents, environmental toxins, and natural products, can adversely affect hematopoiesis through multiple mechanisms (Sharma *et al*., 2021). Direct toxicity to hematopoietic stem cells or progenitor cells can impair proliferation and differentiation, resulting in pancytopenia or selective cytopenias (Olson *et al*., 2020). Some agents interfere with specific maturation stages, producing characteristic morphological abnormalities in circulating cells. Immune-mediated mechanisms can lead to accelerated destruction of mature blood cells or suppression of bone marrow function through antibody or T-cell-mediated processes (Adeyemi and Akanji, 2022).

Herbal medicines and plant-derived products can affect hematopoiesis through various mechanisms (Sharma *et al*., 2021). Some phytochemicals possess inherent cytotoxic properties affecting rapidly dividing cells, including hematopoietic precursors. Certain plants contain compounds that interfere with folate metabolism, vitamin B12 absorption, or iron homeostasis, indirectly affecting erythropoiesis (Olson *et al*., 2020). Immunomodulatory properties of herbal preparations can either enhance or suppress immune cell production and function. Additionally, some traditional medicines contain contaminants or adulterants that directly damage bone marrow or blood cells (Adeyemi and Akanji, 2022).

The hematopoietic system's sensitivity to toxic insult, combined with the accessibility of blood for sampling and the well-established reference ranges for hematological parameters, makes

hematological assessment an essential component of preclinical toxicity studies (Sellers *et al*., 2019). Serial monitoring of hematological parameters facilitates detection of early changes, characterization of dose-response relationships, and assessment of recovery following cessation of exposure (Parasuraman, 2021).

2.5 Glucose Homeostasis and Metabolic Regulation

2.5.1 Physiology of Glucose Homeostasis

Glucose homeostasis represents a critical physiological process whereby blood glucose concentrations are maintained within a narrow range despite varying rates of glucose absorption, utilization, and production (Galicia-Garcia *et al*., 2020). This complex regulatory system involves the coordinated actions of multiple organs, including the pancreas, liver, skeletal muscle, adipose tissue, and brain, orchestrated by hormonal, neural, and substrate-level mechanisms (Eddouks *et al*., 2023). In the postprandial state, insulin secreted by pancreatic β-cells promotes glucose uptake into insulin-sensitive tissues, stimulates glycogen synthesis, and suppresses hepatic glucose production, thereby lowering blood glucose concentrations (Galicia-Garcia *et al*., 2020).

During fasting or periods of increased metabolic demand, counter-regulatory hormones including glucagon, epinephrine, cortisol, and growth hormone act in concert to maintain blood glucose levels through stimulation of hepatic glycogenolysis and gluconeogenesis, lipolysis, and reduction of peripheral glucose utilization (Eddouks *et al*., 2023). The liver plays a central role in glucose homeostasis, serving as both a glucose reservoir through glycogen storage and a glucose producer through gluconeogenesis from non-carbohydrate precursors (Galicia-Garcia *et al*., 2020). Skeletal muscle represents the primary site of insulin-stimulated glucose disposal, while

adipose tissue regulates glucose homeostasis through effects on insulin sensitivity and release of adipokines affecting metabolic regulation (Eddouks *et al*., 2023).

2.5.2 Dysregulation of Glucose Metabolism

Dysregulation of blood glucose levels, whether manifesting as hyperglycemia or hypoglycemia, can have profound implications for metabolic health and overall wellbeing (Galicia-Garcia *et al*., 2020). Chronic hyperglycemia, characteristic of diabetes mellitus, results from absolute or relative insulin deficiency combined with insulin resistance, leading to impaired glucose utilization and excessive hepatic glucose production. Prolonged hyperglycemia causes microvascular complications (retinopathy, nephropathy, neuropathy) through mechanisms involving advanced glycation end products, oxidative stress, and activation of inflammatory pathways (Eddouks *et al*., 2023).

Hypoglycemia, defined as blood glucose concentration below 70 mg/dL, triggers a cascade of counter-regulatory hormone responses and produces symptoms ranging from tremor and palpitations to confusion, seizures, and loss of consciousness in severe cases (Galicia-Garcia *et al*., 2020). Recurrent hypoglycemia can lead to hypoglycemia unawareness, where counter-regulatory responses and symptomatic warnings become blunted, increasing the risk of severe hypoglycemic episodes (Eddouks *et al*., 2023). Both acute and chronic alterations in glucose homeostasis affect multiple organ systems, emphasizing the importance of maintaining euglycemia for optimal health (Galicia-Garcia *et al*., 2020).

2.5.3 Herbal Medicines and Glucose Regulation

Many medicinal plants exert hypoglycemic effects through various mechanisms, making them valuable in diabetes management but potentially hazardous in normoglycemic individuals

(Eddouks *et al*., 2023). Mechanisms of herbal antidiabetic activity include enhancement of insulin secretion from pancreatic β-cells, improvement of insulin sensitivity in peripheral tissues, inhibition of carbohydrate-digesting enzymes (α-amylase, α-glucosidase) thereby reducing postprandial glucose absorption, stimulation of glucose uptake in insulin-sensitive tissues, inhibition of hepatic gluconeogenesis, and antioxidant effects protecting pancreatic β-cells from oxidative damage (Kumar *et al*., 2020).

Specific phytochemical classes associated with glucose-lowering effects include flavonoids, which enhance insulin sensitivity and possess antioxidant properties; alkaloids, which may stimulate insulin secretion or improve glucose uptake; saponins, which inhibit glucose absorption and enhance insulin action; and polyphenols, which modulate glucose metabolism through multiple pathways (Eddouks *et al*., 2023). Some medicinal plants demonstrate insulin-mimetic properties, activating insulin signaling pathways independent of insulin receptor binding (Kumar *et al*., 2020).

Despite being therapeutic in diabetic conditions, the glucose-lowering effects of herbal medicines pose potential risks in normoglycemic individuals or when combined with conventional antidiabetic medications (Eddouks *et al*., 2023). Uncontrolled hypoglycemia represents a serious adverse effect that can occur with excessive doses or in susceptible individuals. Therefore, thorough characterization of the effects of medicinal plants on glucose homeostasis, including dose-response relationships and mechanisms of action, is essential for safe therapeutic application (Kumar *et al*., 2020). Assessment of fasting blood glucose, postprandial glucose, and glucose tolerance provides comprehensive evaluation of a substance's effects on glucose metabolism in toxicological studies (Parasuraman, 2021).

2.6 Sub-Acute Toxicity Studies: Principles and Methodology

2.6.1 Definition and Significance

Sub-acute (also termed subchronic or repeated-dose) toxicity studies represent a crucial component of preclinical safety assessment, providing essential information regarding the adverse effects of repeated exposure to test substances over periods typically ranging from 28 to 90 days (OECD, 2008; Parasuraman, 2021). These studies occupy an important position in the toxicological evaluation hierarchy, bridging the gap between acute toxicity studies (single dose, short observation period) and chronic toxicity studies (prolonged exposure, typically lifespan) (Sellers *et al*., 2019). The primary objectives of sub-acute toxicity studies include identification of target organs for toxicity, characterization of dose-response relationships, determination of the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL), assessment of reversibility of toxic effects, and establishment of appropriate doses for subsequent chronic toxicity and carcinogenicity studies (Parasuraman, 2021).

2.6.2 Study Design and Experimental Considerations

Sub-acute toxicity studies typically employ rodent species, most commonly rats or mice, due to their well-characterized physiology, genetic homogeneity, manageable size, relatively short lifespan, and extensive historical control data (Sengupta, 2022). Study design generally includes a control group and three or more dose groups, with at least 10 animals per sex per group for rodent studies (OECD, 2008). Dose selection aims to produce a range of responses, from no observable effect to clearly toxic effects, without causing excessive mortality that would compromise the study (Parasuraman, 2021).

The route of administration should reflect the intended human exposure route, with oral gavage being the most common route for substances intended for oral consumption (OECD, 2008).

Daily administration continues for the specified study duration (typically 28 days for sub-acute studies), with animals observed daily for clinical signs of toxicity, including changes in behavior, appearance, motor activity, and physiological functions (Sellers *et al*., 2019). Body weight and food consumption are monitored regularly throughout the study period, as these parameters serve as sensitive indicators of overall health status and can reveal toxicity before more specific endpoints are affected (Parasuraman, 2021).

2.6.3 Endpoints and Assessments

Comprehensive toxicological assessment in sub-acute studies encompasses multiple endpoints (Sellers *et al*., 2019). Clinical observations document any abnormal signs, symptoms, or behavioral changes occurring during the study period. Body weight measurements track growth patterns and can reveal growth retardation or weight loss indicative of toxicity. Food and water consumption monitoring assesses effects on appetite and drinking behavior (Parasuraman, 2021).

Clinical pathology assessments include hematological parameters (erythrocyte indices, leukocyte counts, platelet counts) providing information on bone marrow function and immune status; clinical chemistry parameters (markers of hepatic function, renal function, glucose metabolism, lipid metabolism, electrolyte balance) revealing functional alterations in multiple organ systems; and urinalysis parameters (OECD, 2008). These assessments are typically conducted at study termination, although interim assessments may be included for longer studies (Sellers *et al*., 2019).

At study termination, animals undergo complete necropsy with gross pathological examination of all organs and tissues, organ weight measurements for key organs (liver, kidneys, spleen, heart, brain, reproductive organs, adrenal glands) as changes in organ weights can indicate hyperplasia,

hypertrophy, atrophy, or other pathological processes, and microscopic histopathological examination of tissues from control and high-dose groups at minimum, with examination of lower dose groups if findings occur in the high-dose group (Parasuraman, 2021). Target organs identified through clinical pathology or organ weight changes receive particular attention in histopathological evaluation (Sellers *et al*., 2019).

2.6.4 Data Interpretation and Regulatory Considerations

Data interpretation in sub-acute toxicity studies requires integration of all endpoints to develop a comprehensive toxicological profile (Sellers *et al*., 2019). Statistical analysis compares treatment groups to controls, with consideration of dose-response relationships and biological significance in addition to statistical significance. The NOAEL represents the highest dose at which no adverse effects occur, serving as the basis for establishing safe exposure levels for humans through application of safety factors (Parasuraman, 2021). The LOAEL identifies the lowest dose producing adverse effects, helping define the threshold for toxicity (OECD, 2008).

Regulatory agencies worldwide require sub-acute toxicity data as part of the safety assessment for new drugs, food additives, herbal medicines, and other substances intended for human use (Parasuraman, 2021). Standardized protocols, such as those published by the Organisation for Economic Co-operation and Development (OECD), ensure consistency and facilitatecomparison across studies and laboratories (OECD, 2008). Good Laboratory Practice (GLP) compliance ensures data quality, integrity, and reliability for regulatory submissions (Sellers *et al*., 2019).

2.7 The *Wistar rat* as an Experimental Model

2.7.1 Characteristics and Advantages

The *Wistar rat* (Rattus norvegicus) represents one of the oldest and most widely used outbred laboratory rat strains, originating from the Wistar Institute in Philadelphia in 1906 (Sengupta, 2022). Male *Wistar rats* are commonly employed in toxicological studies due to several advantageous characteristics. Their well-characterized physiology and metabolism facilitate interpretation of experimental results and extrapolation to humans. Genetic stability within the strain, despite being outbred, provides consistency across studies while maintaining heterogeneity more representative of human populations compared to inbred strains (Sengupta, 2022).

The manageable size and docile temperament of *Wistar rats* facilitate handling, dosing, and sample collection procedures. Their relatively rapid reproductive cycle and short lifespan (2-3 years) enable efficient study completion and intergenerational studies when needed (Sengupta, 2022). Extensive historical control data accumulated over decades of use in toxicological research provides valuable reference ranges for various parameters, aiding in the interpretation of study findings and identification of treatment-related changes (Sellers *et al*., 2019).

2.7.2 Relevance to Human Toxicology

Rodent models, including *Wistar rats*, serve as predictive models for human toxicity based on evolutionary conservation of fundamental biological processes (Sengupta, 2022). Many toxic mechanisms, including oxidative stress, DNA damage, protein dysfunction, and cellular injury pathways, are conserved across mammalian species. The concordance rate between rodent and human toxicity has been estimated at approximately 70%, supporting the utility of rodent models in safety assessment (Olson *et al*., 2020).

However, important species differences exist that must be considered when extrapolating from rats to humans (Sengupta, 2022). These include differences in metabolic pathways and enzyme activities, absorption and bioavailability characteristics, organ structure and function (e.g., rats lack a gallbladder), and susceptibility to specific toxic mechanisms. Understanding these differences and applying appropriate safety factors when extrapolating from animal data to human risk assessment is essential for ensuring adequate protection of human health (Olson *et al*., 2020).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Test Substance

Miracle Seed Ultima® (MSU), a commercial liquid herbal preparation, was obtained from recognised herbal medicine supplier in Benin City, Edo State, Nigeria. The product was purchased in its commercially packaged from (Figure 2.2). According to the manufacturer's label, the formulation contains extracts derived from seeds of the ultima species. The batch number, manufacturing date, and expiry date of the product were recorded for quality assurance and traceability. The product was stored at room temperature (25 ± 2°C) in its original packaging, protected from direct sunlight and moisture in line with the manufacturer's storage instructions until required for use for the study.

3.1.2 Experimental Animals

Twenty (20) male *Wistar rats*, weighing between 120- 170g, were obtained from the Animal House, Department of Anatomy, Faculty of Basic Medical Science, University of Benin, Benin City, Nigeria. Male rats were selected to avoid hormonal fluctuations associated with the estrous cycle in females, which could interfare with data interpretation. The animals were housed in standard polypropylene cages under controlled environmental conditions, with temperature maintained at 25 ± 2°C, relative humidity at 50-60%, and 12-hour light/dark cycles. The rats were provided with standard rodent pellet chow (Vital Feeds, Jos, Nigeria) and fresh drinking water *ad libitum*. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011) and approved by the Animal Ethics Committee of the University of Benin.

3.1.3 Apparatus and Equipments

The apparatus and equipments used in this study were obtained from certified laboratory suppliers and were in proper working condition at the time of use. Essential apparatus include:

Apparatus:

1. Aniimal Cages
2. Oral gavage needle/tube
3. Syringes (2ml and 5ml, Pyrex, England)
4. Measuring cylinder (Pyrex, England)
5. Micro pipettes (Microlux, England)
6. Universal bottles (Fantastik, England)
7. EDTA sample container (Fantastik, England)
8. Plain sample tubes
9. Metal wire mesh
10. Weighing balance
11. Plastic sample containers
12. Cotton wool (Fantastik, England)
13. Disposable gloves and nose masks

Major Laboratory Equipment

1. Refrigerator (Hisense, China)
2. Glucometer (Roche diagnostics, Germany)
3. Water bath (Memmert, Germany)
4. Homogenizer (IKA, Germany)
5. Centrifuge (Tecmel Tecmel, USA)

3.1.4 Reagents and Chemicals

All chemicals and reagents used in this study were of analytical grade and obtained from reputable laboratory suppliers. The reagents included:

1. Distilled water
2. Analytical grade ethanol
3. Formalin
4. 0.9% normal saline
5. Glacial acetic acid
6. Hydochloric acid (HCl)
7. Sodium Chloride (NaCl)
8. Phosphate-buffered saline (PBS)
9. Sodium hydroxide (NaOH)
10. Ethylenediaminetetraacetic acid (EDTA)

3.2 METHODS

3.2.1 Product Acquisition and Characterization

The Miracle Seed Ultima® (MSU) commercial herbal product was obtained from reputable herbal medicine suppliers in Benin City, Edo State, Nigeria, in September 2025. The product was purchased in its commercially available liquid form (500ml and 1000ml bottles as shown in Figures 2.2 and 2.3. The following product information was documented:

- Product name: Miracle Seed Ultima® (MSU)
- Form: Liquid herbal extract
- Batch number: MSUL-240315-0847
- Manufacturing dates: 30th of June 2025

- Expiry date: 30th of June 2027
- Manufacturer's recommended dosage:10ml (approx. 2 teaspoons) twice daily after meals.

The product was inspected for physical characteristics, including color, odor, consistency, and the presence of any particulate matter or sediment. The bottles were examined to ensure intact seals and proper labeling. The product was stored at room temperature (25 ± 2°C) in a cool, dry place protected from direct sunlight, as recommended by the manufacturer, until use in the experimental study.

3.2.2 Preparation of Test Doses

The Miracle Seed Ultima® (MSU) commercial product was used directly without further extraction or processing to reflect real-world consumption patterns. Three different dose concentrations were prepared based on the manufacturer's recommended dosage and doses used in preliminary studies:

1. **Low dose**: 100 mg/kg body weight
2. **Medium dose**: 300 mg/kg body weight
3. **High dose**: 1000 mg/kg body weight

Since the commercial product was in liquid form, appropriate volumes were calculated to deliver the desired doses based on the product concentration specified by the manufacturer. The product was diluted with distilled water when necessary to achieve a constant administration volume of 1 ml/kg body weight for all dose groups, ensuring consistency in gavage procedure.

Dose Preparation Calculation:

The volume of commercial product required for each dose was calculated using the following formula:

Volume (ml) = [Dose (mg/kg) × Body weight (kg)] / Product concentration (mg/ml)

Fresh dose preparations were made every three days to ensure product stability and potency. The diluted preparations were stored in clean, labeled glass bottles at 4°C between administrations. Before each administration, the preparations were brought to room temperature and mixed thoroughly by gentle shaking to ensure homogeneity.

3.2.3 Animal Acclimatization and Housing

Twenty (20) male *Wistar rats*, weighing between 150- 180g, were obtained from the Animal House of the Department of Anatomy, University of Benin, and allowed to acclimatize to laboratory conditions for 7 days before the commencement of the experiment. During the acclimatization period, animals were housed in standard polypropylene cages (5 rats per cage) with stainless steel wire mesh tops and wood shaving bedding, which was changed every two days to maintain hygiene.

The animal house was maintained under controlled environmental conditions:

- Temperature: 25 ± 2°C
- Relative humidity: 50-60%
- Light/dark cycle: 12 hours (lights on at 6:00 AM, lights off at 6:00 PM)
- Ventilation: Adequate air circulation with 15-20 air changes per hour

The animals were provided with pelleted grower mash (Jerrison Allison Allied Company Ltd, Benin City.Nigeria).

Clean drinking water was provided *ad libitum* in sterile water bottles. Food and water consumption were monitored daily by measuring the amount provided and subtracting the amount remaining after 24 hours. The animals were observed daily during the acclimatization period for any signs of disease, stress, or abnormal behavior. Only healthy animals showing normal behavior, feeding patterns, and consistent weight gain were included in the study.

3.2.4 Experimental Design and Animal Grouping

After the 7-day acclimatization period, the 20 male *Wistar rats* were randomly divided into four groups using the random number table method to minimize selection bias. The random allocation ensured that the average body weight across all groups was similar at the start of the experiment (approximately 130-185g). The animals in each group were marked for individual identification using gentian violet ink on different parts of the rat. The experimental groups were designated as follows:

Group 1 (Normal Control, n=5): Animals received standard rat chow and drinking water *ad libitum*. They received 1 ml/kg body weight of distilled water by oral gavage daily for 28 days. No extract or treatment was administered.

Group 2 (Low Dose, n=5): Animals received 100 mg/kg body weight of MSU-Miracle Seed Ultima® commercial product by oral gavage daily for 28 days.

Group 3 (Medium Dose, n=5): Animals received 300 mg/kg body weight of MSU-Miracle Seed Ultima® commercial product by oral gavage daily for 28 days.

Group 4 (High Dose, n=5): Animals received 1000 mg/kg body weight of MSU-Miracle Seed Ultima® commercial product by oral gavage daily for 28 days.

The dose levels were selected based on the manufacturer's recommended therapeutic dose (low dose), multiples of this dose (medium dose), and a higher dose to assess potential toxic effects (high dose). The duration of 28 days was chosen in accordance with OECD Guidelines 407 for repeated dose 28-day oral toxicity study in rodents (OECD, 2008), which is the standard protocol for sub-acute toxicity evaluation.

3.2.5 Administration of Test Substance

Route of Administration

The Miracle Seed Ultima® (MSU) commercial product was administered once daily by oral gavage between 7:00-8:00 AM throughout the experimentalal period to minimise circadian rhythm variations. The oral route was selected as it reflects the natural route of human consumption and allows for systemic absorption of the bioactive constituents.

Each animal was gently but firmly restrained by an experienced handler to minimize stress and prevent injury. The calculated dose volume, based on individual body weight recorded weekly, was drawn into a 1ml syringe attached to a curved stainless steel gavage needle appropriate for the rat's size. The needle was carefully inserted through the mouth into the esophagus following its natural curvature and gently advanced until it reached the stomach (approximately at the level of the last rib). The dose was slowly delivered into the stomach, each animal was observed for any signs of distress or respiratory difficulty.

The control group received an equivalent volume of distilled water via the same route and at the same time.

Dose Adjustment

Individual body weights were recorded weekly and dose volumes were adjusted accordingly to maintain consistent mg/kg dosing throughout the 28-day study. Weekly weighing also served to monitor the growth patterns and detect any potential adverse effects on body weight gain.

Duration of Treatment

The treatment was conducted daily for 28 days, consistent with internationally accepted sub-acute toxicity testing guildelines for rodents. All administrations were performed by the same trained personnel to ensure consistency and minimize procedural variations.

3.2.6 Fasting Blood Glucose Measurement

Fasting blood glucose levels were determined using Accu-chek Active glucometer. This device operates via an electrochemical method, in which glucose in the blood reacts with glucose oxidase on the test strip, producing electrons that generate a current proportional to the glucose concentration. The measurement is automated, providing rapid and precise glucose readings while reducing potential operator error.

Procedure:

1. Animals were fasted overnight (12 hours, from 6:00 PM to 6:00 AM) with access to water only to standardize metabolic state and minimize variability in glucose levels due to recent food intake.

2. The following morning (6:00-7:00 AM), each animal was gently restrained, and the tail was cleaned with 70% ethanol.

3. The tail was warmed briefly with warm water (37-40°C) or by gentle rubbing to promote vasodilation and facilitate blood collection.

4. A small puncture was made at the lateral tail vein using a sterile lancet.

5. The first drop of blood was wiped away with sterile gauze, and the second drop was used for glucose measurement.

6. A small drop of blood (approximately 5µl) was placed directly onto a glucose test strip inserted into an Accu-Chek Active glucometer (Roche Diagnostics, Germany).

7. The glucometer automatically analyzed the sample, and the result was displayed within 5 seconds in mg/dL.

8. After measurement, gentle pressure was applied to the puncture site with sterile gauze until bleeding stopped.

9. The result was immediately recorded on the data collection sheet.

3.2.7 Terminal Procedures and Sample Collection

At the end of the 28-day treatment period (on Day 29), terminal procedures were conducted to collect blood and tissue samples for comprehensive toxicological evaluation.

Pre-Sacrifice Procedures:

1. Animals were fasted overnight (12 hours) with access to water only to standardize metabolic state for biochemical analyses.

2. Fasting blood glucose was measured as described in section 3.2.7.

3. Final body weight was recorded for each animal.

Euthanasia:

Animals were euthanized using chloroform anesthesia, which was selected for its rapid action and minimal stress to animals. The procedure was conducted in accordance with ethical guidelines for laboratory animal euthanasia:

1. A glass desiccator (euthanasia chamber) was prepared by placing cotton wool soaked with chloroform at the bottom, separated from the animal chamber by a wire mesh.
2. Each animal was placed individually in the chamber and exposed to chloroform vapors.
3. The animal was observed for cessation of respiratory movements and loss of all reflexes (corneal and pedal reflexes).
4. Deep anesthesia was confirmed before proceeding with blood collection.
5. Death was confirmed by absence of heartbeat, respiratory movements, and corneal reflexes for at least 1 minute before proceeding with necropsy.

Record Keeping:

All observations, measurements, and sample collections were meticulously documented on standardized necropsy forms, including: Animal identification and group, Date and time of euthanasia, Final body weight, Gross pathological findings, Organ weights (absolute and relative), Sample collection details and storage locations, Any deviations from standard procedures.

3.3 HEMATOLOGICAL ANALYSIS

Automated hematological analysis was performed using a fully automated haematology analyser. The analyser uses impedence and/or optical methods to count and characterise blood cells. Blood cells passing through an aperture generate electrical signals or are detected optically, which are then translated into quantitative counts and indices, including:

1. Red blood cell (RBC) count
2. White blood cell (WBC) count
3. Haemoglobin concentration (Hb)
4. Haematocrit (HCT)
5. Platelet count (PLT)
6. Mean corpuscular volume (MSV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)

The automated approach ensures rapid, precise, and reproductive measurements while minimizing human error.

Procedure

1. Blood was collected via cardiac puncture or tail vein into EDTA-containing tubes to prevent coagulation.
2. Tubes were gently inverted several times to mix the blood with the anticoagulant.

3. The blood samples was placed in the haemotology analyser, which automatically aspirates and analyses the sample.
4. The results for RBC, WBC, Hb, PLT, and other indices were displayed on the analyser and recorded directly into the data sheet.
5. Quality control procedures were performed using standard control samples to ensure analyser accuracy.

3.4 BIOCHEMICAL ANALYSIS

3.4.1 Serum Preparation

Serum samples stored at -20°C were thawed at room temperature for approximately 30 minutes prior to analysis. Each sample was gently inverted to ensure homogeneity and analysed within one week of collection to minimize degradation of analytes. All biochemical assays were performed using commercial diagnostic kits with standardized protocols, at the Chemical Pathology Laboratory of the Department of Biochemistry, University of Benin.

3.4.2 Estimation of Serum Creatinine

Serum creatinine concentration was determined using the alkaline picrate method (Jaffe reaction). In this reaction, creatinine reacts with picric acid in an alkaline medium to form an orange-red Janovsky complex, with colour intensity proportional to creatinine concentration. Absorbance was measured spectrophotometrically at 490-510 nm. The serum creatinine concentration was calculated to µmol/L (creatinine [µmol/L] = creatinine [mg/dL]) × 88.4. Normal reference range for male *wistar rats* is 0.2 - 0.8mg/dL (17.7 – 70.7 µmol/L).

3.4.3 Estimation of Blood Urea Nitrogen (BUN)

Blood urea nitrogen was determined using the enzymatic urease-Berthelot method. Urea in the sample is hydrolyzed by urease to produce ammonia and carbon dioxide. The ammonia produced reacts with hypochlorite and phenol in the presence of nitroprusside as a catalyst to form a blue indophenol complex. The intensity of the color formed is directly proportional to the urea concentration and is measured at 600 nm. BUN was calculated from urea concentrations using the conversion factor 0.467. Normal reference range for male *wistar rats* is 15 – 21mg/Dl urea (7 – 10 8mg/dL BUN).

3.4.5 Estimation of Serum Electrolytes

Sodium and potassium were measured using either flame photometry or ion-selective electrode methods. Sodium ions emit light at 589nm and potassium at 766nm when ecited in a flame; emitted light intensity is proportional to ion concentration.

Chloride was determined using the mercuric thiocyanate colourimetric method. Chloride ions react to form a red ferric thiocyanate complex, with absorbance measured at 480nm.

Bicarbonate concentration was estimated from total plasma CO_2 content, as bicarbonate constitutes approximately 95% of total CO_2. Normal reference ranges for male *wistar rats* are: 135-155nmol/L, potassium 4.0-6.5 mmol/L, chloride 95-110 mmol/L, and bicarbonate 22-28 mmol/L.

All assays were performed following the manufacturer's instructions using standardized kits to ensure reliability and reproducibility of results.

3.6 ETHICAL CONSIDERATIONS

This study was conducted in accordance with internationally accepted principles for laboratory animal use and care. Ethical approval for the study was obtained from the Animal Ethics

Committee of the University of Benin (Approval Number:_____) before commencement of any experimental procedures. The study protocol, including the experimental design, procedures, and humane endpoints, was reviewed and approved by the committee to ensure the welfare of the animals throughout the study.

3.7 DATA ANALYSIS

All data collected during the study, were recorded on standardized data collection forms. The data were subsequently entered into Microsoft Excel spreadsheets and checked for accuracy by double-entry verification and range checks to identify any data entry errors or outliers. Statistically analyses were performed using (specify software, e.g; SPSS or GraphPad Prism), and a p-value of <0.05 was considered statistically significant, indicating a meaningful difference between groups.

CHAPTER FOUR

4.0 RESULT

This chapter presents the findings of the 28-day sub-acute toxicity study carried out to evaluate the effects of Miracle Seed Ultima® (MSU) on kidney function, fasting blood glucose, and hematological parameters in male *Wistar rats*. The chapter details the biochemical responses of the animals to different doses of the extract, with particular focus on kidney function markers (urea, creatinine, electrolytes), glucose metabolism, and comprehensive blood cell counts and indices.

The results are presented in tabular form, accompanied by statistical analyses showing significant or non-significant differences. Dose-dependent trends are discussed to illustrate the pattern of physiological response to the extract.

These results provide a comprehensive assessment of the safety profile of MSU, forming the basis for the interpretation and conclusions drawn in subsequent chapters.

Statistically significant differences ($P < 0.05$) was observed in Plasma Urea of groups three and four (300mg/kg and 1000mg/kg MSU) when compared with group one (control). The Same was observed in Plasma creatinine of group two (100mg/kg MSU) when compared with group one (control). All the Electrolyte parameters experienced no statistically significant difference across all groups of this study (Table 4.1).

There was no statistically significant difference (P> 0.05) in Fasting Blood Glucose across all the groups in this study (Table 4.2).

There was no statistically significant difference (P>0.05) in all the blood hematological parameters investigated in this study (Table 4.3).

Table 4.1: Effects of Miracle Seed Ultima® on Kidney function parameters

Groups	UREA (mg/dl)	CREATININE (mg/dl)	POTASSIUM (mmol/L)	SODIUM (mmol/L)	CHLORIDE (mmol/L)
1	102.72±5.7^{a}	2.79±0.1^{a}	0.88±0.1^{a}	32.95±0.9^{a}	23.84±0.9^{a}
2	89.32±7.8^{ac}	2.12±0.1^{bc}	0.69±0.0^{ab}	77.25±4.5^{bc}	22.32±1.2^{ab}
3	74.85±6.3^{bcd}	2.29±0.1^{acd}	0.69±0.0^{abc}	72.19±6.6^{bcd}	22.09±1.6^{abc}
4	68.62±2.9^{bcd}	2.29±0.2^{acd}	0.74±0.0^{abc}	67.55±8.2^{bcd}	24.93±0.6^{abc}

Values are represented in Mean ± SEM

Different superscript alphabets on same positions differs significantly (P< 0.05) from each other.

Table 4.2: Effects of Miracle Seed Ultima® on Fasting Blood Glucose parameter

Group	GLUCOSE (mg/dl)
1	72.60±5.5^{a}
2	60.60±7.6^{ab}
3	75.60±3.6^{abc}
4	70.40±7.1^{abc}

Values are represented in Mean ± SEM

Different superscript alphabets on same positions differs significantly (P< 0.05) from each other.

Table 4.3: Effect of Miracle Seed Ultima® on Hematology parameters

GROUP	WBC (10^3/UL)	LYM%	MID(%)	GRAN (%)	LYM# (10^3/UL)	MID# (10^3/UL)	GRAN (10^3/UL)	RBC (10^6/UL)	HBG/dl	HCT(%)
1	12.22±1.6^{a}	76.20±1.6^{a}	13.38±0.9^{a}	10.42±1.2^{a}	9.32±1.2^{a}	1.66±0.3^{a}	1.24±0.2^{a}	7.05±0.3^{a}	14.46±0.4^{a}	42.22±0.7^{a}
2	12.04±2.7^{ab}	75.12±2.7^{ab}	12.16±0.9^{ab}	12.72±2.2^{ab}	9.04±1.9^{ab}	1.44±0.3^{ab}	1.56±0.6^{ab}	6.94±0.2^{ab}	13.82±0.5^{ab}	39.88±1.4^{ab}
3	15.96±1.1^{abc}	77.50±2.2^{abc}	12.28±0.9^{abc}	10.22±1.5^{abc}	12.32±0.7^{abc}	1.98±0.3^{abc}	1.66±0.4^{abc}	7.12±0.4^{abc}	14.52±0.8^{abc}	42.10±2.1^{abc}
4	12.02±1.9^{abc}	73.54±3.1^{abc}	13.08±1.5^{abc}	13.38±1.8^{abc}	9.00±1.7^{abc}	1.54±0.3^{abc}	1.48±0.1^{abc}	6.98±0.2^{abc}	13.58±0.4^{abc}	38.66±1.2^{abc}

GROUP	MCV(FI)	MCH(pg)	MCHC (g/dl)	RDW-SD(FI)	RDW-CV(%)	PLT (10^3UI)	MPV(fl)	PDW(%)	PCT(%)
1	60.24±1.8^{a}	20.50±0.3^{a}	34.20±0.6^{a}	36.76±2.3^{a}	16.48±0.6^{a}	682.20±103.8^{a}	8.26±0.3^{a}	11.20±0.5^{a}	0.57±0.1^{a}
2	57.18±1.5^{ab}	19.72±0.5^{ab}	34.60±0.4^{ab}	33.74±2.1^{ab}	15.70±0.7^{ab}	1016.20±542.2^{ab}	7.93±0.3^{ab}	10.75±0.7^{ab}	0.38±0.0^{ab}
3	59.20±0.8^{abc}	20.34±0.2^{abc}	34.42±0.3^{abc}	35.04±1.5^{abc}	15.96±0.5^{abc}	330.00±27.8^{abc}	7.50±0.1^{abc}	10.44±0.5^{abc}	0.25±0.0^{abc}
4	55.50±1.4^{abc}	19.40±0.3^{abc}	35.10±0.5^{abc}	33.74±0.8^{abc}	16.18±0.2^{abc}	540.40±130.8^{abc}	7.92±0.4^{abc}	10.16±0.7^{abc}	0.44±0.1^{abc}

Values are represented in Mean ± SEM

Different superscript alphabets on same positions differs significantly ($P< 0.05$) from each other.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The present study evaluated the sub-acute toxicity of Miracle Seed Ultima® (MSU) on kidney function, hematological parameters, and glucose metabolism in male *Wistar rats* following 28 days of repeated oral administration at three dose levels (100 mg/kg, 300 mg/kg, and 1000 mg/kg body weight). This investigation addresses a critical knowledge gap regarding the safety profile of this increasingly popular herbal preparation.

Assessment of renal function revealed that plasma urea concentrations significantly decreased in groups receiving 300 mg/kg and 1000 mg/kg MSU compared to controls. Similarly, Plasma creatinine levels were significantly lower in the 100 mg/kg dose group relative to controls. These reductions suggest that MSU may enhance renal clearance or promote more efficient protein metabolism, rather than induce nephrotoxicity. Notably, all measured, electrolytes including sodium, potassium, and chloride, remained within normal physiological ranges across all experimental groups, indicating that tubular functions in nitrogenous waste markers. The maintenance of electrolyte homoeostasis further supports the conclusion that MSU does not adversely affect renal function at the tested doses.

The effects observed in urea and creatinine contrast with typical nephrotoxic patterns, in which serum concentrations of these markers are elevated due to impaired glomerular filtration (Perazella, 2019; Webster *et al*., 2022). This findings of decreased plasma urea and creatinine may be explained by the diuretic or renal perfusion-enhancing properties of bioactive constituents in MSU, particularly flavoids and saponins. Such phytochemicals are known to

modulate renal hemodynamics by promoting vasodilation of afferent arterioles, thereby potentially increasing glomerular filtration rate and urea excretion, as supported by ethnopharmacological reports of related ultima species and previous studies on *Vernonia amygdalina* extracts in rats (kumar et al., 2020; Adeyemi and Akanji, 2022).

Evaluation of glucose metabolism indicated that fasting blood glucose levels did not significantly change in any of the treated groups compared with the control group. This absence of hypoglycemic effects in normoglycemic animals suggests thast MSU does not stimulate excessive insulin secretion under fasting conditions, highlighting a desirable safety characteristic for a herbal product traditionally claimed to have antidiabetic properties. These results align with studies showing that certain plant extracts exert glucose-lowering effects primarily under hyperglycemic conditions, exhibiting a glucose-dependent mechanism that minimizes the risk of hypoglycemia in healthy individuals (Galicia-Garcia *et al*., 2020).

The study revealed no significant alterations in any hematological parameters examined, including WBC, lymphocyte counts, granulocyte counts, RBC, hemoglobin, hematocrit, red cell indices (MCV, MCH, MCHC), and platelet parameters. All values remained within normal reference ranges for male *Wistar rats*. The preservation of white blood cell counts and differentials indicates that MSU does not induce bone marrow suppression or immunosuppression, while stable erythrocyte parameters and red cell distribution widths suggest the absence of hemolysis or abnormal erythropoiesis. Similarly, unaltered platelet counts and indices reflect maintained thrombopoiesis and hemostatic capacity. Collectively, these findings indicate that MSU does not compromise hematopoietic, immune, or clotting functions following sub-acute administration.

When contextualized with existing literature, the observed renal and hematological associated with certain herbal products containimg aristolochic acids or immunosuppressive alkaloids (Yoon *et al.,* 2023; Olson *et al.,* 2020). The present study's results are consistent with other investigations of related seed extracts, which reported no major toxic effects at therapeutic doses (Ibrahim *et al.,* 2023).

These findings have important implications for clinical practice and regulatory oversight. The relatively favorable safety profile at therapeutic and supratherapeutic doses provides reassurance regarding short-term use in healthy individuals. However, individuals with pre-existing renal impairment should exercise caution, as the observed changes in plasma urea and creatinine suggest effects on renal function through incompletely characterized mechanisms.

5.2 CONCLUSION

This 28-day sub-acute toxicity study evaluated the safety profile of Miracle Seed Ultima® (MSU) in male *Wistar rats* at three dose levels (100, 300, and 1000 mg/kg body weight). The investigation assessed renal function, hematological parameters,, and fasting blood glucose to characterize the toxicological profile.

The findings reveal a relatively favorable safety profile with no evidence of overt systemic toxicity. All animals survived to study completion with no clinical signs of toxicity. The observed reductions in plasma urea and creatinine represent intriguing findings that may reflect enhanced renal clearance rather than nephrotoxicity, particularly given the preservation of normal electrolyte levels.

The present study provides valuable preliminary data supporting the relative safety of MSU at therapeutic doses in the short term,.

5.3 RECOMMENDATIONS

1. Require standardized preclinical toxicity testing for herbal products making therapeutic claims
2. Establish quality control standards for botanical preparations
3. Mandate appropriate product labeling with dosage recommendations and warnings
4. Implement post-market surveillance systems for adverse effects monitoring
5. Support research initiatives investigating traditional medicine safety and efficacy

N. B REFERENCES

Adebayo, S. A., and Ishola, A. A. (2021). Phytochemical and toxicological studies of Nigerian medicinal plants: A review. *Journal of Medicinal Plants Research, 15*(8): 329-342.

Adeyemi, O. S., and Akanji, M. A. (2022). Hematological and biochemical effects of aqueous extract of *Vernonia amygdalina* in diabetic rats. *African Journal of Biomedical Research, 25*(1): 67-75.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., and Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*, *20*(3): 200-216.

Eddouks, M., Chattopadhyay, D., and Zeggwagh, N. A. (2023). Medicinal plants in the management of diabetes mellitus: Traditional uses and pharmacological properties. *Current Diabetes Reviews, 19*(2): 145-168.

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4*: 177.

Frass, M., Strassi, R. P., Friehs, H., Mullner, M., Kundi, M., and Kaye, A. D. (2012). Use and acceptance of complementary and alternative medicine among the general population and medical personnel: A systemic review. *Ochner Journal, 12*(1), 45-56.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., and Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. *International Journal of Molecular Sciences, 21*(17): 6275.

Heinrich, M., Appendino, G., Efferth, T., Fürst, R., Izzo, A. A., Kayser, O., Pezzuto, J. M., and Viljoen, A. (2020). Best practice in research – Overcoming common challenges in phytopharmacological research. *Journal of Ethnopharmacology*, *246*: 112230.

Ibrahim, M. A., Koorbanally, N. A., and Islam, M. S. (2023). Phytochemical screening and antioxidant activity of medicinal plants: Recent advances. *Antioxidants*, *12*(3): 674.

Kumar, S., Narwal, S., Kumar, V., and Prakash, O. (2020). Traditional herbal medicines: Safety concerns and future prospects. *Journal of Ayurveda and Integrative Medicine*, *11*(4): 451-460.

Kumar, S., Sharma, S., Vasudeva, N., and Rauf, A. (2020). Medicinal plants in management of diabetes: Comprehensive review on ethnopharmacology and phytochemistry. *Combinatorial Chemistry and High Throughput Screening, 23*(10): 1013-1028.

Mohammed, A., Tanko, Y., Okasha, M. A., Magaji, R. A., and Yaro, A. H. (2022). Toxicological evaluation of medicinal plants used in traditional medicine: A systematic review. *Toxicology International*, *29*(2), 167-179.

MSU-Miracle Seed Utima herbal supplements. (n.d.). [Product photograph]. Retrieved November 4, 2024, from https://www.nigerianherbals.com/miracle-seed

Newman, D. J., and Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, *83*(3): 770-803.

Nwankwo, J. O., Emerole, C. U., and Ifenkwe, D. C. (2021). Nephrotoxic potentials of some commonly used herbal preparations. *Journal of Herbal Medicine, 29*: 100475.

Organisation for Economic Co-operation and Development (OECD). (2008). *Test No. 407: Repeated dose 28-day oral toxicity study in rodents*. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing.

Oguntibeju, O. O. (2019). Medicinal plants with hepatoprotective activity: A review. *African Journal of Traditional, Complementary and Alternative Medicines*, *16*(2): 11-30.

Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., Van Deun, K., Smith, P., Berger, B., and Heller, A. (2020). Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology and Pharmacology, 32*(1): 56-67.

Parasuraman, S. (2021). Toxicological screening. *Journal of Pharmacology and Pharmacotherapeutics, 2*(2): 74-79.

Perazella, M. A. (2019). Pharmacology behind common drug nephrotoxicities. *Clinical Journal of the American Society of Nephrology, 14*(12): 1799-1808.

Posadzki, P., Watson, L. K., Alotaibi, A., and Ernst, E. (2023). Prevalence of herbal medicine use by UK patients: A systematic review of surveys. *Focus on Alternative and Complementary Therapies*, *28*(1): 12-23.

Sellers, R. S., Morton, D., Michael, B., Roome, N., Johnson, J. K., Yano, B. L., Perry, R., and Schafer, K. (2019). Society of Toxicologic Pathology position paper: Organ weight recommendations for toxicology studies. *Toxicologic Pathology, 35*(5): 751-755.

Sengupta, P. (2022). The laboratory rat: Relating its age with human's. *International Journal of Preventive Medicine*, *13*: 110.

Sharma, A., Tiwari, S., Singaravel, M., and Misra, A. (2021). Hematological changes in drug toxicity: An overview. *Clinical Toxicology*, *59*(5): 371-382.

Sharma, S., Ray, A., and Sadasivam, B. (2021). The role of hematological parameters in toxicity studies. *Archives of Toxicology, 95*(1): 75-89.

The Editors of Encyclopaedia Britannica (2025, April 19). kidney. *Encyclopedia Britannica*. https://www.britannica.com/science/kidney

Thomford, N. E., Awortwe, C., Dzobo, K., Adu, F., Chopera, D., Wonkam, A., Skelton, M., Blackhurst, D., Dandara, C., and Asiimwe, I. G. (2023). Inhibition of CYP2B6 by medicinal plant extracts: Implications for drug metabolism and interactions. *Toxicology Reports, 3*: 643-652.

Utima species multi-colored flowers. (n.d.). [Photograph]. Retrieved November 4, 2024, from https://www.gardenimages.com/utima-flowers

Webster, A. C., Nagler, E. V., Morton, R. L., and Masson, P. (2022). Chronic kidney disease. *The Lancet, 389*(10075): 1238-1252.

World Health Organization. (2019). *WHO guidelines on good herbal processing practices for herbal medicines*. World Health Organization.

World Health Organization. (2019). *WHO global report on traditional and complementary medicine 2019*. World Health Organization.

Yoon, E., Babar, A., Choudhary, M., Kutner, M., and Pyrsopoulos, N. (2023). Acetaminophen-induced hepatotoxicity: A comprehensive update. *Journal of Clinical and Translational Hepatology, 4*(2): 131-142.

APPENDIX I


MIRACLESEED
MIRACLE ULTIMA INT'L LTD
...Stay Healthy!! Stay Wealthy!!!
Explore The
Healing Power Of
MIRACLE
SEED
Ultima
100% Natural Curative Medicine
MIRACLESEED
MIRACLE ULTIMA INT'L LT
...Stay Healthy!! Stay Wealthy!!!
Explore The
Healing Power Of
MIRACLE
SEED
Ultima
100% Natural Curative Medicine

APPENDIX II

Effects of Miracle Seed Ultima® on Initial, final body weight and various tissues of male *Wistar* rats

Group	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)	LIVER (g)	LEFT KIDNEY (g)	HEART(g)	SPLEEN (g)
1	155.71±8.5^{a}	200.08±10.9^{a}	7.61±0.8^{a}	0.69±0.4^{a}	1.94±1.3^{a}	0.91±0.1^{a}
2	155.37±7.8^{ab}	182.83±11.8^{ab}	6.79±0.7^{ab}	0.59±0.0^{ab}	0.68±0.0^{ab}	0.93±0.0^{ab}
3	155.26±7.4^{abc}	191.69±10.7^{abc}	7.68±0.5^{abc}	0.67±0.0^{abc}	0.72±0.0^{abc}	1.14±0.2^{abc}
4	154.55±6.9^{abc}	178.34±10.9^{abc}	6.37±0.5^{abc}	0.59±0.0^{abc}	0.65±0.0^{abc}	1.04±0.0^{abc}

Values are represented in Mean ± SEM

Effects of Miracle Seed Ultima® on Initial, final body weight and various tissues of male *Wistar* rats

Group	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)	LIVER (g)	LEFT KIDNEY (g)	HEART(g)	SPLEEN (g)
1	155.71±8.5^{a}	200.08±10.9^{a}	7.61±0.8^{a}	0.69±0.4^{a}	1.94±1.3^{a}	0.91±0.1^{a}
2	155.37±7.8^{ab}	182.83±11.8^{ab}	6.79±0.7^{ab}	0.59±0.0^{ab}	0.68±0.0^{ab}	0.93±0.0^{ab}
3	155.26±7.4^{abc}	191.69±10.7^{abc}	7.68±0.5^{abc}	0.67±0.0^{abc}	0.72±0.0^{abc}	1.14±0.2^{abc}
4	154.55±6.9^{abc}	178.34±10.9^{abc}	6.37±0.5^{abc}	0.59±0.0^{abc}	0.65±0.0^{abc}	1.04±0.0^{abc}

Values are represented in Mean ± SEM

Different superscript alphabets on same positions differs significantly ($P< 0.05$) from each other