HISTOMORPHOLOGICAL ASSESSMENT OF *Manihot esculenta* LEAF EXTRACT EFFECT ON RENAL AND HEPATIC TISSUE OF LEAD-INDUCED TOXICITY IN ALBINO RATS

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SCHOOL OF BASIC MEDICAL SCIENCES

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UNIVERSITY OF BENIN

BENIN CITY.

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THIS PROJECT IS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE

SUPERVISED BY

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OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by **IKOSINI USERHUMU ESTHER** with matriculation number: **BMS2001167** in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree**.**

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DEDICATION

I dedicate this project work to God Almighty, for making this work a great success, to my lovely parents and family members for their constant support throughout the process.

ACKNOWLEDGEMENTS

I give thanks to God Almighty for His grace upon my life and for seeing me through this project work.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES** 55

ABSTRACT

Lead is a toxic heavy metal associated with renal and hepatic injury through oxidative stress and biochemical disruption. This study evaluated the histomorphological and protective effects of *Manihot esculenta* leaf extract on lead-induced renal and hepatic toxicity in albino rats. Literature highlights lead's mechanisms of toxicity and the antioxidant, anti-inflammatory, and organoprotective properties of *M. esculenta.* Twenty-five albino rats were divided into five groups: control, lead only (60 mg/kg), lead plus extract (500 mg/kg), lead plus extract (1000 mg/kg), and extract only (1000 mg/kg). Treatments were administered orally for 28 days, after which body weights, organ weights, biochemical parameters, and histological changes were assessed. Body and kidney weights showed no significant differences ($p = 0.177$; $p = 0.753$), but liver weights varied significantly ($p = 0.010$), with hepatomegaly in the lead plus extract (500 mg/kg) group. Biochemical markers (urea, creatinine, electrolytes, bilirubin, AST, ALT, ALP) were largely unchanged ($p > 0.05$), except chloride levels ($p = 0.035$), which were elevated in the lead plus extract (1000 mg/kg) group. Histological analysis revealed hepatocellular degeneration and renal tubular necrosis in the lead-only group, while extract-treated groups showed preserved hepatic cords, intact glomeruli, and reduced inflammatory changes. In conclusion, *M. esculenta* leaf extract demonstrated dose-dependent protective effects against lead-induced renal and hepatic toxicity, with moderate doses (500 mg/kg) offering optimal benefit. These findings support its potential as a complementary therapeutic agent in managing heavy metal toxicity.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Lead has been recognized as a potent environmental toxin for centuries, with its widespread use since the industrial revolution leading to significant contamination of air, water, and soil (Jaishankar *et al.,* 2017). Human exposure to lead can occur through various routes, including inhalation of lead-containing dust and fumes, ingestion of contaminated food and water, and dermal contact (Centers for Disease Control and Prevention, 2017; World Health Organization, 2017; Jaishankar *et al.*, 2017). Once absorbed, lead enters the bloodstream and is distributed to various organs, where it exerts its toxic effects (Obasi, and Akudinobi, 2020).

The mechanisms of lead toxicity are complex and multifaceted. Lead does not have any known biological role in the human body and primarily affects several biological activities by disturbing the hematopoietic, hepatic, renal, and central nervous systems (Jaishankar *et al.*, 2017). One primary mechanism involves the generation of reactive oxygen species (ROS), including hydrogen peroxide and singlet oxygen, leading to augmented oxidative stress (Jaishankar *et al.*, 2017). This oxidative stress causes free radical damage, enhancing membrane lipid peroxidation and affecting the function of membrane-bound proteins, receptors, and enzymes (Obasi, and Akudinobi, 2020). Lead also directly depletes antioxidants, contributing to this imbalance (Obasi, and Akudinobi, 2020). Another key mechanism is the inhibition of enzyme activities. Lead has a high affinity for sulfhydryl (-SH) groups, and its binding to these groups inhibits the function of enzymes such as delta-aminolevulinic acid dehydratase (δ-ALAD), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) (Jaishankar *et al.,*

2017; Obasi, and Akudinobi, 2020). This inhibition disrupts vital metabolic pathways and antioxidant defenses. Lead can interfere with essential metal ions by mimicking or competing with them, such as calcium, zinc, and iron, thereby disrupting cellular processes that rely on these ions (Flora *et al.,* 2012; Jaishankar *et al.,* 2017). For instance, lead can act as a calcium analogue, interfering with ion channels and disrupting neurotransmitter release (Flora *et al.,* 2012).

The kidneys are highly vulnerable to lead toxicity due to their role in filtering blood and concentrating toxins. Chronic lead exposure can lead to nephropathy, characterized by progressive tubular, glomerular, and interstitial damage, ultimately progressing to chronic kidney disease (El-Sayed *et al.,* 2017). Histomorphologically, tubular changes often appear earlier than glomerular and interstitial ones (Obasi, and Akudinobi, 2020). Specific alterations observed in renal tissue include anisokaryosis (variation in nuclear size), nuclear pyknosis (condensed nuclei), karyomegaly (enlarged nuclei), and the development of intranuclear and cytoplasmic inclusions (El-Sayed *et al.,* 2017). Tubular damage can manifest as dilation, necrosis (cell death), vacuolization, and tubular hyperplasia (El-Sayed *et al.,* 2017). Glomerular alterations include mesangial hypercellularity, segmental proliferation, focal and segmental glomerulosclerosis, glomerular hyalinization, and glomerular tuft alterations. These changes indicate significant histological and histochemical damage that can lead to severe complications (El-Sayed *et al.,* 2017).

Similarly, the liver, being the primary organ for detoxification and metabolism, is a major target for lead-induced damage (Basha *et al.,* 2019). Lead can cause hepatocellular necrosis, inflammation, and steatosis (fatty change), leading to impaired liver function (Afridi *et al.,* 2018). The histopathological manifestations in the liver often include disorganized hepatic cords, sinusoidal congestion, cellular

degeneration, and inflammatory cell infiltration (Navas *et al.,* 2017). Specific hepatic changes observed include anisokaryosis, nuclear vesiculation, binucleation, cytoplasmic inclusions, cytoplasmic swelling, hydropic degeneration, necrosis, and a reduction in glycogen content (Jing *et al.,* 2020). Additionally, chronic portal triaditis (inflammation of the portal areas), Kupffer cell hyperplasia (increase in liver macrophages), and occasional fatty changes have been reported. These structural damages can lead to significant functional impairments in the liver (Miranda *et al.*, 2017).

The search for effective and safe interventions against heavy metal toxicity has gained considerable momentum. While conventional chelating agents are effective in removing heavy metals from the body, they are often associated with negative side effects such as gastrointestinal problems or kidney injury, and can be expensive (World Health Organization, 2017). Some chelating agents may not fully restore related adverse effects or prevent toxicant redistribution. This has spurred interest in natural products, particularly medicinal plants, as potential sources of less toxic and more accessible therapeutic agents (Muzanila *et al.,* 2023). *Manihot esculenta* (cassava) is a plant of significant socioeconomic importance globally, widely cultivated as a staple food crop (Montagnac *et al.,* 2017). Beyond its role as a food source, its leaves have been traditionally used in various folk medicine systems to treat a range of ailments (Montagnac *et al.,* 2017). Modern scientific investigations have begun to validate some of these traditional uses, attributing the observed therapeutic effects to the presence of bioactive compounds such as flavonoids, phenolic acids, carotenoids, and vitamins (Montagnac *et al.,* 2017). These phytochemicals are known for their antioxidant, anti-inflammatory, and metal-chelating properties, which could potentially offer protection against heavy metal-

induced organ damage (Adeyemi *et al.,* 2020). For example, rutin, a flavonoid found in many plants, exhibits protective effects on the liver and kidneys by counteracting organ toxicity through its antioxidant, anti-inflammatory, and antiapoptotic properties. It enhances antioxidant enzymes, inhibits inflammatory mediators, and reduces apoptosis (Amadi and Nzeh, 2018). While cassava roots contain Cyanogenic glycosides that can be toxic if not properly processed, traditional processing methods aim to reduce these levels (Montagnac *et al.,* 2017). The focus of this study is on the potential benefits of the leaf extract, which is rich in beneficial phytochemicals. This study will therefore delve into the specific histomorphological changes induced by lead in the renal and hepatic tissues and assess how the intervention with *Manihot esculenta* leaf extract might mitigate these deleterious effects.

1.2 Statement of Problem

Though its toxic properties are widely known, there are few expensive or side effect-prone treatment options available for reducing lead-induced toxicity in renal and hepatic tissue. Trado-medicinal therapies are becoming increasingly popular as possible protective agents against heavy metal poisoning. *Manihot esculenta* (cassava) leaves are commonly ingested in many African countries and are known to have antioxidant, anti-inflammatory, and cytoprotective qualities (Boukhers *et al*., 2022). However, there have been few experimental research examining the histomorphological effect of *Manihot esculenta* on lead-induced renal and hepatic tissue.

1.3 Aim of Study

To assess the histomorphological effects of *Manihot esculenta* leaf extract on lead-induced renal and hepatic tissue toxicity in albino rats, in other to determine its ameliorating effect following toxic exposure.

1.4 Objectives

1. To assess the histomorphological effects of lead-induced toxicity on the renal and hepatic tissues of albino rats.
2. To assess the protective function of *Manihot esculenta* leaf extract against lead-induced renal and hepatic tissues.
3. To compare the histomorphology of renal and hepatic tissues between untreated, lead-exposed, and *Manihot esculenta* leaf extract treated rats.
4. To determine whether *Manihot esculenta* leaf extract can restore normal renal and heaptic histomorphology following lead exposure.

1.5 Research Questions

1. What are the histomorphological changes in the renal and hepatic tissues of albino rats exposed to lead toxicity?
2. Can *Manihot esculenta* leaf extract protect the liver and kidney of albino rat against histological damage from lead-induced toxicity.
3. How do the renal and hepatic tissues of lead-exposed rats appear compare to those of rats treated with *Manihot esculenta* leaf extract?
4. Does Manihot esculenta leaf extract restore normal renal and hepatic histomorphology following lead exposure?

1.6 Significance of Study

This study is significant because it investigates the possibility of using leaf extract from *Manihot esculenta* as a natural treatment for renal and hepatic damage caused by lead. Alternatives to standard therapies that are accessible and cost-effective are needed since lead toxicity continues to be a serious public health concern, particularly in areas with limited resources. This research may support the use of cassava leaf extract in treating heavy metal toxicity and encourage more

research on plant-based therapies in toxicology by offering histological proof of the extract's protective properties.

CHAPTER TWO

LITERATURE REVIEW

2.1 Lead Toxicity: Sources, Exposure, and General Mechanisms of Toxicity

Lead (Pb), a non-essential heavy metal, is an insidious environmental contaminant that poses a profound global public health challenge due to its widespread distribution and persistent nature. Unlike essential trace elements, lead serves no beneficial biological function in the human body, yet its pervasive presence leads to involuntary exposure for populations worldwide (Lanphear *et al*., 2018).

Though efforts have reduced primary sources like leaded gasoline and paint in many developed nations, exposure remains a major issue worldwide. Contemporary sources of lead include contaminated water, old paint, certain cosmetics, traditional remedies, and occupational exposure. Children are particularly vulnerable due to hand-to-mouth behaviors and the long-term reservoir of lead in soil and dust. Secondary exposure can occur when workers inadvertently carry lead dust on their clothing or shoes from the workplace to their homes, exposing family members (AAP 2016).

General Mechanisms of Lead Toxicity

Lead's toxicity is due to its ability to disrupt a wide range of biological processes at a cellular and molecular level.

Mimicking Essential Cations: Lead mimics essential divalent cations, primarily calcium (Ca^{2+}), but also zinc (Zn^{2+}) and iron (Fe^{2+}). Its similar size and charge allow it to substitute for calcium in critical biological processes. This can aberrantly activate or inhibit calcium-dependent enzymes, disrupting cell signaling, neurotransmitter release, and gene expression, which is particularly damaging to the nervous system (Amadi and Nzeh, 2018).

Enzyme Inhibition: Lead has a high affinity for sulfhydryl (-SH) groups found in proteins and enzymes. By binding to these groups, it inactivates a wide array of enzymes, including those involved in heme synthesis (Kim *et al*., 2018). A key example is its inhibition of delta-aminolevulinic acid dehydratase (ALAD), an enzyme in the heme synthesis pathway. This can lead to the accumulation of neurotoxic substances and result in lead-induced anemia (Sharma and Bahacharya, 2017).

Oxidative Stress: A cornerstone of lead's toxicity is its ability to cause oxidative stress. It directly generates reactive oxygen species (ROS) and simultaneously compromises the body's natural antioxidant defense system (Lanphear *et al*., 2018). Lead depletes key antioxidants like glutathione (GSH) and inhibits important antioxidant enzymes, leading to an imbalance that damages cell membranes, proteins, and DNA (WHO, 2021).

Mitochondrial Dysfunction and Altered Gene Expression: Lead disrupts the function of mitochondria, the cell's energy producers, which further exacerbates oxidative stress and can trigger programmed cell death (EPA 2024). Additionally, lead can alter gene expression by competing with essential metals for binding to transcription factors, modulating the transcription of genes related to inflammation and stress (Reese and glass, 2022). These mechanisms collectively contribute to widespread systemic damage, particularly to organs like the kidneys and liver (Jha *et al*., 2018).

2.2 Epidemiology and Public Health Significance of Lead Poisoning

Lead poisoning remains a significant global health crisis, disproportionately affecting vulnerable populations (Roy *et al*., 2021). The staggering statistic that an estimated one in three children worldwide has elevated blood lead levels underscores that this issue is far from resolved (CDC, 2017).

Vulnerability of Children: Children are particularly susceptible to lead's harmful effects due to a combination of behavioral and physiological factors. They are more likely to ingest lead from contaminated dust and soil due to hand-to-mouth behaviors, and their bodies absorb lead more efficiently (CDC, 2019). The most devastating impact is on their developing nervous systems, with even low levels of exposure leading to irreversible neurodevelopmental deficits. These include reduced IQ, learning disabilities, and behavioral problems like ADHD. The long-term consequences of this damage are immense, affecting educational attainment and economic productivity (Flora *et al*., 2012).

Impact on Adults: While children are the most vulnerable, lead poisoning also affects adults. Chronic exposure is linked to a range of serious health problems, including hypertension, cardiovascular disease, kidney damage, and reproductive issues (Al-malki and Abo-omar, 2021). Occupational exposure is a major concern, with workers in industries like battery manufacturing and mining facing a higher risk of lead-related illnesses (Jha *et al*., 2020).

Global Disparities and Public Health Response: The burden of lead poisoning is not uniform. In developing nations, informal practices such as rudimentary battery recycling and artisanal mining are major sources of exposure (Nwokocha *et al*., 2023). Even in developed countries, legacy lead from old paint and water pipes continues to be a problem, often disproportionately affecting low-income communities (Gurer and Ercal, 2020).

Effective public health strategies must be multi-pronged, focusing on prevention. This includes:

Primary Prevention: Eliminating lead sources through regulations, replacing old infrastructure, and public education (Navas *et al*., 2017).

Secondary Prevention: Proactive screening of high-risk children to detect elevated lead levels early (UNICEF, 2020).

Tertiary Prevention: Medical management, such as chelation therapy for severe cases, though this cannot reverse existing damage (Nwokocha *et al*., 2023).

2.3 Renal Anatomy and Physiology

The kidneys are paired, bean-shaped organs located retroperitoneally from T12–L3, with the right kidney slightly lower due to the liver (Dibona, 2018). Each measures about 10–12 cm long, 5–7 cm wide, and 3 cm thick, weighing 125–170 g. They are protected by a fibrous capsule, adipose tissue, and renal fascia, with the hilum serving as the entry/exit for vessels, nerves, and the ureter (Pallak and sedor, 2017). Internally, the kidney has an outer cortex (with renal corpuscles and tubules) and an inner medulla (containing pyramids that drain urine into calyces, the renal pelvis, and then the ureter) (Sands and Layton, 2017).

The nephron, about one million per kidney, is the functional unit. Each nephron includes a renal corpuscle (glomerulus and Bowman's capsule) and a renal tubule (PCT, loop of Henle, DCT, and collecting duct). Blood filtration at the glomerulus produces filtrate, which is modified through reabsorption and secretion along the tubules. Juxtamedullary nephrons with vasa recta support countercurrent exchange for urine concentration (Pollak and sedor, 2017).

The kidneys perform an astonishing array of physiological functions:

Filtration of Blood: This is the initial step in urine formation, occurring at the glomerulus. Blood plasma, excluding proteins and blood cells, is forced from the glomerular capillaries into Bowman's capsule, forming the glomerular filtrate (Sands and Layton, 2017).

Regulation of Blood Volume and Blood Pressure: The kidneys precisely control extracellular fluid volume by regulating water excretion. This, in turn, directly impacts blood volume and blood pressure. The renin-angiotensin-aldosterone system (RAAS) is a primary hormonal mechanism. When blood pressure or renal blood flow decreases, the juxtaglomerular cells in the kidney release renin, initiating a cascade that leads to the production of angiotensin II and aldosterone. Angiotensin II causes vasoconstriction and stimulates aldosterone release, while aldosterone promotes sodium and water reabsorption in the tubules, increasing blood volume and pressure (Obeten *et al*., 2017).

Regulation of Electrolyte Balance: The kidneys are vital for maintaining the balance of key electrolytes, including sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), phosphate (PO_4^{3-}), and chloride (Cl^-). Specific transporters and channels in different segments of the renal tubule are responsible for the precise reabsorption and secretion of these ions, adjusting their excretion rates to match dietary intake and metabolic needs (Dibona, 2018).

Regulation of Acid-Base Balance: The kidneys play a critical role in maintaining the pH of body fluids within a narrow, physiological range (7.35-7.45). They achieve this by excreting excess acids (primarily hydrogen ions, H^+) and conserving bicarbonate ions (HCO_3^-), the body's primary buffer (Basha *et al*., 2019).

Excretion of Metabolic Wastes and Foreign Substances: The kidneys are the primary route for eliminating metabolic end products that are no longer needed by the body, such as urea (from protein metabolism), creatinine (from muscle metabolism), uric acid (from nucleic acid metabolism), and bilirubin (from hemoglobin breakdown) (Good, 2017).

Hormone Production: Beyond their excretory and regulatory roles, the kidneys function as endocrine organs, producing several important hormones (Afridi *et al*., 2024).

Erythropoietin (EPO): A glycoprotein hormone that stimulates the bone marrow to produce red blood cells (erythropoiesis) in response to hypoxia.

Renin: As mentioned, an enzyme involved in the RAAS, crucial for blood pressure regulation (Pollak and Sedor, 2017).

Calcitriol (active Vitamin D): The kidneys convert 25-hydroxyvitamin D, produced in the liver, into its active form, 1, 25-dihydroxyvitamin D. Calcitriol is essential for calcium and phosphate homeostasis, promoting their absorption from the gut and influencing bone mineralization (WHO, 2021).

Gluconeogenesis: During prolonged fasting or starvation, the kidneys can synthesize glucose from non-carbohydrate precursors (e.g., amino acids, glycerol) in a process called gluconeogenesis, contributing significantly to maintaining blood glucose levels, particularly when hepatic glycogen stores are depleted (Reese and glass, 2022).

The intricate anatomy of the kidney, from its macroscopic organization to the microscopic architecture of the nephron, is perfectly adapted to its multifaceted physiological roles. These roles extend far beyond simple waste removal to encompass the precise regulation of fluid and electrolyte balance, acid-base homeostasis, blood pressure control, red blood cell production, and bone metabolism, all of which are vital for overall organismal health and survival. Disruption of these delicate renal functions, as can occur with toxic exposure like lead, has profound systemic consequences (El-sayed *et al*., 2017).

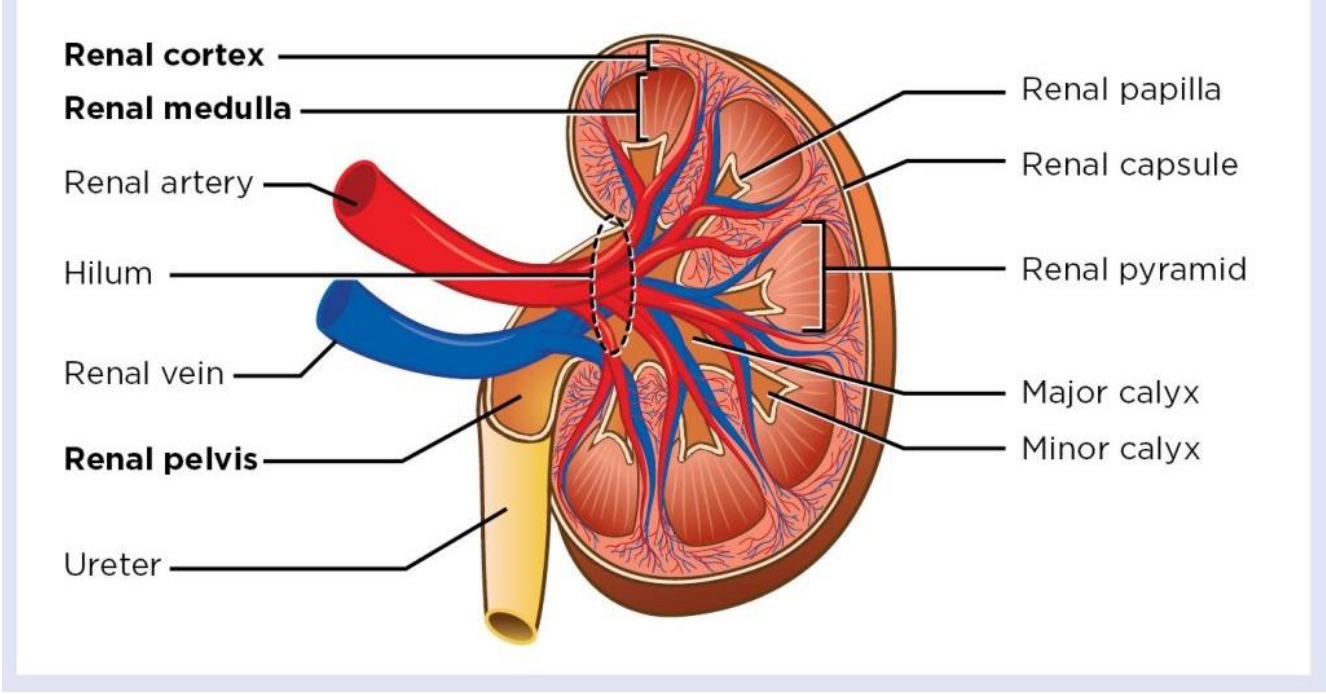

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Fig. 2.1 Anatomy of the Kidney (Obeten *et al.*, 2017).

2.4 Histology of Normal Kidney Tissue

The kidney is divided into the cortex and medulla, each with specialized microscopic structures that underpin its physiological roles (El- Sayed *et al*., 2017).

Renal Corpuscle: This is the initial filtering unit of the nephron and consists of two main components: (Pollak and Sedor, 2017)

Glomerulus: A tuft of specialized capillaries, the glomerulus is characterized by a unique fenestrated endothelium, which allows for the rapid filtration of plasma while retaining blood cells and large proteins. The capillary loops are supported by a gelatinous mesangium, containing mesangial cells (Dibona, 2018).

Bowman's Capsule: A double-layered epithelial cup that surrounds the glomerulus. The inner visceral layer consists of highly specialized cells called podocytes (Ojo *et al*., 2021).

Filtration Barrier: The crucial filtration barrier is formed by three layers: the fenestrated endothelium of the glomerular capillaries, the glomerular basement membrane (GBM), and the slit diaphragms between the podocyte foot processes. This selective barrier permits the passage of water and small solutes but restricts the passage of larger molecules, particularly proteins (Good, 2017).

Renal Cortex: Contains renal corpuscles and convoluted tubules.

Proximal convoluted tubule (PCT): Abundant, with brush border, mitochondria, and high reabsorptive activity (Sands and Layton, 2017).

Distal convoluted tubule (DCT): Smaller, cleaner lumen, fewer mitochondria, involved in ion balance (Sands and Layton, 2017).

Collecting ducts (cortical): Straight tubules with pale cuboidal cells, regulated by ADH (Sands and Layton, 2017).

Renal Medulla: Appears striated due to parallel tubules and vessels (Dibona, 2018).

Loops of Henle: Thin limbs (squamous cells, water/solute permeability) and thick ascending limb (cuboidal, ion transport, forms macula densa) (Dibona, 2018).

Collecting ducts: Larger, columnar cells deeper in medulla, concentrate urine under ADH (Dibona, 2018).

Vasa recta: Straight capillaries maintaining the medullary osmotic gradient (Obeten *et al*., 2017).

Juxtaglomerular Apparatus (JGA): Specialized structure at the vascular pole (Oben *et al*., 2017).

Macula densa (NaCl sensors in DCT), juxtaglomerular cells (renin-secreting smooth muscle),and extraglomerular mesangial cells (supportive/communicative role) (Basha *et al*., 2019).

The rich vascular supply of the kidney is also evident histologically. The renal artery branches extensively within the kidney, forming segmental, interlobar, arcuate, and interlobular arteries, which give rise to the afferent arterioles supplying the glomeruli. The efferent arterioles then branch into the peritubular capillaries (in the cortex) or vasa recta (in the medulla), before venous drainage parallels the arterial supply.

The normal histology of the kidney is a testament to its functional sophistication. Each cellular type and structural arrangement within the glomerulus, tubules,collecting system,and specialized juxtaglomerular apparatus is precisely designed to facilitate the complex processes of filtration, reabsorption, secretion, and concentration,ensuring the maintenance of fluid, electrolyte, and acid-base homeostasis (El- Sayed *et al*., 2017). Any deviation from this normal architecture,such as cellular damage, inflammation, or structural disorganization, as

might be induced by toxins like lead, can severely compromise renal function and lead to overt kidney disease (Good, 2017).

2.5 Pathophysiology of Lead-Induced Nephrotoxicity

The kidney is one of the primary target organs for lead toxicity, owing to its crucial role in filtering and excreting substances from the blood, making it highly susceptible to accumulation of circulating lead. Lead-induced nephrotoxicity, ranging from acute reversible dysfunction to chronic irreversible kidney disease, is a well-documented consequence of lead exposure. The pathophysiology is complex, involving multiple cellular and molecular mechanisms that ultimately compromise renal structure and function (Abas *et al*., 2021).

Acute lead nephropathy is typically observed following high-level, short-term lead exposure. Histologically, it is characterized by distinct morphological changes, most notably the appearance of intranuclear inclusion bodies within the renal tubular epithelial cells, particularly in the proximal convoluted tubules (PCTs) (Abas *et al*., 2021). These inclusion bodies, composed of lead-protein complexes, are acid-fast and pathognomonic for lead poisoning. While initially considered a protective mechanism to sequester lead, their presence signifies significant cellular lead burden (Mishra *et al*., 2021). Functionally, acute lead exposure can lead to a syndrome resembling Fanconi's syndrome, characterized by generalized dysfunction of the PCTs. This manifests as aminoaciduria (excretion of amino acids), glucosuria (excretion of glucose despite normal blood glucose levels), hyperphosphaturia (excessive phosphate excretion), and bicarbonate wasting, due to impaired reabsorption mechanisms in the PCT. This acute phase is often reversible upon cessation of lead exposure and chelation therapy (Abas *et al*., 2021).

Chronic lead nephropathy, however, is a more severe and often irreversible condition resulting from prolonged or repeated low-level lead exposure. It is characterized by progressive interstitial fibrosis, tubular atrophy, and glomerulosclerosis, leading to a gradual decline in renal function (Mahammadi *et al*., 2019). The key pathophysiological mechanisms contributing to chronic lead-induced kidney damage include:

Oxidative Stress: This is a central mechanism in lead nephrotoxicity. Lead significantly enhances the production of reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, within renal cells. This occurs through several pathways, including the uncoupling of electron transport chains in mitochondria and the direct participation of lead in redox cycling. Simultaneously, lead depletes the kidney's endogenous antioxidant defenses. It inhibits the activity of key antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and reduces the levels of non-enzymatic antioxidants like glutathione (GSH) by forming stable complexes with its sulfhydryl groups. The resulting imbalance between pro-oxidants and antioxidants leads to oxidative damage to lipids (lipid peroxidation of cell membranes), proteins (protein carbonylation and enzyme inactivation), and DNA, compromising cellular integrity and signaling pathways in renal tubular and glomerular cells. This sustained oxidative assault contributes to inflammation and progressive renal injury (Jing *et al*., 2020).

Mitochondrial Dysfunction: Renal tubular cells, particularly those in the PCT, are rich in mitochondria due to their high energy demands for active transport processes. Lead preferentially accumulates in mitochondria, disrupting their structure and function. It impairs oxidative phosphorylation, reducing ATP production and

consequently hindering energy-dependent transport mechanisms essential for reabsorption and secretion in the tubules. Mitochondrial damage also exacerbates ROS generation, creating a vicious cycle of oxidative stress and energy depletion that contributes to tubular cell injury and necrosis (Jing *et al*., 2020).

Inflammation and Fibrosis: Chronic lead exposure triggers a persistent inflammatory response within the renal interstitium. Activated immune cells and renal resident cells release pro-inflammatory cytokines (e.g., TNF-alpha, IL-1, IL-6) and chemokines, leading to infiltration of inflammatory cells. This chronic inflammation drives the synthesis and deposition of extracellular matrix components, such as collagen, by activated fibroblasts. This process, known as renal interstitial fibrosis, stiffens the kidney, compresses tubules, and ultimately leads to tubular atrophy and loss of renal parenchyma, irreversibly impairing kidney function. Transforming growth factor-beta (TGF-β) is a key mediator in lead-induced renal fibrosis, promoting fibroblast activation and collagen synthesis (Mishra *et al*., 2021).

Disruption of Ion Transport and Enzyme Activity: Lead can directly interfere with the function of various ion transporters and enzymes critical for renal homeostasis. For instance, it inhibits Na+/K+-ATPase, a vital enzyme for maintaining cellular ionic gradients and driving many reabsorptive processes in the tubules. Lead's mimicry of calcium can also disrupt calcium-dependent signaling pathways and transport proteins, leading to dysregulation of calcium and phosphate handling. This impairment of ion transport contributes to the functional deficits observed in lead nephropathy, such as altered fluid and electrolyte balance (Mahammadi *et al*., 2019).

Glomerular Damage: While tubular damage is often more prominent in lead nephropathy, prolonged exposure can also affect the glomeruli. This can manifest as increased glomerular permeability, leading to proteinuria (excess protein in urine),

and in severe cases, glomerulosclerosis. Lead's ability to induce oxidative stress and inflammation within the glomerulus, as well as its effects on mesangial cells, can contribute to these changes, ultimately reducing the kidney's filtering capacity (Jing *et al*., 2020).

Vascular Effects: Lead is a known vasoconstrictor and can contribute to renal vascular disease. Chronic lead exposure is associated with hypertension, which itself is a major risk factor for kidney disease. Lead can directly damage renal arterioles, leading to nephrosclerosis, characterized by thickening of arterial walls and narrowing of the lumen, further impairing renal blood flow and contributing to ischemia and progressive damage (Al-Malki and Abo-Omar, 2021).

The long-term consequence of chronic lead nephrotoxicity is a progressive decline in glomerular filtration rate (GFR) and the development of chronic kidney disease (CKD), which can eventually progress to end-stage renal disease (ESRD), requiring dialysis or kidney transplantation. The insidious nature of chronic lead exposure, often occurring at low levels without overt symptoms until significant renal damage has occurred, makes it a significant public health challenge (Obeten *et al*., 2017).

2.6 Hepatic Anatomy and Physiology

The liver, the largest internal organ in the human body, is a vital and remarkably versatile organ located primarily in the upper right quadrant of the abdominal cavity, beneath the diaphragm and superior to the stomach and intestines. Weighing approximately 1.4-1.6 kg (3-3.5 lbs) in an adult, its reddish-brown appearance reflects its rich blood supply. The liver is enveloped by a thin connective tissue capsule, Glisson's capsule, and is loosely attached to the diaphragm and abdominal wall by various peritoneal folds. Its unique anatomical position allows it to serve as a critical

intermediary between the digestive system and the rest of the body (Chroskek and Janicka, 2017).

Grossly, the liver is divided into four main lobes: a large right lobe, a smaller left lobe, and two smaller lobes on the posterior surface, the caudate and quadrate lobes. The falciform ligament, a peritoneal fold, separates the right and left lobes anteriorly. The porta hepatis, or "gate to the liver," is a deep fissure on the inferior surface of the liver, serving as the point of entry and exit for the liver's dual blood supply (hepatic artery and hepatic portal vein), bile ducts, lymphatic vessels, and nerves (Okon *et al*., 2022).

The liver's unique vascular arrangement is fundamental to its physiological functions. It receives blood from two distinct sources:

Hepatic Artery: A branch of the celiac trunk, it supplies oxygenated blood to the liver parenchyma, accounting for about 25% of the total blood flow to the liver (Okwu *et al*., 2017).

Hepatic Portal Vein: This vein carries nutrient-rich, deoxygenated blood directly from the gastrointestinal tract, spleen, and pancreas to the liver, accounting for approximately 75% of the liver's blood supply. This direct access to absorbed nutrients, toxins, and waste products from the digestive system positions the liver as the body's primary metabolic and detoxification center (Okwu *et al*.,2017).

Within the liver, these two blood supplies mix and flow through a specialized vascular system, the hepatic sinusoids, before eventually draining into the hepatic veins, which empty into the inferior vena cava (Good, 2017).

Microscopically, the functional unit of the liver is traditionally considered the hepatic lobule. Classically, this is described as a hexagonal prism with a central vein (a terminal hepatic venule) at its core and portal triads at its six corners (Okon *et al*., 2022). A portal triad typically consists of three main structures:

A branch of the hepatic artery (arteriole)

A branch of the hepatic portal vein (venule)

A bile ductule

Radiating outwards from the central vein are plates of liver cells, or hepatocytes, which are the primary functional cells of the liver. Between these plates are the hepatic sinusoids, specialized capillaries lined by fenestrated endothelial cells. The fenestrations in the sinusoidal endothelium are much larger than those in other capillaries, allowing for direct contact between the blood plasma and the surface of the hepatocytes, facilitating efficient exchange of substances (Roy *et al*., 2021).

Within the sinusoids, or lining their walls, are specialized macrophages called Kupffer cells. These are resident phagocytic cells that remove old red blood cells, bacteria, toxins, and other foreign materials from the blood, playing a crucial role in the liver's immune defense. Also found in the space of Disse (the perisinusoidal space between the sinusoidal endothelium and hepatocytes) are hepatic stellate cells (Ito cells), which store vitamin A and, in pathological conditions, can transform into myofibroblast-like cells, contributing to liver fibrosis (Trefts *et al*., 2017).

Bile, produced by hepatocytes, flows in the opposite direction to blood, moving from bile canaliculi (small channels between adjacent hepatocytes) towards the bile ductules in the portal triads. The bile ductules then merge to form larger bile ducts, eventually forming the common hepatic duct (Abarbanel and Hato, 2019).

The diverse and extensive physiological functions of the liver are critical for maintaining overall bodily homeostasis:

Metabolic Regulation: The liver is the body's metabolic powerhouse, playing a central role in the metabolism of carbohydrates, lipids, and proteins (Afridi *et al*., 2018).

Carbohydrate Metabolism: It regulates blood glucose levels by performing glycogenesis (glucose to glycogen synthesis), glycogenolysis (glycogen breakdown to glucose), and gluconeogenesis (synthesis of glucose from non-carbohydrate sources like amino acids and lactate) when blood glucose is low (Reese and glass, 2022).

Lipid Metabolism: The liver synthesizes cholesterol, lipoproteins (e.g., VLDL, HDL), and phospholipids. It also oxidizes fatty acids to produce energy (ATP) and synthesizes triglycerides for storage. (Reese and glass, 2022).

Protein Metabolism: The liver is the primary site for the synthesis of most plasma proteins, including albumin (maintains osmotic pressure), clotting factors (e.g., fibrinogen, prothrombin), and transport proteins. It also deaminates amino acids (removing the amino group) for energy production or conversion to glucose/fat, producing ammonia, which is then converted to urea in the urea cycle. (Afridi *et al*., 2018).

Detoxification and Biotransformation: This is one of the liver's most critical functions. It metabolizes and detoxifies endogenous waste products (e.g., ammonia, bilirubin, hormones) and exogenous substances (e.g., drugs, alcohol, environmental toxins, pesticides). This process involves two main phases:

Phase I Reactions: Introduction or exposure of functional groups (e.g., oxidation, reduction, hydrolysis) to make the compound more polar, often by cytochrome P450 enzymes (Trefts *et al*., 2017).

Phase II Reactions: Conjugation reactions, where polar groups (e.g., glucuronic acid, sulfate, glutathione) are added to the compound, making it more water-soluble and easier to excrete via bile or urine (Trefts *et al*., 2017).

Bile Production and Secretion: Hepatocytes continuously produce bile, a yellowish-green fluid essential for the digestion and absorption of fats and fat-soluble vitamins

in the small intestine. Bile contains bile salts, bilirubin (a breakdown product of hemoglobin), cholesterol, phospholipids, and electrolytes (Trefts *et al*., 2017).

Storage: The liver acts as a significant storage organ for various substances, including:

Glycogen (as an energy reserve)

Vitamins (A, D, E, K, and B12)

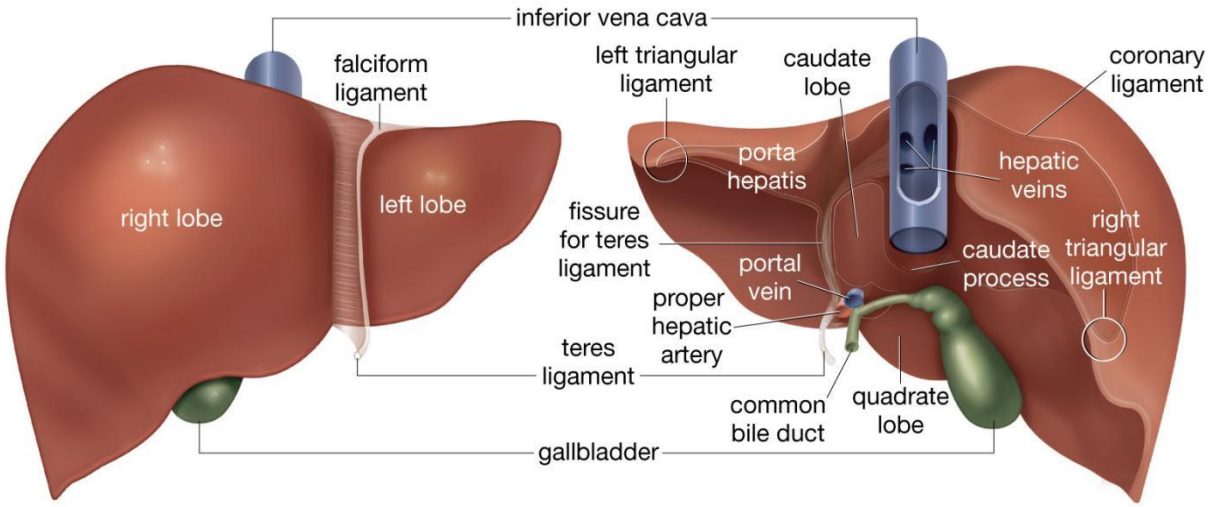
Iron (as ferritin) and copper

Synthesis of Plasma Proteins: As mentioned, the liver synthesizes almost all plasma proteins, which are crucial for maintaining osmotic pressure, blood clotting, immune function, and transport of various substances in the blood (Kim *et al*., 2018).

Immune Function: The Kupffer cells within the liver sinusoids constitute a major component of the reticuloendothelial system, actively engulfing bacteria, viruses, and other foreign particles that enter the portal circulation, thus acting as a crucial immunological barrier (Afridi *et al*., 2018).

Hormone Metabolism: The liver plays a role in the metabolism and inactivation of various hormones, including insulin, glucagon, cortisol, estrogen, and aldosterone, thereby regulating their levels in the bloodstream (Basha *et al*., 2019).

The liver's intricate anatomical organization, with its dual blood supply, unique sinusoidal architecture, and diverse cell types (hepatocytes and Kupffer cells), is perfectly designed to support its unparalleled range of metabolic, synthetic, detoxification and excretory functions. These roles are indispensable for maintaining metabolic balance, purifying the blood, aiding digestion, and supporting overall systemic health. Given its central role in processing absorbed substances, the liver is particularly vulnerable to the toxic effects of environmental pollutants like lead, which can significantly impair its multifaceted functions (Abarbanel and Hato, 2019).



.Fig. 2.2 Anatomy of the Liver (Okon *et al.*, 2022)

2.7 Histology of Normal Liver Tissue

The liver's microscopic architecture is highly specialized to support its central roles in metabolism, detoxification, and bile production. Its primary parenchymal cells, hepatocytes, account for about 80% of liver mass (Okon *et al*., 2022). These large, polyhedral cells typically contain one or two nuclei and are packed with organelles reflecting their intense metabolic activity: rough and smooth endoplasmic reticulum for protein synthesis, lipid metabolism, and drug detoxification; abundant mitochondria for energy production; lysosomes and peroxisomes for waste processing and fatty acid oxidation; and cytoplasmic reserves of glycogen and lipids (Okwu *et al*.,2017). On H&E staining, hepatocyte cytoplasm appears eosinophilic due to its protein content (Okwu *et al*., 2017).

Hepatocytes are arranged into cords or plates, one to two cells thick, radiating outward from a central vein (Reese and glass,2022). These are separated by hepatic sinusoids, specialized capillaries with fenestrated endothelium and no basement membrane, allowing direct exchange between blood plasma and hepatocytes. Within the sinusoidal lining reside Kupffer cells, the liver's resident macrophages, which phagocytose pathogens, debris, and aged blood cells. Adjacent to sinusoids lies the Space of Disse, where hepatocyte microvilli extend to enhance exchange. This perisinusoidal space also houses hepatic stellate (Ito) cells, which normally store vitamin A but can transform into fibrogenic myofibroblasts during chronic injury, contributing to fibrosis and cirrhosis (Ojo *et al*., 2021).

The liver's structural unit, the classical hepatic lobule, is hexagonal in concept, centered around a central vein (Trefts *et al*.,2017). At each corner lies a portal triad, composed of a branch of the portal vein (large, thin-walled, nutrient-rich), a branch of the hepatic artery (smaller, muscular, oxygenated), and a bile ductule (cuboidal

epithelium) (Upadhyay and Singh, 2018). Lymphatics and nerves may also be present. Blood flows from the triads through sinusoids toward the central vein, ensuring hepatocytes receive a mixture of oxygenated and nutrient-rich blood. In contrast, bile flows in the opposite direction, from hepatocytes into bile canaliculi, then into ductules of the portal triads, eventually forming larger bile ducts. This arrangement highlights the delicate balance between blood and bile flow; disturbances can expose hepatocytes to bile acid toxicity (Basha *et al*., 2019).

In addition to the lobule, the hepatic acinus (Rappaport acinus) provides a more functional view of liver organization, emphasizing metabolic zonation and susceptibility to injury. The acinus is diamond-shaped, spanning between two central veins and two portal triads. It is divided into three zones:

Zone 1 (periportal): Closest to arterial blood supply, receiving oxygen- and nutrient-rich blood. Hepatocytes here perform oxidative metabolism (gluconeogenesis, urea synthesis). They resist hypoxia but are more vulnerable to toxins requiring metabolic activation (Chrostek and Janicka , 2017).

Zone 2 (midzonal): Intermediate between zones 1 and 3.

Zone 3 (centrilobular): Closest to the central vein and least oxygenated. These hepatocytes are rich in cytochrome P450 enzymes, favoring detoxification and lipid synthesis, but are highly vulnerable to hypoxic damage and toxin accumulation (Chrostek and Janicka, 2017).

The normal histology of the liver is an exemplar of form following function. The highly efficient and systematic arrangement of hepatocytes into cords, seamlessly interspersed with fenestrated sinusoids containing specialized Kupffer cells, all meticulously organized around a central vein and portal triads, underpins the liver's unparalleled capacity for metabolism, detoxification, nutrient storage, and bile

production (Abarbanel and Hato, 2019). Any deviation from this precisely maintained normal architecture whether it manifests as cellular damage, inflammation, or alterations in vital blood flow patterns can severely compromise the liver's multifaceted functions and pave the way for various forms of hepatic pathology, as is frequently observed in cases of toxic injury, such as that induced by lead poisoning (Okwu *et al*., 2017).

2.8 Pathophysiology of Lead-Induced Hepatotoxicity

The liver, as the body's central organ for metabolism and detoxification, is highly vulnerable to toxic insults, with lead exposure posing a serious threat (Ojo *et al*., 2021). Lead accumulates in hepatocytes, triggering a cascade of cellular and molecular injuries that culminate in profound hepatic dysfunction (Kim *et al*., 2018).

Oxidative Stress: Lead indirectly promotes the generation of reactive oxygen species (ROS) by disrupting mitochondrial electron transport and displacing essential metal cofactors (Basha *et al*., 2019). It depletes glutathione (GSH) by binding sulfhydryl groups and inhibits key antioxidant enzymes (SOD, CAT, GPx) (Haefliger *et al*., 2017). The result is lipid peroxidation, protein oxidation, and DNA damage, which drive inflammation and cell death (Trefts *et al*., 2017).

Mitochondrial Dysfunction: Lead accumulates in mitochondria, impairs ATP synthesis, and increases electron leakage, fueling further ROS generation. This vicious cycle induces apoptosis and hepatocyte loss (Obasi and Akudinobo, 2020).

Metabolic Disruption: Lead inhibits enzymes of the heme synthesis pathway (ALAD, ferrochelatase), causing buildup of toxic precursors like δ-aminolevulinic acid. It also impairs carbohydrate and lipid metabolism (Gurer and Ercal, 2020) (altering gluconeogenesis, glycogenolysis, and fatty acid oxidation), leading to dyslipidemia

and hepatic steatosis. Protein synthesis, including albumin and clotting factors, is also compromised (Afridi *et al*., 2018).

Inflammation and Fibrosis: Persistent hepatocyte injury activates Kupffer cells and recruits immune cells, releasing cytokines (TNF-α, IL-1, IL-6) (UNICEF, 2020). This inflammatory environment stimulates hepatic stellate cells to produce excess collagen, driving fibrosis and, with progression, cirrhosis. TGF-β plays a central role in this fibrogenic process (Kasten and Jolly, 2022).

Impaired Detoxification: Lead suppresses cytochrome P450 enzymes and conjugating pathways, weakening the liver's ability to metabolize xenobiotics and drugs (Good, 2017). This increases the burden of toxins, worsening liver injury (OSHA, 2023).

The pathophysiology of lead-induced hepatotoxicity unfolds as a complex, self-propagating cascade primarily ignited by overwhelming oxidative stress, profound mitochondrial dysfunction, and direct, multifaceted interference with vital metabolic pathways (Aikhuahayri *et al*., 2024). These initiating events cascade into widespread hepatocyte damage, triggering a sustained chronic inflammatory response, ultimately leading to progressive fibrosis, and a precipitous decline in the liver's critical, life-sustaining functions. This intricate interplay of detrimental mechanisms starkly underscores the severe and often devastating implications of lead exposure on hepatic health (Jaishanker *et al*., 2017).

2.9 *Manihot esculenta* (Cassava) Leaf: Phytochemistry and Traditional Medicinal Uses

Manihot esculenta Crantz (cassava), a major staple crop in tropical and subtropical regions, is primarily cultivated for its starchy roots but its leaves are also widely consumed, particularly in Africa and Asia. Beyond their role as a source of protein,

vitamins, and minerals, cassava leaves hold long-standing medicinal value that has drawn increasing scientific interest, especially regarding their phytochemistry and therapeutic potential (Adeyemi *et al*., 2020).

Phytochemical Profile:

Cassava leaves are chemically rich, with both nutritive and non-nutritive bioactive compounds. A key feature is the presence of cyanogenic glycosides (linamarin and lotaustralin), which can release toxic hydrogen cyanide when hydrolyzed. While this makes proper processing essential, traditional techniques such as boiling, soaking, fermentation, and sun-drying effectively reduce cyanide to safe levels (Obeten *et al*., 2017). Besides these compounds, the leaves contain:

Flavonoids (quercetin, kaempferol, rutin): potent antioxidants that scavenge free radicals, reduce lipid peroxidation, and chelate metals, with particular relevance to heavy metal detoxification (Amadi and Nzeh, 2018).

Phenolic acids (caffeic, gallic, chlorogenic acids): strong antioxidants contributing to cellular protection (Adeyemi *et al*., 2020).

Saponins and tannins: with anti-inflammatory, cholesterol-lowering, antimicrobial, and immune-modulating properties (AAP, 2016), though excessive tannins may hinder nutrient absorption (Muzanila *et al*., 2022).

Alkaloids: present in small amounts, adding specific biological activities (Muzanila *et al*., 2022).

Vitamins: abundant Vitamin C (powerful antioxidant), beta-carotene (provitamin A for vision and immunity), and B vitamins (thiamine, riboflavin, niacin, folate) vital for energy metabolism (Afridi *et al*., 2018).

Minerals: iron, calcium, magnesium, potassium, and zinc, all essential for physiological functions and relevant to interactions with heavy metals (Reese and glass, 2022).

In traditional medicine systems across various cultures where cassava is cultivated, the leaves are employed for a wide range of ailments. Cassava leaves are deeply rooted in folk medicine. They are employed for anti-inflammatory and analgesic purposes, such as treating arthritis, headaches, fevers, and swellings (Montagnac *et al*., 2022). Their antioxidant and detoxifying effects are recognized in traditional practices that describe them as "cleansing" or strengthening tonics (Amadi and Nzeh, 2018). They are also used for antimalarial and antipyretic care, not as direct cures but as supportive remedies. Wound healing applications involve topical use to reduce infection and inflammation (Amadi and Nzeh, 2018). Additionally, their fiber content supports digestive health, while high iron levels make them valuable in treating anemia and promoting blood health (Adeyemi *et al*., 2020).

Relevance to Toxicology and Health

The synergy of antioxidants, anti-inflammatory agents, and metal-chelating compounds positions cassava leaves as promising candidates for mitigating oxidative stress and organ injury caused by toxic agents such as lead (Miranda *et al*., 2017). Their dual role as nutrient-rich food and therapeutic agent highlights the importance of validating traditional knowledge through pharmacological research, potentially advancing their use in managing heavy metal toxicity and related oxidative disorders (Nweze and Nwabueze, 2019).

2.10 Antioxidant, Anti-inflammatory, and Organoprotective Properties of *Manihot esculenta* Extracts

Cassava (Manihot esculenta) leaves, traditionally used for healing, contain a rich phytochemical profile particularly flavonoids (quercetin, kaempferol, rutin), phenolic acids, vitamin C, and beta-carotene that provide strong antioxidant and anti-inflammatory properties (Ezeani and okoro, 2021).

Antioxidant effects: They scavenge free radicals, chelate pro-oxidant metals, protect and enhance antioxidant enzymes (SOD, CAT, GPx), and prevent lipid peroxidation, reducing oxidative stress markers such as MDA while boosting glutathione (GSH) (Arogundade and Agbon, 2017).

Anti-inflammatory effects: They suppress cytokines (TNF-α, IL-1, IL-6), inhibit COX-2 and iNOS, block NF-κB signaling, and stabilize lysosomal membranes, thereby reducing tissue injury (Montargnac *et al*.,2019).

Renal protection: Cassava leaf extracts shield kidney tubules from oxidative injury, preserve mitochondrial and membrane integrity, reduce interstitial inflammation, and prevent fibrosis (Nweze and Nwabueze, 2019). They normalize serum creatinine/urea and improve kidney histology in lead-induced nephrotoxicity.

Liver protection: They counter oxidative stress, restore antioxidant defenses, stabilize hepatocyte membranes, and suppress Kupffer cell and stellate cell activation, thus preventing fibrosis. Studies show reductions in liver enzymes (ALT, AST, ALP) and improved histopathology in heavy metal toxicity (Muzanila *et al*., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area

This study was conducted at the Department of Medical Laboratory Science, (Histopathology Unit Laboratory), School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City: Edo State, Nigeria.

3.2. Ethical Approval

All experimental procedures involving animals was conducted in strict compliance with the internationally accepted principles and standard ethical protocols for laboratory animal use and care

3.3. Materials

3.3.1. Experimental Animals

A total of 25 healthy adult male Wister albino rats, weighing between 150-200 grams, was used for this study. Cages were cleaned daily, and bedding was changed every two days to maintain hygiene.

3.3.2. Chemicals and Reagents

Lead Acetate: Analytical grade lead acetate trihydrate ($Pb(CH_3COO)_2 \cdot 3H_2O$) was purchased from [Specify supplier, e.g., Sigma-Aldrich, Merck].

Ethanol: Absolute ethanol (99.9%) for extraction.

Formalin: 10% neutral buffered formalin for tissue fixation.

Paraffin Wax: Histological grade paraffin wax.

Hematoxylin and Eosin (H&E) Stains: For routine histological staining.

Xylene: For tissue clearing.

Graded Alcohols: For tissue dehydration (70%, 80%, 90%, 95%, 100%).

Distilled Water: For solution preparation.

Other reagents: Eosin Y, Mayer's hematoxylin, mounting medium (DPX), coverslips, microscope slides. All chemicals were of analytical grade.

3.3.3. Plant Identification and Extraction

Manihot esculenta leaf was collected and authenticated by a certified Botanist. Lyophilization method was used for leaf extraction.

3.4 Methods

3.4.1 Administration

Following the acclimatization period, the rats were randomly divided into five (5) groups (A to E), with five (5) rats per group (n=5). The experimental period lasted for 28 days during which body weights were monitored weekly. At the end of the treatment period, animals were sacrificed under mild anesthesia.

Group A (Control): Received distilled water orally daily.

Group B (Lead Acetate Only): Received lead acetate at a dose of 60mg/kg.

Group C (Lead Acetate + *Manihot esculenta* Extract): Received lead acetate at a dose of 60mg/kg + 500mg/kg of *Manihot esculenta* orally.

Group D (Lead Acetate + *Manihot esculenta* Extract): Received lead acetate at a dose of 60mg/kg + 1000mg/kg of *Manihot esculenta* orally.

Group E (*Manihot esculenta* Extract): Received 1000mg/kg of *Manihot esculenta* orally

3.4.2. Sample Collection and Histological Analysis

Rats were euthanized humanely. Liver and renal tissues were harvested, fixed in formalin, processed, sectioned, and stained for microscopic analysis. Histomorphological changes were assessed using light microscopy

3.4.3. Histopathological Examination

The fixed kidney and liver tissues undergo standard tissue processing for paraffin embedding:

1. Dehydration: Tissues were dehydrated through a series of ascending grades of alcohol (70%, 80%, 90%, 95%, 100% twice) for approximately 60-90 minutes in each concentration.
2. Clearing: Dehydrated tissues were cleared in xylene (two changes, 60-90 minutes each) to remove alcohol.
3. Infiltration: Tissues were infiltrated with molten paraffin wax in a hot air oven at 60^0C (three changes, 60-90 minutes each).
4. Embedding: Infiltrated tissues were embedded in fresh paraffin wax in molds to form tissue blocks.
5. Sectioning: The tissue blocks were sectioned at 5 micrometers (µm) thickness using a rotary microtome.
6. Mounting: The sections were floated on a warm water bath and carefully picked up onto clean, albumin-coated glass slides.
7. Drying: The slides were dried overnight in an oven at 37^0C.

Staining (Hematoxylin and Eosin - H&E):

1. Dewaxing: Slides were dewaxed in xylene (two changes).
2. Rehydration: Sections were rehydrated through descending grades of alcohol (100%, 95%, 70%) to distilled water.
3. Hematoxylin Staining: Slides were stained in Mayer's Hematoxylin for 5-10 minutes.
4. Washing: Washed thoroughly under running tap water to blue.

5. Differentiation: Differentiated in 1% acid alcohol (1% HCl in 70% ethanol) for a few seconds.
6. Washing: Washed again under running tap water.
7. Eosin Staining: Stained in 1% Eosin Y for 1-3 minutes.
8. Dehydration: Dehydrated through ascending grades of alcohol (70%, 95%, 100% twice).
9. Clearing: Cleared in xylene (two changes).
10. Mounting: Mounted with a few drops of DPX mounting medium and covered with a coverslip.

Microscopic Examination and Photomicrography:

The stained tissue sections were examined under a high-power light microscope (Olympus CX21 or equivalent) at various magnifications (×100, ×400). Key histopathological features were observed and noted for both kidney and liver tissues, including but not limited to:

Kidney: Glomerular architecture (e.g., congestion, swelling, atrophy), tubular integrity (e.g., degeneration, necrosis, presence of inclusion bodies, brush border integrity), interstitial changes (e.g., inflammation, edema, fibrosis), vascular changes.

Liver: Hepatocyte morphology (e.g., vacuolation, necrosis, steatosis, ballooning degeneration), sinusoidal integrity, central vein congestion, portal triad inflammation (e.g., presence of inflammatory cells), presence of fibrosis.

Representative photomicrographs will be taken from each group using a digital camera attached to the microscope for documentation and presentation of findings.

3.5. Statistical Analysis

All quantitative data (e.g., body weight, organ weight, organ-to-body weight ratio) were expressed as Mean ± Standard Error of the Mean (SEM). Statistical analysis was

performed using GraphPad Prism software (version 9.0 or later). One-way Analysis of Variance (ANOVA) was used to compare the means among the different experimental groups.

CHAPTER FOUR

RESULTS

This study investigated the histomorphological effects of lead toxicity on the renal and hepatic tissues of albino rats and further evaluated the ameliorative or protective role of *Manihot esculenta* leaf extract intervention. The findings are presented in Tables 4.1 and 4.2. and graphically represented in figures 4.1 to 4.4

Table 4.1 and figure shows the effects of different treatment groups on final body weight, liver weight, kidney weight, and stomach weight. The mean final body weight varied across the groups, with the highest value observed in Group B (Lead + Extract 500 mg/dL, 175.00 ± 5.13 g) and the lowest in the Control group (139.40 ± 9.85 g). Although body weight appeared higher in lead exposed and extract treated groups, the differences were not statistically significant (ANOVA $p = 0.177$). This indicates that neither lead exposure nor extract intervention significantly altered overall growth performance within the study period.

For liver weight, significant group differences were observed ($p = 0.010$). The post hoc analysis revealed that Groups B (Lead + Extract 500 mg/dL, 9.10 ± 0.55 g) and D (Extract only, 7.28 ± 0.96 g) had significantly higher liver weights compared to the Control (5.56 ± 0.53 g) and Group C (Lead + Extract 1000 mg/dL, 5.68 ± 0.33 g). This suggests that lead exposure in combination with moderate extract administration (500 mg/dL) may have led to hepatic enlargement, while higher extract concentration (1000 mg/dL) appeared to restore liver weight toward baseline values, pointing to a possible dose-dependent protective role of *Manihot esculenta*.

Kidney and stomach weights did not show significant variations across groups ($p = 0.753$ and $p = 0.702$, respectively), indicating that lead and extract treatments did not markedly influence renal or gastric organ weights within the experimental timeframe.

Table 4.1: Histomorphological Effects of Lead Toxicity and *Manihot esculenta* Leaf Extract Intervention on Renal and Hepatic Tissues and Final Body Weight in Albino Rats

Group	Treatment Description	N	Final Wt (g) Mean ± SEM	Liver (g) Mean ± SEM	Kidney (g) Mean ± SEM	Stomach (g) Mean ± SEM
Control	No treatment (baseline)	5	139.40 ± 9.85	5.56 ± 0.53[a]	0.50 ± 0.07	1.20 ± 0.11
Group A	Lead only 60 mg/dL	5	163.60 ± 9.52	6.84 ± 0.50[b]	0.50 ± 0.08	1.28 ± 0.13
Group B	Lead + Extract 500 mg/dL	3	175.00 ± 5.13	9.10 ± 0.55[c]	0.60 ± 0.06	1.37 ± 0.12
Group C	Lead + Extract 1000 mg/dL	4	145.75 ± 6.84	5.68 ± 0.33[a]	0.48 ± 0.09	1.43 ± 0.09
Group D	Extract 1000 mg/dL	4	156.50 ± 14.33	7.28 ± 0.96[b]	0.45 ± 0.03	1.18 ± 0.22
ANOVA p-value	-	-	0.177	0.010*	0.753	0.702

Values are mean ± SEM. Superscript letters indicate Tukey HSD post hoc comparisons; groups sharing the same letter are not significantly different ($p > 0.05$).*Significant at $p < 0.05$ (ANOVA).

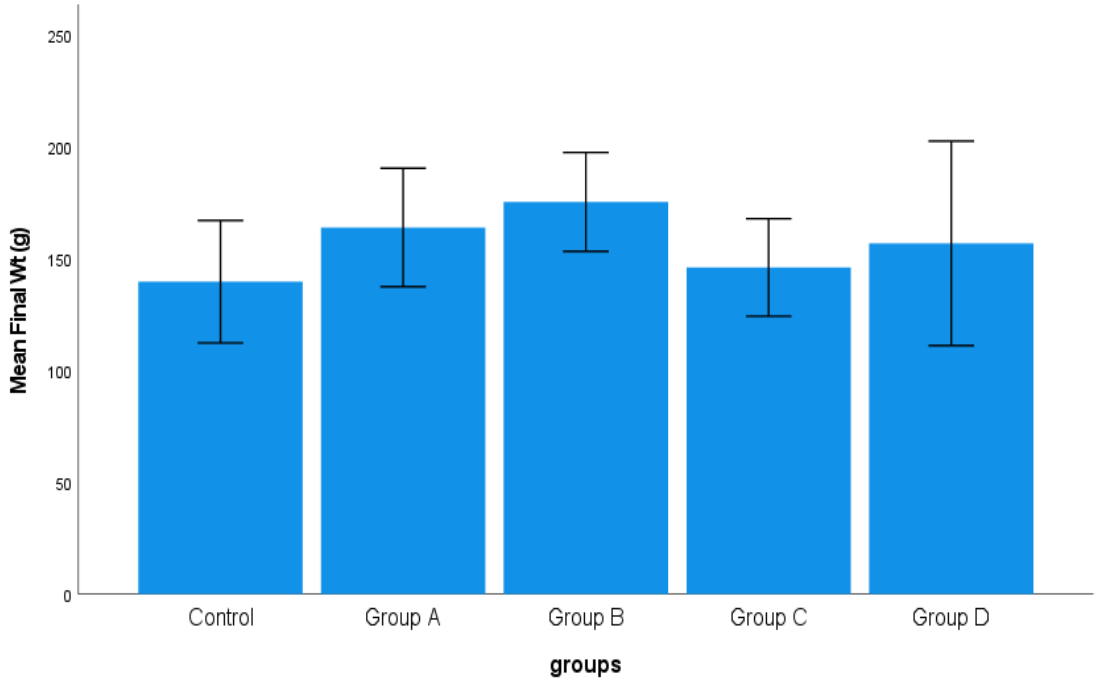


Figure 4.1 Mean final weights of wistar albino rats after exposure to Lead Toxicity and *Manihot esculenta* Leaf Extract Intervention

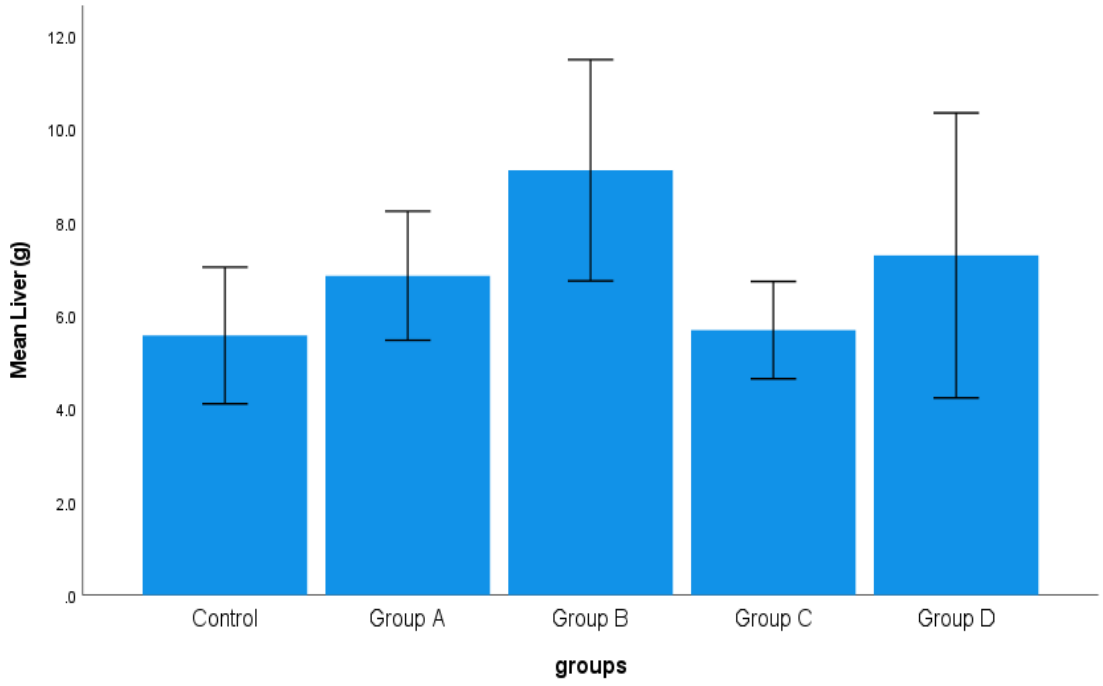


Figure 4.2 Mean weight of liver after exposure to Lead Toxicity and *Manihot esculenta* Leaf Extract Intervention

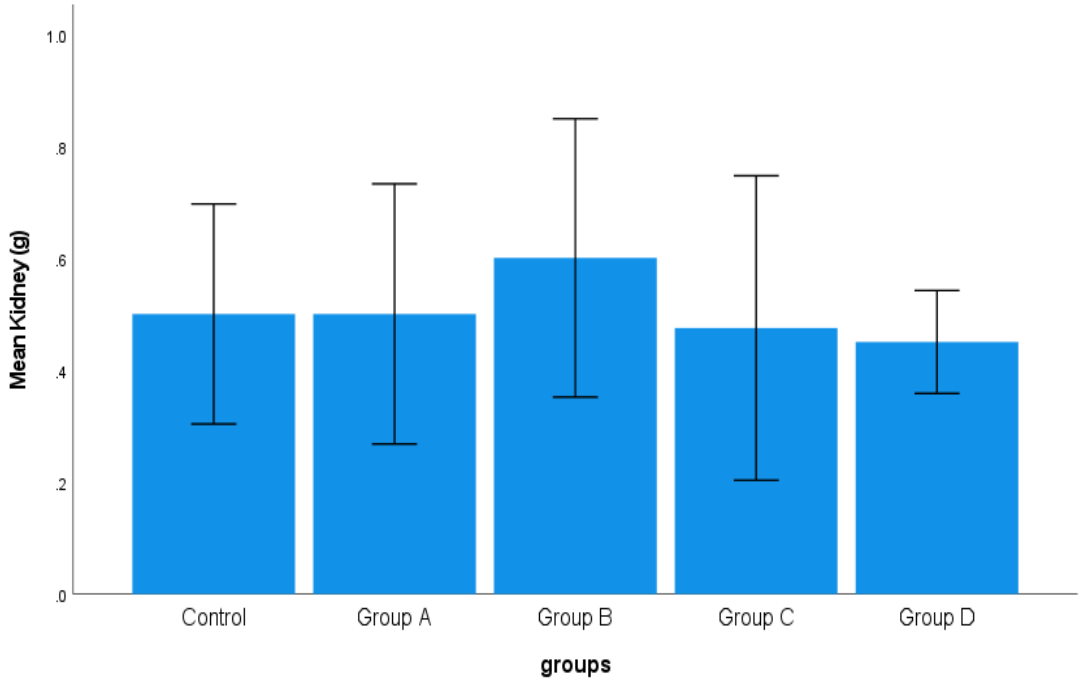


Figure 4.3 Mean weight of kidney after exposure to Lead Toxicity and *Manihot esculenta* Leaf Extract Intervention

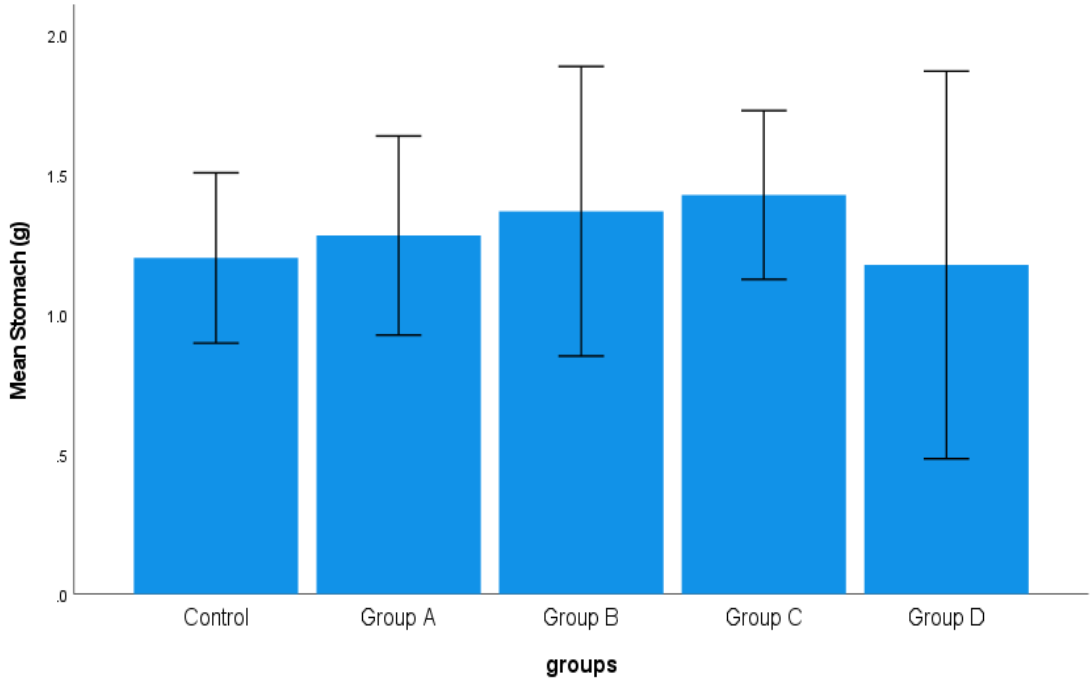


Figure 4.4 Mean weight of stomach after exposure to Lead Toxicity and *Manihot esculenta* Leaf Extract Intervention

Table 4.2 highlights renal and hepatic biochemical parameters across the different experimental groups. Overall, most parameters including urea, creatinine, electrolytes (Na^+, K^+, HCO_3^-), bilirubin (total and conjugated), and hepatic enzymes (AST, ALT, ALP) showed no significant group differences ($p > 0.05$).

The only significant finding was observed in chloride (Cl^-) levels ($p = 0.035$). Post hoc analysis revealed that Group C (Lead + Extract 1000 mg/dL, 98.67 ± 1.20 mmol/L) had significantly higher chloride compared to Group E (Control, 91.50 ± 1.50 mmol/L; $p = 0.044$). This elevation may reflect mild electrolyte imbalance induced by combined lead and extract treatment at high concentration, though the physiological relevance requires further investigation.

Liver function markers (AST, ALT, ALP, and bilirubin levels) did not show significant differences across groups. The absence of pronounced alterations in these enzymes indicates that while lead exposure may predispose to hepatotoxicity, *Manihot esculenta* leaf extract appeared to offer protective or restorative effects, maintaining hepatic enzyme activity within near-normal ranges.

Similarly, renal function markers (urea and creatinine) did not significantly differ among groups.

Table 4.2: Histomorphological Effects of Lead Toxicity and *Manihot esculenta* Leaf Extract Intervention on Renal and Hepatic Parameters in Albino Rats

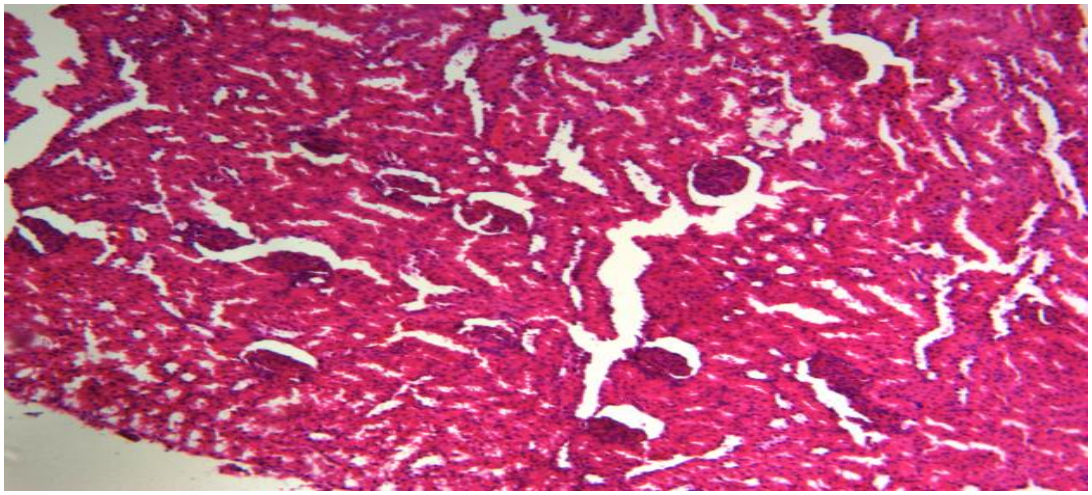
Parameter	Group A (n=2)	Group B (n=2)	Group C (n=3)	Group D (n=3)	Group E (n=2)	p-value
Urea (mmol/L)	24.00 ± 1.00	28.00 ± 2.00	25.67 ± 1.67	23.00 ± 1.53	25.00 ± 1.00	0.336
Na^+ (mmol/L)	135.00 ± 1.00	134.00 ± 1.00	132.67 ± 1.76	134.00 ± 0.58	135.50 ± 0.50	0.553
K^+ (mmol/L)	4.35 ± 0.35	3.75 ± 0.15	3.83 ± 0.09	3.73 ± 0.22	4.05 ± 0.05	0.267
HCO_3^- (mmol/L)	20.00 ± 1.00	20.50 ± 0.50	20.00 ± 0.58	18.33 ± 1.67	19.50 ± 0.50	0.684
Cl^- (mmol/L)	93.00 ± 1.00	98.00 ± 2.00	98.67 ± 1.20*	96.33 ± 1.20	91.50 ± 1.50*	**0.035*
Creatinine (mg/dL)	0.50 ± 0.00	0.55 ± 0.05	0.53 ± 0.07	0.43 ± 0.07	0.50 ± 0.10	0.748
Total Bilirubin (mg/dL)	0.075 ± 0.025	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.311
Conjugated Bilirubin (mg/dL)	0.025 ± 0.00	0.025 ± 0.00	0.025 ± 0.00	0.025 ± 0.00	0.025 ± 0.00	–
AST (U/L)	9.50 ± 0.50	7.00 ± 1.00	9.00 ± 0.58	7.33 ± 0.33	7.00 ± 1.00	0.089
ALT (U/L)	8.50 ± 0.50	6.00 ± 0.00	7.67 ± 1.45	8.00 ± 0.58	6.00 ± 0.00	0.348
ALP (U/L)	10.50 ± 0.50	12.50 ± 3.50	10.00 ± 1.16	11.33 ± 0.88	9.50 ± 1.50	0.737

*values as Mean ± SEM. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. *p < 0.05 considered statistically significant. Post Hoc analysis revealed that only Chloride (Cl^-) showed significant differences, specifically between Group C and Group E (Mean Difference = 7.167, p = 0.044). All other parameters showed no significant pairwise differences (p > 0.05).*

4.1 Histopathological Changes

The histopathological alterations observed in the kidney and liver of Wistar albino rats following *Lead Toxicity* exposure and *Manihot esculenta Leaf Extract Intervention*

A1 KIDNEY X100



A1 KIDNEY X400

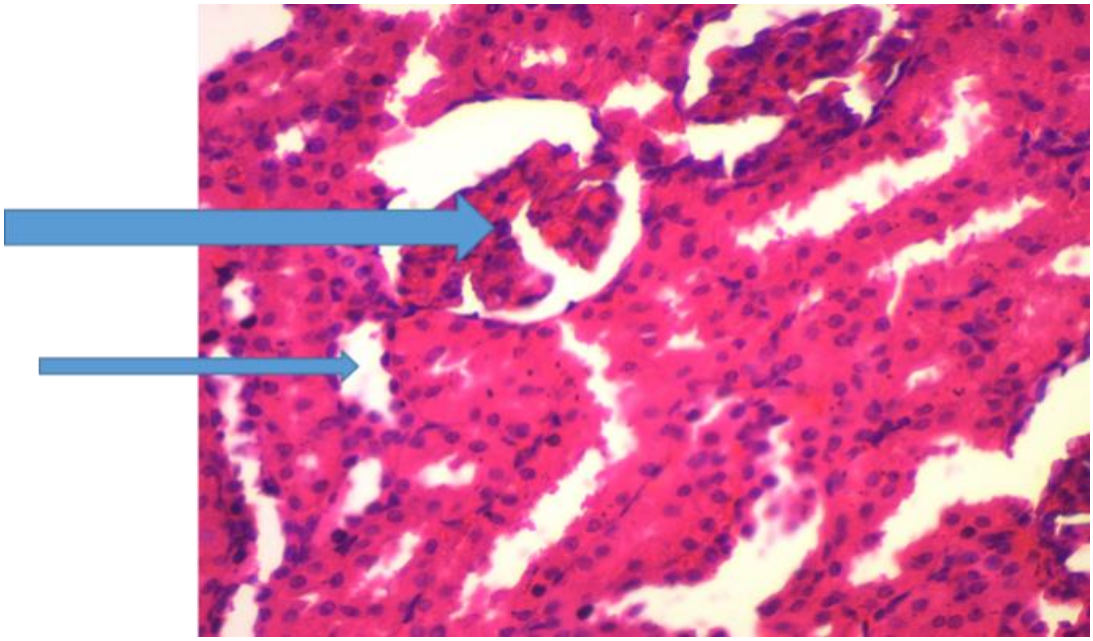
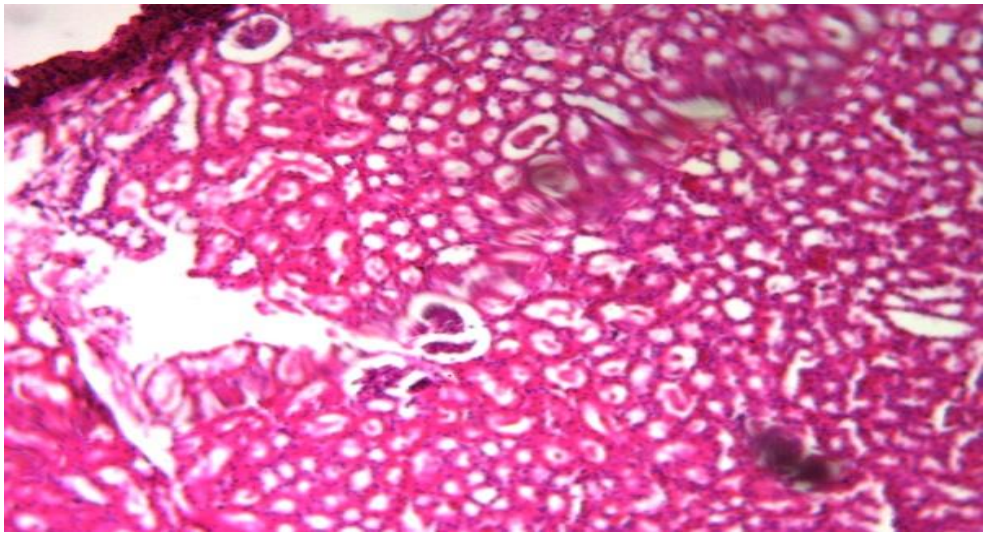


Plate 4.1: Showing kidney Section of group A. the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

B1 KIDNEY X100



B1 KIDNEY X 400

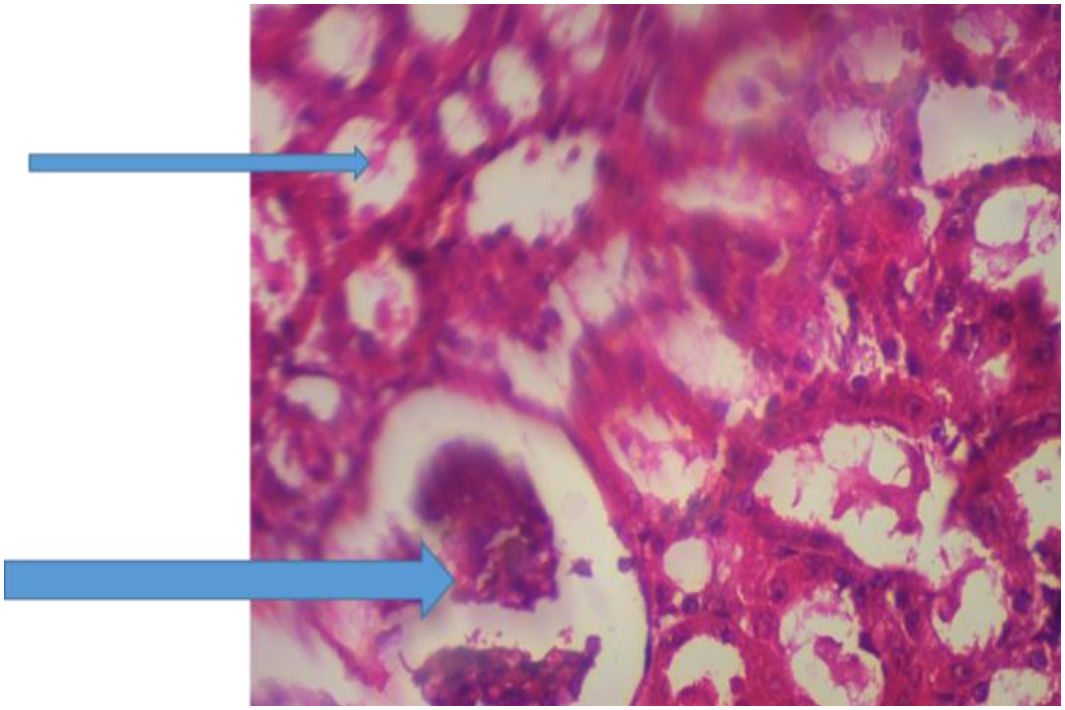
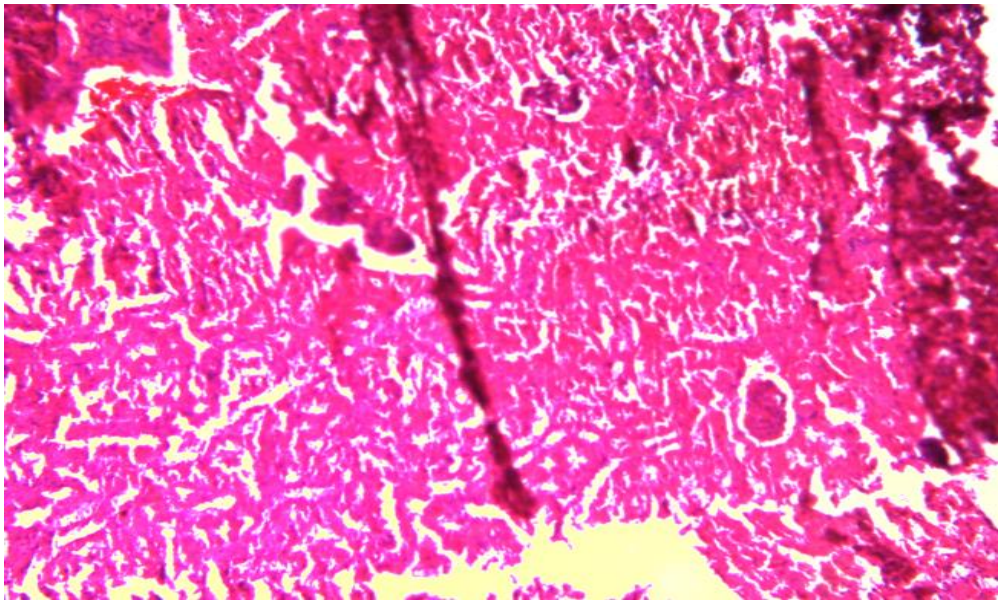


Plate 4.2: Section of the kidney from group B, showing normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

C1 KIDNEY X100



C1 KIDNEY X400

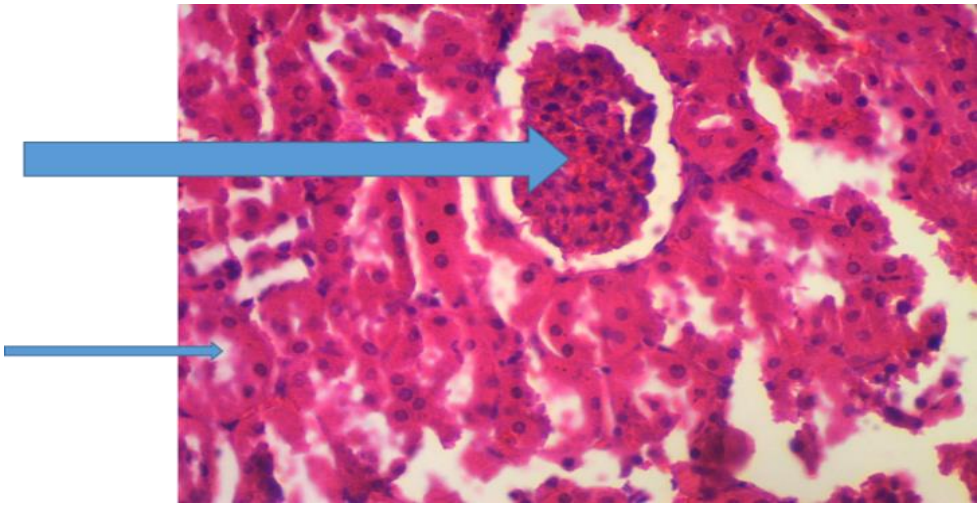
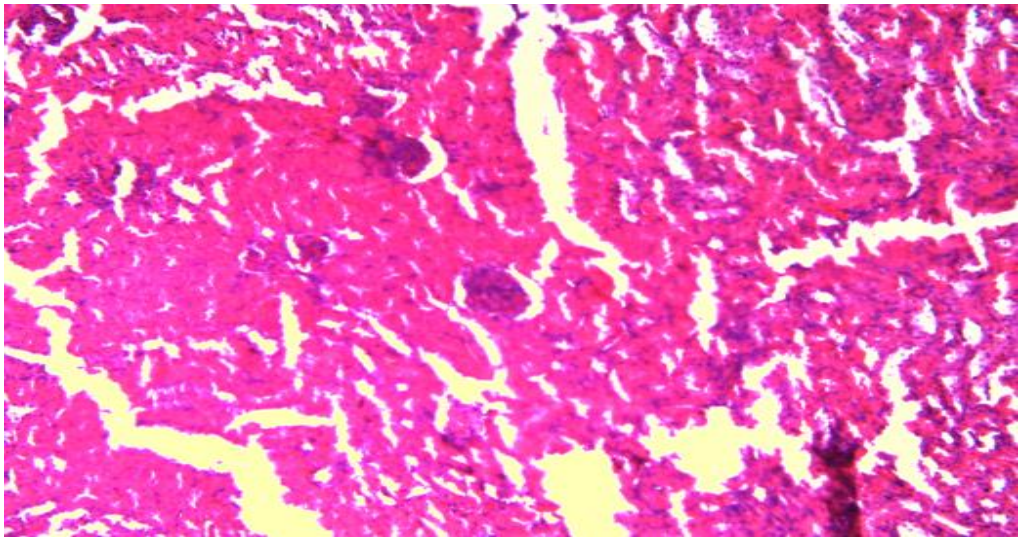


Plate 4.3: Section of the kidney from group C showing normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

D1 KIDNEY X100



D1 KIDNEY X 400

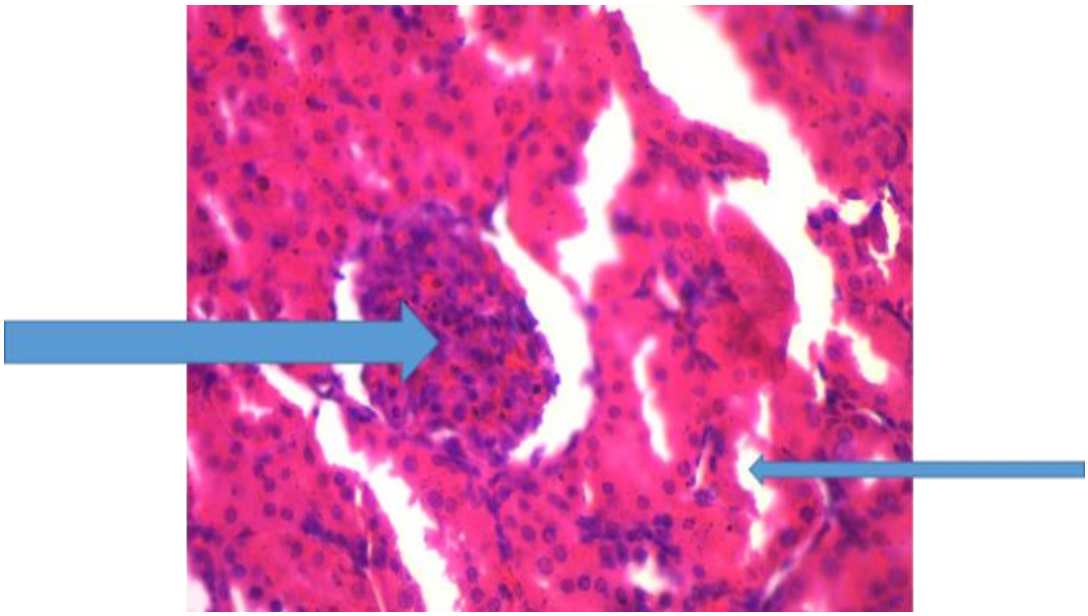
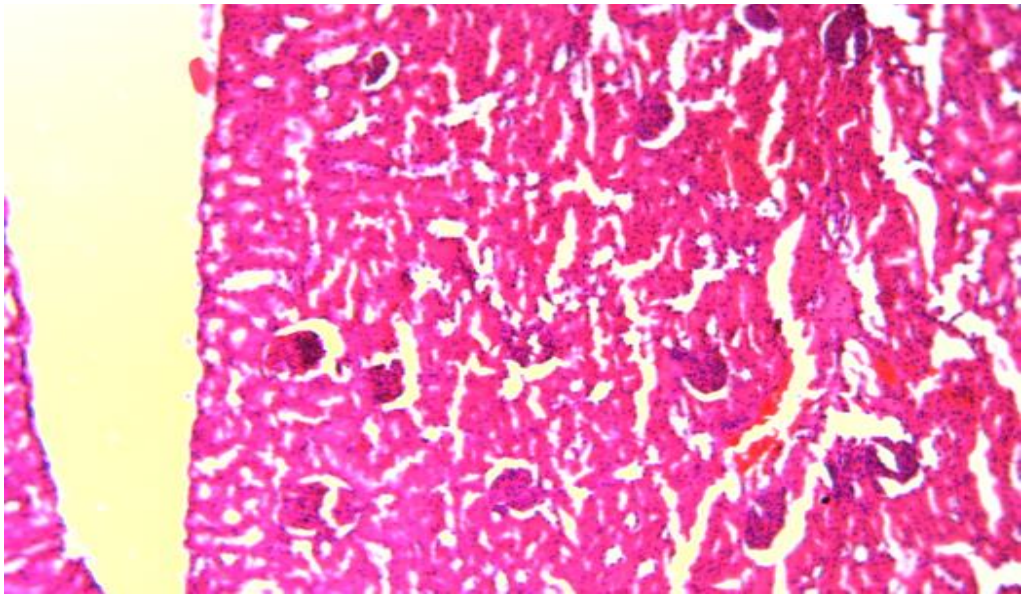


Plate 4.4: Section of the kidney from group D shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

E1 KIDNEY X100



E1 KIDNEY X400

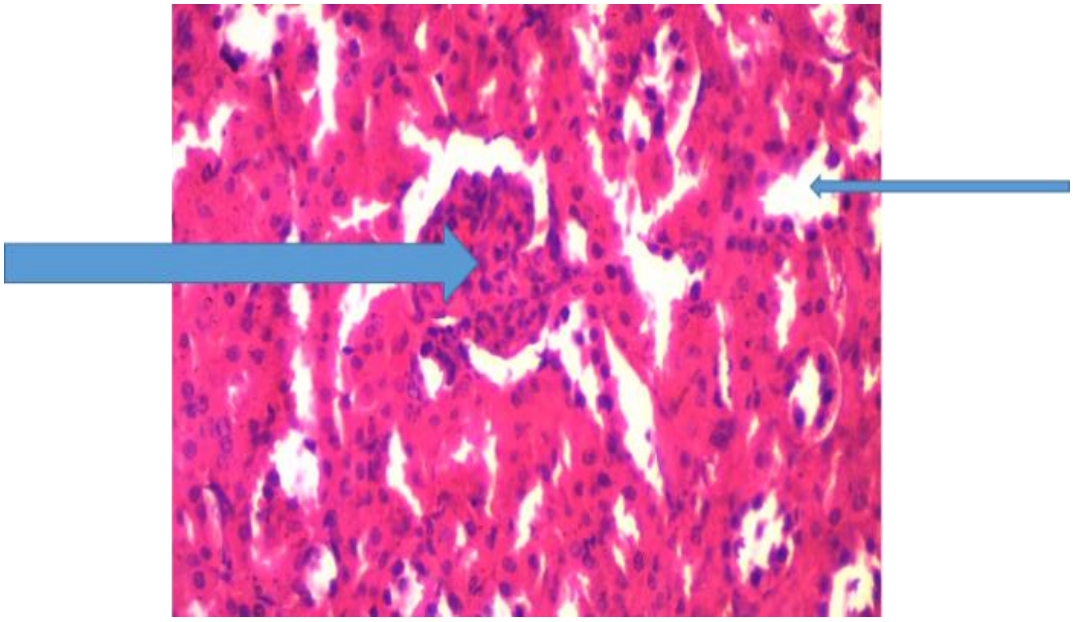
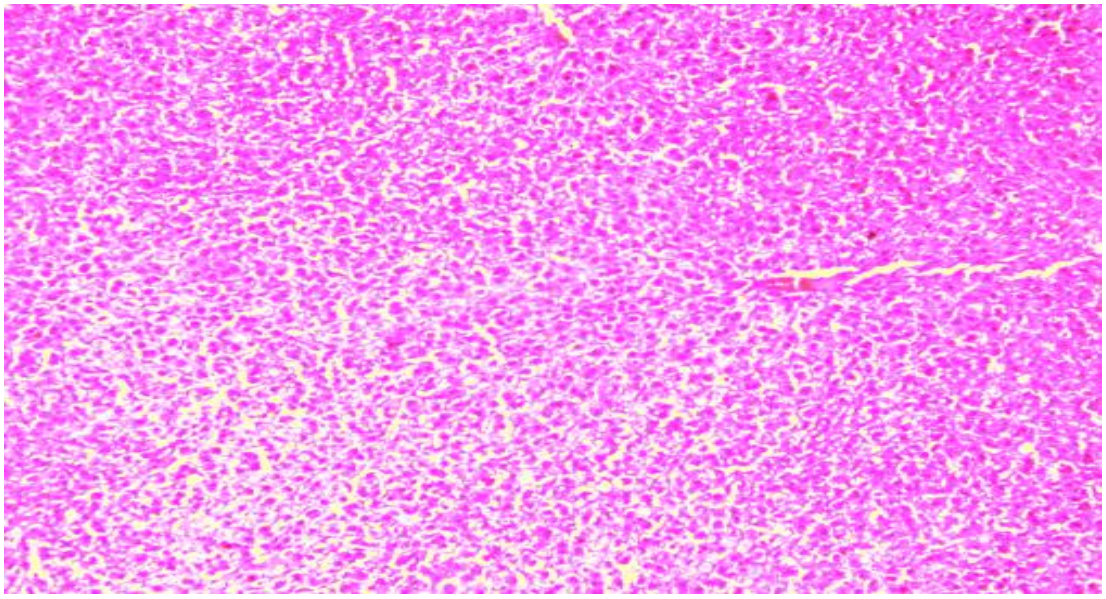


Plate 4.5: Section of the kidney from group E shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

A1 LIVER X100



A1 LIVER X400

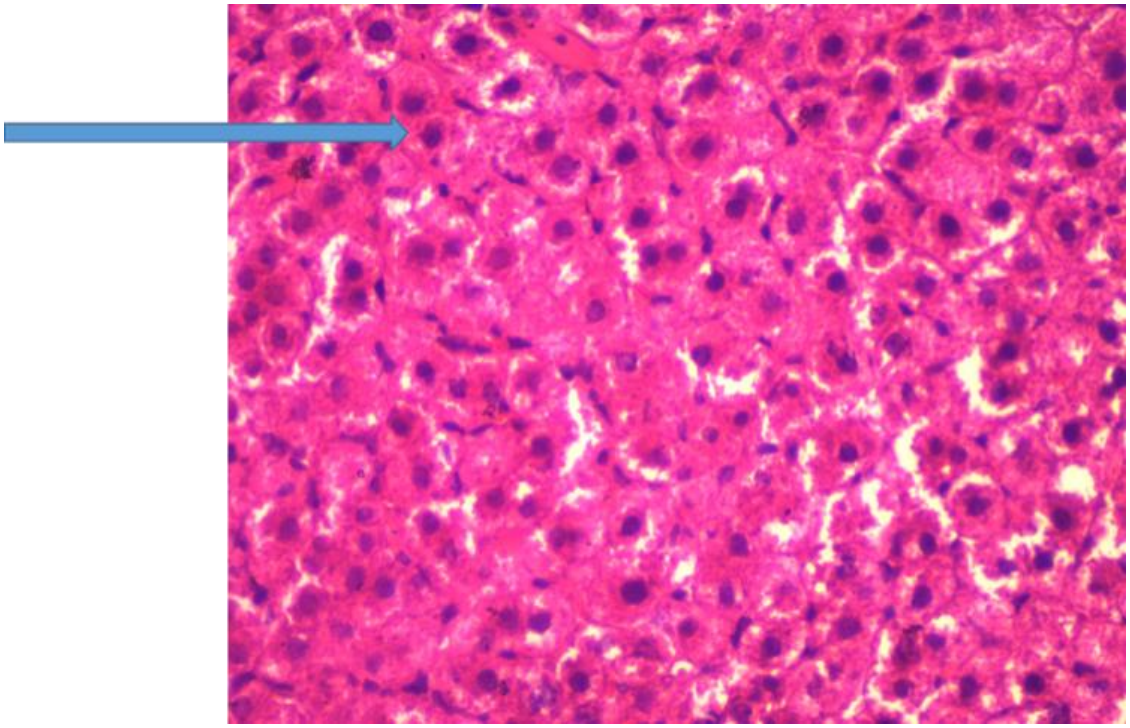
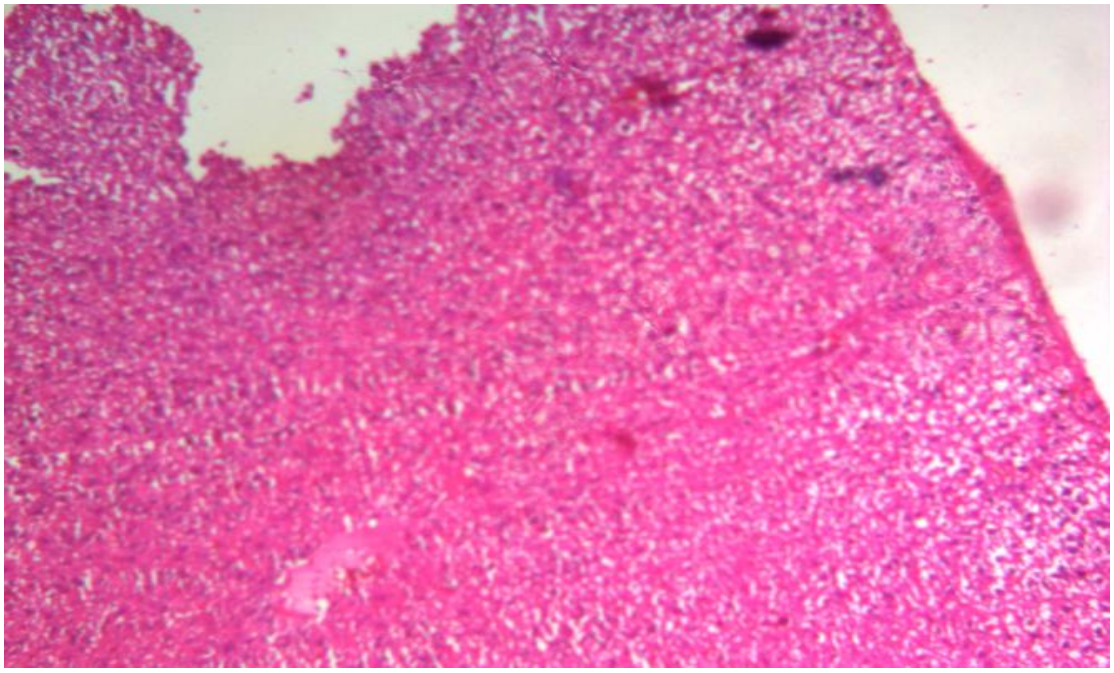


Plate 4.6: Section of the liver from group A shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

B1 LIVER X100



B1 LIVER X400

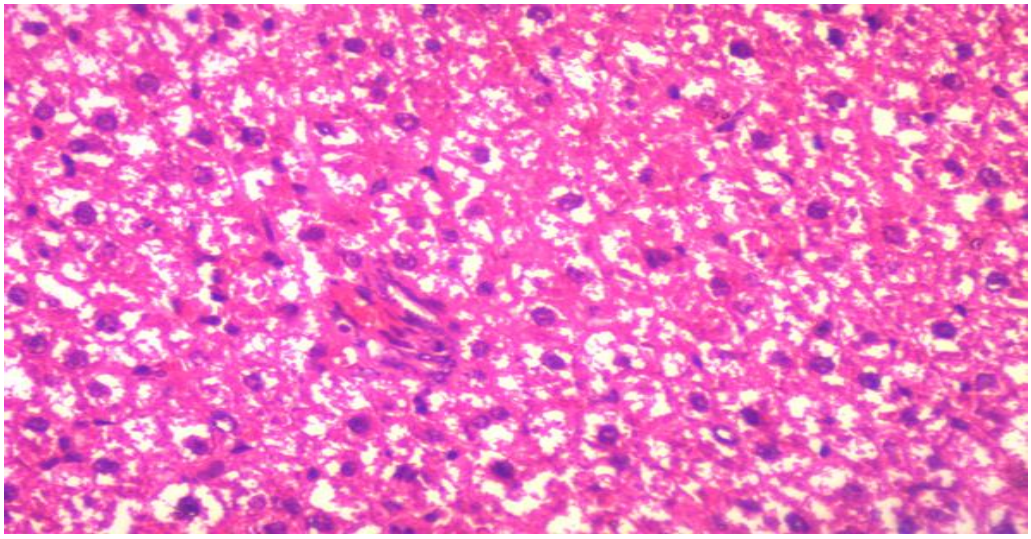
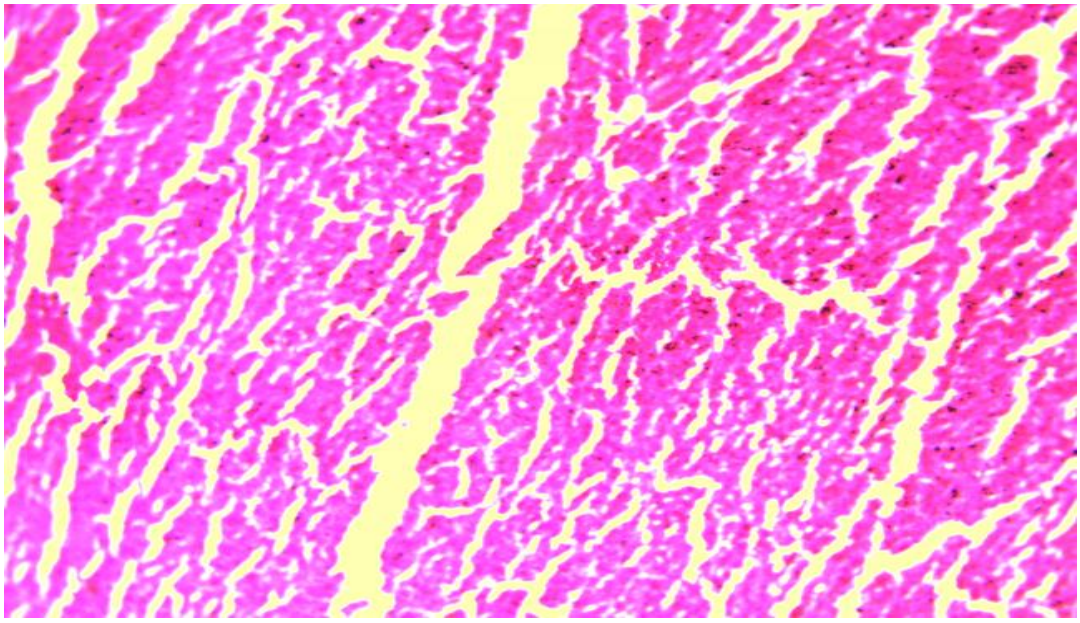


Plate 4.7: Section of the liver from group B shows hepatocytes with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei. **FEATURES IN KEEPING WITH STEATOSIS**

C1 LIVER X100



C1 LIVER X400

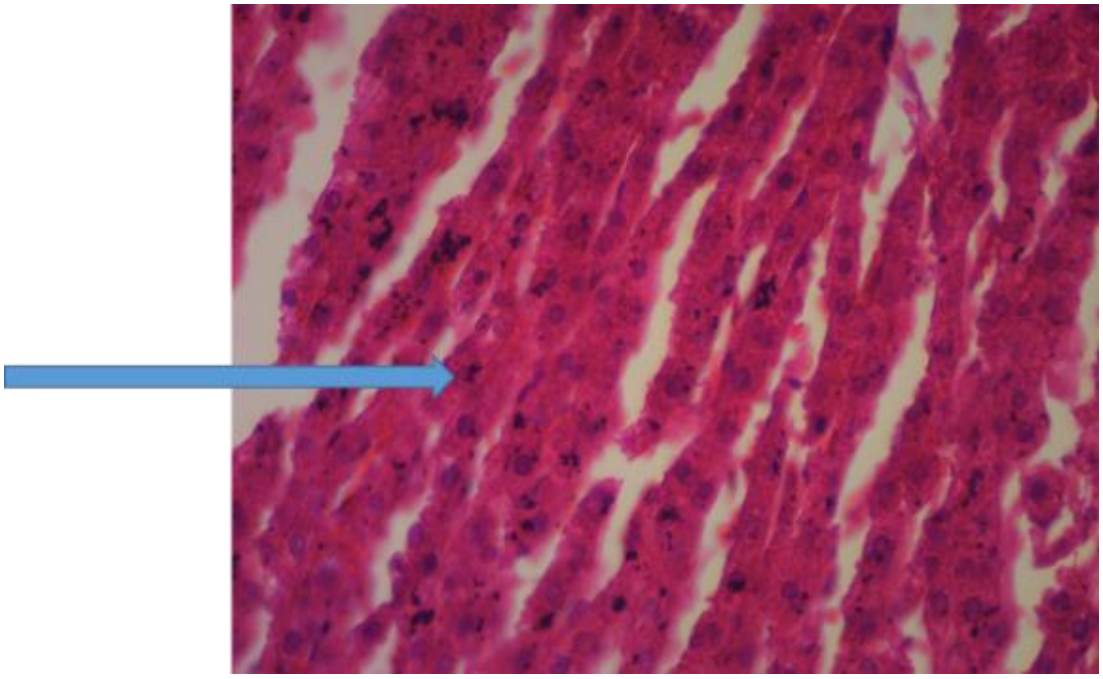
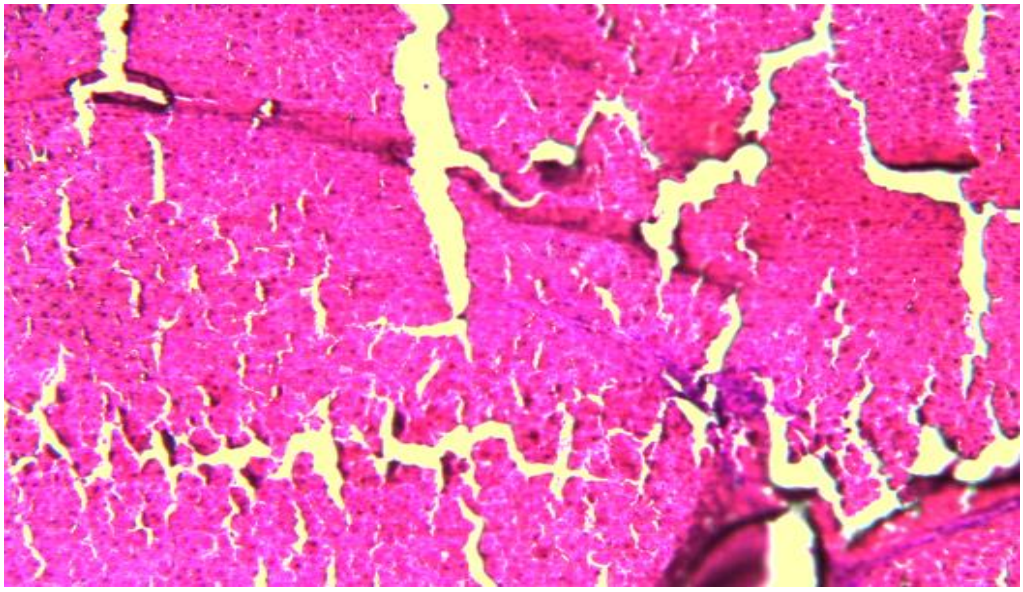


Plate 4.8: Section of the liver from group C shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

D1 LIVER X100



D2 LIVER X400

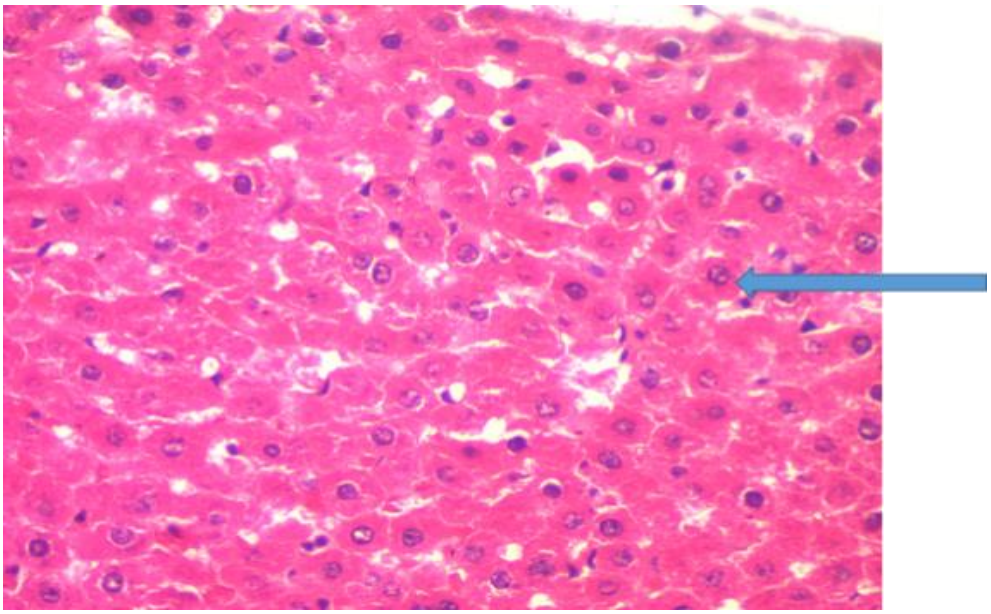
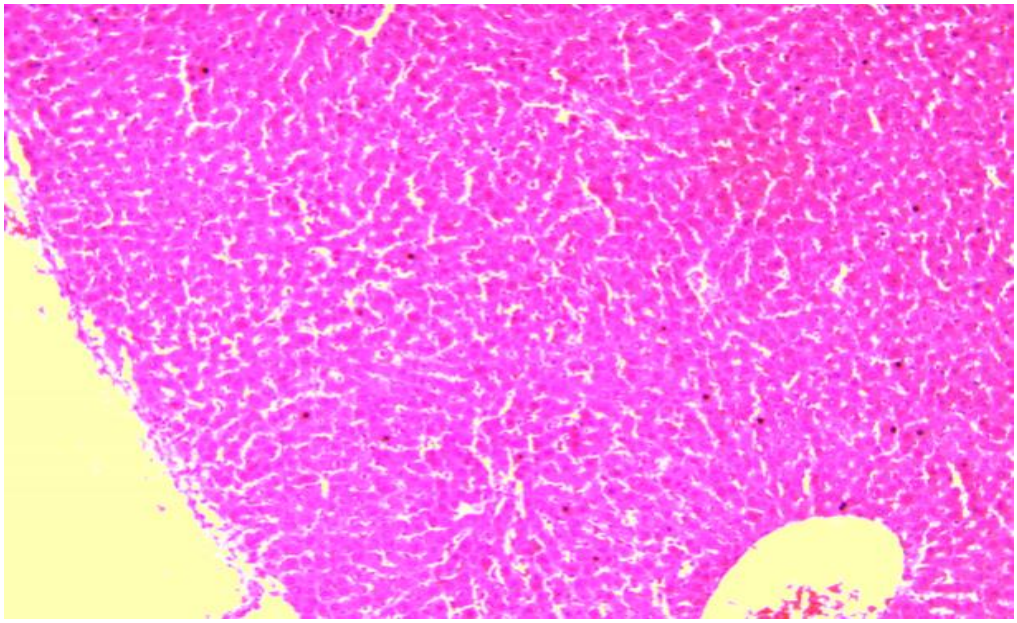


Plate 4.9: Section of the liver from group D shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

E3 LIVER X100



E3 LIVER X400

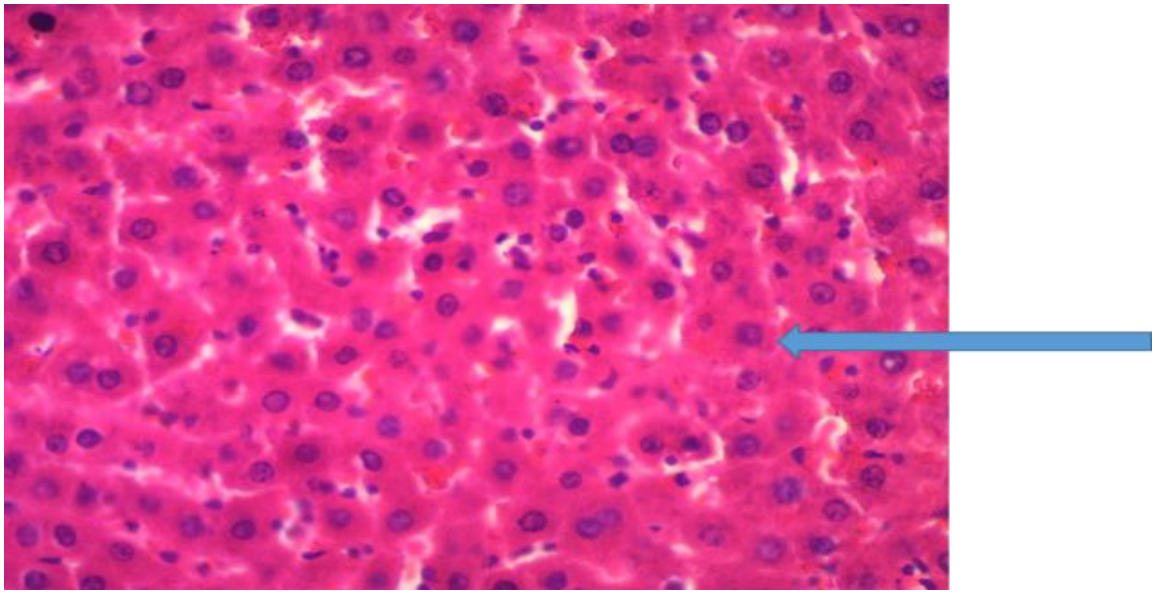


Plate 4.10: Section of the liver from group E shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

This study examined the histomorphological effects of lead toxicity on renal and hepatic tissues of albino rats and assessed the protective potential of Manihot esculenta (cassava) leaf extract.

The final body weights across groups showed no statistically significant differences ($p = 0.177$), though lead exposed and extract-treated rats had slightly higher mean weights. This suggests that short-term lead exposure did not significantly impair growth, possibly due to the limited exposure period or compensatory physiological responses. Similar findings were reported by Jing *et al*. (2020), who observed that early lead exposure may not immediately cause weight loss. Significant differences in liver weight ($p = 0.010$), particularly the enlargement observed in Group B (Lead + Extract 500 mg/dL), indicate hepatic sensitivity to toxic insults (Basha *et al*., 2019; Ojo *et al*., 2021). Restoration of liver weight toward control values in Group C (Lead + Extract 1000 mg/dL) suggests a dose-dependent hepatoprotective effect of cassava leaf extract (Amadi and Nzeh, 2018; Adeyemi *et al*., 2020).

Kidney and stomach weights showed no significant differences ($p > 0.7$), implying minimal gross renal damage under the conditions tested. Abas *et al*. (2021) similarly reported that early-stage lead nephropathy may present without marked changes in kidney size.

Renal and hepatic biochemical markers (urea, creatinine, bilirubin, AST, ALT, ALP, and most electrolytes) remained within normal ranges, suggesting preserved functional integrity. The only significant alteration was elevated chloride levels in

Group C ($p = 0.035$), possibly reflecting mild electrolyte disturbance associated with disrupted ion transport as described by Mahammadi *et al*. (2019). Stable urea and creatinine levels agree with Mishra *et al*. (2021), who noted that low-level lead exposure might not immediately impair glomerular filtration. Similarly, the absence of elevated liver enzymes supports earlier findings that moderate lead exposure induces subclinical hepatic injury before biochemical derangements become evident (Kim *et al*., 2018; Trefts *et al*., 2017).

Renal histology across all groups showed normal glomeruli and tubules, indicating minimal acute damage. Chronic lead nephropathy typically involves tubular atrophy, fibrosis, and glomerulosclerosis (Jing *et al*., 2020; Mishra *et al*., 2021), but these were not observed, possibly due to the short exposure period or protective effects of the extract.

Liver histology revealed steatosis (ballooning degeneration) only in Group B, while Groups C and D showed normal hepatocytes. This confirms that centrilobular hepatocytes (zone 3) are especially susceptible to toxins like lead (Chrostek and Janicka, 2017). Restoration of normal liver structure in Group C underscores the antioxidant and anti-inflammatory properties of cassava leaves, which stabilize hepatocyte membranes and suppress Kupffer cell activation (Montagnac *et al*., 2019; Muzanila *et al*., 2017).

The phytochemicals in cassava leavesflavonoids, phenolic acids, vitamin C, and beta carotene likely mediated the observed protective effects. These compounds are known to scavenge free radicals, chelate metals, and enhance antioxidant enzyme activity (Amadi and Nzeh, 2018; Ezeani and Okoro, 2021). Anti-inflammatory actions, including inhibition of pro-inflammatory cytokines and NF-κB signaling, further

explain the prevention of hepatic steatosis and maintenance of renal integrity (Montagnac *et al*., 2022).

These findings are consistent with prior reports demonstrating the efficacy of plant-based antioxidants in mitigating heavy metal toxicity (Miranda *et al*., 2017; Nweze and Nwabueze, 2019). The dose dependent hepatoprotective effect observed here parallels results from Adeyemi *et al*. (2020), who emphasized the importance of sufficient antioxidant dosing.

5.2 Conclusion

This study confirms that lead exposure produces early hepatic alterations, notably steatosis, without significant renal damage or widespread biochemical disruption within the study's timeframe. The normalization of liver weight and histology in rats treated with high dose cassava leaf extract highlights its potential as a hepatoprotective and nephroprotective agent. These results validate traditional knowledge of cassava leaves' medicinal uses and support their role as a low cost, accessible intervention for populations at risk of lead exposure (Arogundade and Agbon, 2017; Nweze and Nwabueze, 2019).

5.3 Recommendations

Long-term investigations be conducted to evaluate the chronic effects of lead exposure and to confirm the sustained protective potential of Manihot esculenta leaf extract. Further research should focus on optimizing the dosage and preparation methods of the extract, as the higher dose (1000 mg/dL) proved more effective in preventing hepatic alterations. Detailed phytochemical analyses are also necessary to isolate and characterize the active compounds responsible for its antioxidant and anti-inflammatory properties. In addition, cassava leaf extract could be considered as a complementary, low-cost public health intervention in areas with high lead exposure,

alongside broader environmental control strategies. Finally, advanced molecular and histological techniques should be employed to clarify the mechanistic pathways underlying its organoprotective effects.

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APPENDIX I

The instrument used for this research is as follows:

1. Animal House: during the time of feeding.

a. Feeding flat plate

b. Feeding water bottles

c. Feed (pellets)

d. ISOL disinfectant

e. Digital thermometer

f. Plastic cage

g. Weighing balance

h. Indian ink and plate

2. For Sacrificing

a. Hand gloves

b. Sterile Lancet

c. Cotton wool

d. Chloroform

e. Plastic container sterile with a cover

f. Dissenting set

g. Sterile containers

h. Formalin

3. Histology Laboratory

a. Scrape blade

b. Spatula

c. Block holder

d. Automatic tissue processor

e. Molten basket

f. Tissue basket

g. L-shaped mould

h. Rotary type microtome

i. Water bath

j. Hot plate

k. Metal pencil

l. Slides and cover slip

m. Stain (Haematoxylin and eosin)

n. Binocular microscope

o. Dibutylphthalate polysterene xylene (DPX),

p. Xylene, alcohol and water

APPENDIX II

The mould was filled with molten paraffin wax

With a pair of warm blunt-nosed forceps, tissues were transformed from the paraffin bath to the mould

Forceps were warmed and tissues oriented until lying in the desired plane.

Corresponding labels from the paraffin bath were removed and placed against the side of the mould adjacent to the tissues.

Air was blown on the surface until a thin film of wax has solidified.

The mould was transferred to a container of cold water and submerged until wax hardens.

After embedding, the block is to left harden up while placed on the ice for some hours before sectioning.

The Hertz microtome (Cambridge model) was used for trimming and sectioning at varying microns and the block clamp adjusted so that sections at 3-5microns were obtained in a ribbon-like manner, which was floated in a water bath to flatten by gentle heat.

The section or short ribbon was picked using a clean grease-free slide to ensure that the sections were thoroughly dried before staining by placing on a hot plate. After which, slides were stained according to Hematoxylin and Eosin method.

APPENDIX III

PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

1. The section was dewaxed in two changes of xylene for 2minutes each.
2. The section were taken through descending grades of alcohol. From absolute alcohol for 2minutes to 90% alcohol for 1minutes, 70% alcohol for 1minutes
3. The slides were washed in running tap water for one minutes.
4. Tissue sections were stained in hematoxylin for 10minutes
5. The sections was rinsed in distilled water for 30 seconds.
6. The sections was then differentiated in 1% acid alcohol for 15seconds.
7. After that, the sections were rinsed in distilled water for 5minutes.
8. The sections was counterstained with 1% eosin for 5minutes.
9. The sections was washed in running tap water for 30seconds.
10. Sections was dehydrated by passing through ascending grades of alcohol (70%, 90%, and 100%) for 1minutes each.
11. The section was cleared in two changes of xylene for 2minutes each.
12. The section was mounted with DPX and viewed microscopically using the objectives lens.

APPENDIX IV

APPENDIX V

MINISTRY OF AGRICULTURE AND FOOD SECURITY,
ANIMAL ETHICS COMMITTEE (MAFSAEC)

CERTIFICATE
OF ETHICAL APPROVAL

This is to certify that

IKOSINI USERHUMU ESTHER

Has been given MAFSAEC Approval for the Animal Component of the research titled:

HISTOMORPHOLOGICAL ASSESSMENT OF *MANIHOT ESCULENTA* LEAF EXTRACT EFFECT ON RENAL AND HEPATIC TISSUE OF LEAD-INDUCED TOXICITY IN ALBINO RATS.

In accordance with the Animal Disease Control Act, 2022.

Dr L.I Adebudo
Chairman MAFSAEC

Approval No.
MAFSAEC: 025-10/15/0048

Date Of Approval
17th October, 2025

(This Approval is only valid for this study)