INVESTIGATING THE HISTOMORPHOLOGICAL EFFECT OF*Spondias mombin* ROOT EXTRACT ON LIVER AND KIDNEY OF WISTAR ALBINO RATS

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SEPTEMBER, 2025.

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BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILMENT FOR THE REQUIREMENT OF THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE (BMLS)

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SEPTEMBER, 2025.

CERTIFICATION

This is to certify that this research work was carried out by **MODUPEOLUWA FOLASHADE ATOYEBI** with Matriculation Number **BMS2101481** under the supervision of **DR. (MRS). OGEYEMHE B.E.** in the Department Of Medical Laboratory Science, University Of Benin, Benin City, Edo State.

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DEDICATION

I dedicate this work to EL-ROI, for he alone has brought me thus far.

ACKNOWLEDGEMENTS

Words are not enough to express my gratitude to GOD ALMIGHTY for his faithfulness, it is by his mercies and grace that I am not consumed.

To my wonderful supervisor, **DR. (MRS). B.E. OGEYEMHE**, I'd like to say thank you for your guidance, support and feedback throughout this project. Your expertise and encouragements were invaluable.

My appreciation also goes to our amazing Head of Department, Medical Laboratory Science, University of Benin, **DR. (MRS.) OMORUYI Z.**, an amazing woman who cares so much about every student.

My esteemed lecturer, I appreciate your teachings and impact you continue to make in our lives – both academically and personally.

I also want to express my heartfelt gratitude to my beloved father, **MR. R. ATOYEBI**, for his unwavering love, support and encouragement. May you live long to enjoy the bountiful fruits of your labor. To my late mother, **MRS. E. ATOYEBI**, I remain eternally grateful for your love, sacrifices, and the strong foundation you laid in my life.

To my irreplaceable siblings (AUNTY AANU, AUNTY SEUN, & SOLOMON), thank you for being a constant support and motivation.

To amazing people who held my hands throughout the journey: Late MR. YUNUS L.A., MR. AWONYEMI O.I., MRS. ADELEYE C.M., ADEWUYI OMOTOLA, thank you all for being my safe place.

To my amazing senior colleagues: Oreoluwa, Omoye and Michael, sincerely thank you for the genuine love, care and support you have shown to me.

To my friends: Angelica, Daisy, Olawale, Chukwuemerie and my coursemates, thank you for the memories and laughter that made this journey memorable.

And to the House of BSF (Baptist Student Fellowship, UNIBEN/UBTH), thank you for being a family to me, for your prayers, encouragement and the bonds we share.

Thank you all for your contribution, big or small, that have made this project a success.

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ABSTRACT

Spondias mombin, a tropical medicinal plant, is widely employed in ethnomedicine, yet its safety profile on vital organs remains insufficiently defined. This study investigated the histopathological and biochemical effects of graded doses of *Spondias mombin* root extract on the liver and kidney of Wistar rats. Twenty-four male albino rats (150–200 g) were divided into six groups ($n = 4$): a control group and five groups administered 200, 400, 600, 800, and 1000 mg/kg of the extract orally for 28 days. Serum electrolytes, urea, creatinine, and liver enzymes (AST, ALT, ALP) were analyzed using standard biochemical methods, while hematoxylin and eosin staining was employed for histological assessment of liver and kidney tissues. Body weight, organ weight, and tissue integrity were evaluated to determine systemic effects. The results showed significant variation in body weight across groups ($p < 0.05$), with the highest gain at 800 mg/kg and the lowest at 200 mg/kg, though organ weights did not differ significantly ($p > 0.05$). Biochemical parameters remained largely stable across all doses, except for a significant reduction in serum creatinine at 1000 mg/kg ($p = 0.006$). Histological examination revealed normal hepatic and renal architecture up to 600 mg/kg, while higher doses (800–1000 mg/kg) showed hepatic ballooning degeneration and microvesicular steatosis, with kidneys appearing normal in all groups. These findings suggest that *S. mombin* root extract is relatively safe at low to moderate doses but may cause dose-dependent hepatic steatosis at higher concentrations. Further chronic toxicity and mechanistic studies are recommended to establish safe therapeutic limits and identify the bioactive compounds mediating both protective and toxic hepatic effects.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Medicinal plants have a crucial role in traditional medicine and contemporary pharmaceuticals to cure and manage various human disorders (Singh R., 2015). Medicinal plants are herbs or medicinal compounds utilized for medical purposes. According to the World Health Organization (WH O), over 80% of our global population relies on medicinal plants for basic healthcare since they play an important role in sustaining human health and improving quality of life (Shaik *et al*., 2017). *Spondias mombin* Linn, like some other known medicinal plants, serves in a variety of roles, including herbal medicine, where it has many ethnopharmacological properties for the treatment of a variety of ailments implicated in folkloric use across cultures (Ogunro *et al*., 2023). For example, plant parts such as leaves, flowers, fruits, seed, stem bark, pulp, and root are ethnomedicinally viable in managing or treating ailments from leprosy, severe cough, diarrhoea, dysentery, dyspepsia, gastralgia, colic, constipation, hemorrhoids, gonorrhea, leucorrhea, dystocia, to postpartum haemorrhage and inflammation (Ogunro & Yakubu, 2021). *Spondias mombin* is a strictly tropical plant that lives in the rainforest lowlands but has evolved to flourish in increasingly dry areas (Almeida *et al*., 2007). The deciduous fruit tree is extensively spread in Nigeria (particularly in the Southwestern parts), Ivory Coast, Brazil, Bolivia, Mexico, Peru, Venezuela, Colombia, Guianas, as well as a number other worldwide tropical forests across the world(Mattietto & Matta, 2011). *Spondias mombin (Linn)* is of the Sapindales order, genus *Spondias* and essentially a member of family Anacardiaceae, comprising about 73 genera, 850 species (Sameh *et al*., 2018). It is commonly known as Hog plum in English-speaking tropical regions (Mitchell & Daly, 2015). In Nigeria, the tree is known by many tribal names, including Akika (Yoruba), Nsukakara (Efik), Tsardar masar (Hausa), Ijikara (Igbo), Aginiran (Ijaw), Kakka (Tiv), and Chabbuli (Fulani) (Mattietto & Matta, 2011). *Spondias mombin* root are specifically used as oral infusion for treatment of dysentery, oral decoction for treatment of Tuberculosis, diarrhea, childbirth aid, adjunctive with antibiotics, vaginal infections and hemorrhoids, and Gastric analgesic (Ogunro *et al*., 2023). Recently, the root of *S. mombin* was shown to have quantities of carbohydrates, reducing sugar, alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, steroids, oil, and terpenoids in the methanolic extract (Estella & Loveth, 2020).

The liver is the body's largest gland and is well-positioned to receive absorbed nutrients while also detoxifying ingested medications and other unpleasant compounds (Alessandrino *et al*., 2019). The elimination of waste materials like urea and ammonia, electrolyte control, and acid-base balance are just a few of the vital tasks carried out by the kidneys. Through the renin-angiotensin-aldosterone pathway, they are essential for maintaining intravascular volume and controlling blood pressure. In addition to secreting the hormones calcitriol and erythropoietin, they are in charge of the reabsorption of amino acids, electrolytes, calcium, phosphate, water, and glucose (Madrazo-Ibarra & Vaitla, 2025; McMahon *et al*., 2025; Scott & Quaggin, 2015).

1.2 Statement of Problem

Medicinal plants such as *Spondias mombin* had been used in treatment of various disease such as oral infusion for treatment of dysentery, oral decoction for treatment of Tuberculosis, diarrhea, childbirth aid, adjunctive with antibiotics, vaginal infections and hemorrhoids, and Gastric analgesic (Duke, 2018). The root had been found to contain quantities of carbohydrates, reducing sugar, alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, steroids, oil, and terpenoids in the methanolic extract (Estella & Loveth, 2020). There is limited scientific evidence regarding its safety profile, particularly concerning its structural effect on organs like the liver and kidney. This study aims to investigate the potential effects of*Spondias mombin* tree root extract on histology of the liver and kidney of Wistar albino rats, giving explanations to its therapeutic potential and contributing to the development of safer renal and hepatic protective agents.

1.3 Justification of Study

Spondias mombin root extract had gained wide attention due to their antibacterial, antidiabetic, antioxidant, haematinic, oxytotic effect (Nworu *et al*., 2023). It has a high concentration of vitamins, minerals, and phytochemicals, all of which have potential health benefits. According to the World Health Organization (W.H.O., 2023), over 80% of our global population relies on medicinal plants for basic healthcare since they play an important role in sustaining human health and improving quality of life (Shaik *et al*., 2017). However, there is limited scientific evidence regarding its safety profile, particularly concerning its structural effect on organs like the liver and kidney. This study is justified by its potential to authenticate the effects of *Spondias mombin* root extract in preclinical model, giving scientific evidence for its use as a natural therapeutic agent.

1.4. Significance of Study

The methanolic extract of *Spondias mombin* root included carbohydrates, reducing sugar, alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, steroids, oil, and terpenoids (Estella & Loveth, 2020). The histological effect of this root extract at diverse dosages on the liver and kidney is unclear, and there is little to no scientific data to support the many applications of this extract. This study will give critical information on the root extract's toxicity or protective effect on the liver and kidney at various doses. The study will encourage the long-term usage of medicinal plants, notably *Spondias mombin*, and help to conserve medicinal plant species (W.H.O., 2019).

1.5 Aim of Study

The study aimed to investigate the histomorphological effect of *Spondias mombin* root extract on the liver and kidney of adult male Wistar rat.

1.6 Objectives of Study

The specific objectives of this study are:

1. To assess the effects of *Spondias mombin* root extract on the body weight of adult Wistar rat.
2. To assess the effects of *Spondias mombin* root extract on organ weight of adult Wistar rat.
3. To assess histopathological changes in the liver and kidney tissues of Wistar rats treated with *Spondias mombin* root extract.

1.7 Research Questions

1. How does *Spondias mombin* root extract affect the body weight of adult Wistar rat?
2. How does *Spondias mombin* root extract affect the organ weight of adult Wistar rat?
3. How does *Spondias mombin* root extract affect the liver and kidney histology of adult Wistar rat?

1.8 Research Hypothesis

1.8.1 Null Hypothesis

1. There is no changes in the body weight of adult Wistar rat.
2. There is no changes in the organ weight of adult Wistar rat.

3. There is no histological changes in the liver and kidney of adult Wistar rat.

1.8.2 Alternate Hypothesis

1. There are changes in the body weight of adult Wistar rats.
2. There are changes in the organ weight of adult Wistar rats.
3. There are histological changes in the liver and kidney of adult Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

There are an estimated 350,000 to almost half a million species of vascular plants, and 10 percent of all vascular plants are employed as medical plants (Fonnegra, 2007). Plants have been used in medicine since ancient times and continue to be used today (Grover *et al.*, 2002). The usage of these plants has evolved throughout time, and it is now referred to as traditional medicine in various situations. Traditional medicine is defined by the FDA as "the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses" (W.H.O., 2000). It is true that all civilizations have evolved this type of medicine (Gurib-Fakim, 2006) based on the plants in their natural environment (Houghton, 1995). Some scholars even argue that this transferred knowledge is the beginning of medicine and pharmacy. Even today, hundreds of higher plants are cultivated across the world to provide important compounds for medicine and pharmacy (Kinghorn & Seo, 2020). Plants' therapeutic characteristics inspired the development of medical medicines derived from specific plants that provide these advantages (Jones *et al*., 2006). According to the countries Health Organization (WHO), 80% of people in the developing countries receive their treatment through traditional medicine. The use of complementary or alternative medicine (CAM), especially herbal medicines, has increased in the developed world during the past several decades (Chintamunnee & Mahomoodally, 2012). Herbs, herbal materials, herbal preparations, and completed herbal products using plant parts or other plant components as active ingredients are all considered herbal medicines. Even though 90% of Ethiopians rely on herbal treatments for their main healthcare, studies conducted in wealthy nations like Canada and Germany often reveal that at least 70% of their citizens have tried complementary and alternative medicine (CAM) at least once (Chintamunnee & Mahomoodally, 2012; Gurib-Fakim, 2006).

Of all the therapeutic systems, African traditional medicine is the oldest and maybe the most diverse (Abdullahi, 2011). With its great ecological and cultural variety and regional variations in healing customs, Africa is regarded as the cradle of humanity(Gurib-Fakim, 2006). There are two main explanations for the African healthcare system's ongoing interest in traditional medicine (W.H.O., 2003). The first is the lack of access to western therapies and allopathic drugs, which most Africans cannot afford due to the high expense of contemporary medical care or the absence of sufficient medical service providers (Yenet *et al*., 2023). Second, some diseases like HIV/AIDS and malaria, which are widely distributed yet disproportionately affect Africa more than other parts of the world, lack adequate contemporary medical treatment (Gupta *et al*., 2008). The usage of medicinal plants is the most widely used traditional medicine throughout the African continent (Mahomoodally, 2013). Medicinal plants are the most readily available health resource for the population in many regions of Africa (Mahomoodally, 2013). Africa is really endowed with a wealth of biological resources; it is thought to have between 40 and 45,000 plant species with potential for growth, of which 5,000 are utilized medicinally (Abdullahi, 2011). Given that Africa has a tropical and subtropical climate and that plants naturally acquire significant secondary metabolites as a method of surviving in harsh environments, this is not surprising (Manach *et al*., 2004).

Herbal medicine practitioners (HMPs) in Nigeria have been using medicinal plants and herbal methods to treat infectious and non-infectious ailments for many years. Actually, a number of studies have shown the use of plants in ethnomedicine to cure conditions including bacterial infections, cancer, and malaria (Amujoyegbe *et al*., 2016; Dike *et al*., 2012; Odoh *et al*., 2018; Olorunnisola *et al*., 2013).

Traditional medicine is most frequently used because it is more economical, more in line with the patient's beliefs, allays worries about the negative effects of chemical (synthetic) medications, satisfies a need for more individualized treatment, and makes health information more widely available to the general public (Ekpor *et al*., 2024). Herbal remedies are mostly used to treat chronic illnesses rather than life-threatening ones and to promote wellness (ElSayed *et al*., 2023). However, when contemporary medicine fails to cure an illness, such as advanced cancer or in the face of emerging infectious diseases, the use of traditional medicines rises (Qato *et al*., 2008).

Numerous chemical molecules with a wide range of structures and functions that exhibit significant biological activities and are associated with numerous advantageous properties, including antimicrobial, anticancer, antiviral, antioxidant, and enzyme inhibitory effects, as well as anti-aging, anti-inflammatory, antihypertensive, neuroprotective, and anticoagulant effects, can be found in medicinal plants (El-Saadony *et al*., 2025). Around the world, medicinal plants are very important, both on their own and in conjunction with conventional medicine (Fig. 2.0). Growing interest in the use of natural goods is a result of both the extensive experience in folk medicine and the astounding number of studies on the therapeutic qualities of medicinal plants (Tlili & Sarikurkcu, 2020). Today, the investigation of novel bioactive chemicals is even more important due to the rise of germs that are resistant to antibiotics. Various sources claim that 25–50% of presently manufactured pharmaceuticals used in healthcare are derived from medicinal plants (Mahmood *et al*., 2019; Sinan *et al*., 2020), and researchers are searching all over the world for novel bioactive chemicals from both common and unusual plants (Fettach *et al*., 2019). Bioactive chemicals are abundant in the leaves, stems, roots, seeds, flowers, and fruits of medicinal plants(Knez Hrnčič *et al*., 2020). Plant-derived bioactive compounds have been shown to have similar protective and health-promoting effects on both humans and animals. They are also very appealing to consumers and many industries due to their widespread perception as natural, safe, and having fewer side effects than chemical drugs.

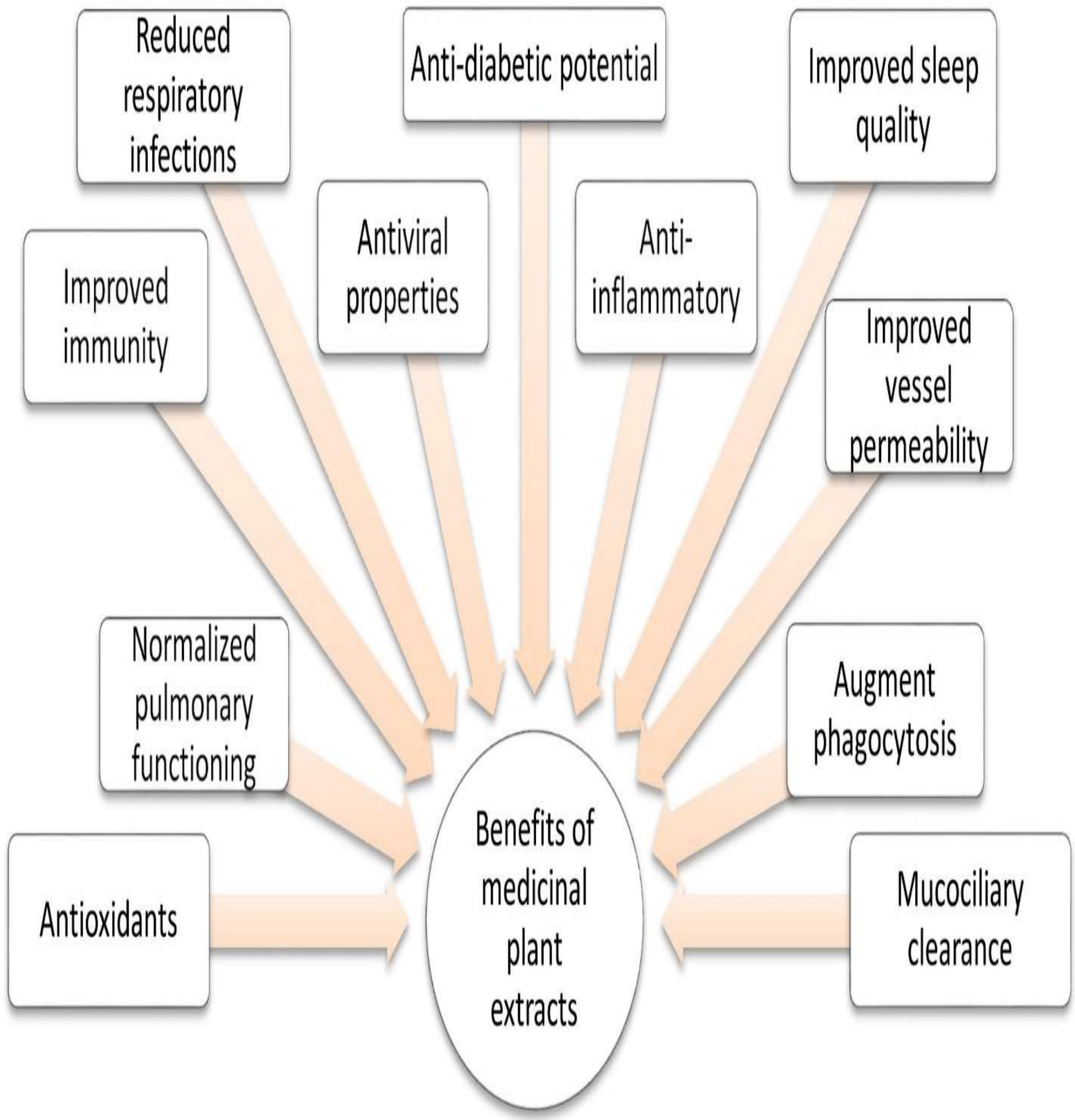


Figure. 2.1 Schematic representation of the potential beneficial effects of medicinal plant extracts in the prevention and treatment of many diseases (Knez Hrnčič *et al*., 2020).

2.2 *Spondias mombin* (Linn)

Originally from the rainforest lowlands, *Spondias mombin* is a stringently tropical plant that has evolved to grow in drier climates. The deciduous fruit tree is found in many tropical woods across the world, as well as in Nigeria (particularly in the southwest), Ivory Coast, Brazil, Bolivia, Mexico, Peru, Venezuela, Colombia, and Guiana (Mattietto & Matta, 2011; Fadimu *et al*., 2012). Contrary to earlier beliefs that the plant was imported to Africa, an evidence-based research and appraisal of African origin in contrast to the American area revealed that *S. mombin* can be disseminated naturally (Duvall, 2006). The tropical tree may reach heights of 20 to 30 m and has a trunk that is 60 to 75 cm in diameter. It grows upright, with a thick, rough bark covering it(de Vasconcelos *et al*., 2016). The tree's leaves are 20–45 cm long, hairy underside, and have 5–11 pairs of petiolate leaflets and short petioles. The blooms are typically white and produced in large quantities in tiny, fragrant panicles (Orwa *et al*., 2009).

The midrib has a bulging symmetry with supporting vascular bundles in a closed arc shape, and the leaf's tetracytic/anomocytic stomata are dispersed on the dorsal or abaxial surface(de Vasconcelos *et al*., 2016). The fruits have a stiff but thin exterior and a luscious flesh. They are 3–4 cm in length and 2.5 cm in breadth (Orwa *et al*., 2009). Depending on the carotenoid content, these fruits are often green at first and become golden-yellow when mature (Orwa *et al*., 2009). The endocarp, a pentagonal structure coated in tubercles, contains the noticeably large and gritty seeds (Orwa *et al*., 2009). The parenchymatous and locular masses are responsible for the endocarp's status as the most characteristic fruit component(Mattietto & Matta, 2011). According to a research on the morphology of *S. mombin* trees, typical morphology includes 15.2% without seed production, 52.8% with four loculi, and 44.8% with one-seeded endocarp (Souza *et al*., 2000).

Spondias mombin (Linn) is of the Sapindales order and genus *Spondias* (Fadimu *et al*., 2012. It is essentially a member of family Anacardiaceae, comprising about 73 genera, 850 species. It is often referred to as Hog plum in the English-speaking tropical regions (Orwa *et al*., 2009). In Nigeria, the tree is known by many tribal names, including Akika (Yoruba), Nsukakara (Efik), Tsardar masar (Hausa), Ijikara (Igbo), Aginiran (Ijaw), Kakka (Tiv), and Chabbuli (Fulani). The fruit is also called Iyeye (Yoruba), Oheeghe (Edo), Ngulungwu (Igbo), Isada (Hausa) (Sacramento & Souza, 2000; Mattietto & Matta, 2011; Fadimu *et al*., 2012).

The fruits of *S. mombin* are tropical, have a unique flavor and scent, and are high in nutrients. Low pH and reducing sugars describe the nutritional value, which includes rich mineral and vitamin contents, high and low moisture, and fat levels, respectively. Additionally, it contains phenolic chemicals and carotenoids (Tiburski *et al*., 2011; de Freitas *et al*., 2024).

High levels of vitamin A and carotenoids in the epicarp of *S. mombin* fruits give them a distinctive appearance (Pinheiro *et al*., 2019). Similarly, the pulp of *S. mombin* has a considerable quantity (mg/100 g) of manganese (0.025), iron (0.327), copper (0.118), aluminum (0.394), barium (0.069), sodium (5.551), magnesium (15.095), phosphorous (32.849), potassium (288.276), and calcium (11.038) (Njoku & Akumefula, 2007). Additionally, up to 37% of the recommended daily amount (RDA) for vitamin A may be obtained from 100 g of *S. mombin* pulp(Tiburski *et al*., 2011). Furthermore, due to its high content of carbohydrates, ash, crude fiber, moisture, crude protein, crude fat, calcium, iron, magnesium, manganese, zinc, and copper, the seed kernel was shown to be a valuable source of nutrient-dense food with potential for haematinic and anti-diabetic effects (40.56%, 8.09%, 31.86%, 8.48%, 7.73%, 3.28%, 1317 mg/kg, 839.08 mg/kg, 494.71 mg/kg, 17.93 mg/kg, 15.27 mg/kg, and 7.68 mg/kg) (Esua *et al*., 2016). Due to its high iron and chromium content, *S. mombin* leaves have also been suggested as effective antidiabetic and haematinic agents

(Ayoola *et al*., 2010). The moisture content of the root and leaf was high, at 35.78% and 32.59%, respectively. The leaf has the highest percentage of carbohydrates (32.08%), whereas the stem had the highest percentage of ash (4.52%). Vitamins B1 and C are abundant in each root, stem, and leaf in amounts greater than 2 mg/100 ml and 1 mg/100 ml, respectively. In a similar vein, the leaf and stem are rich in minerals, particularly salt and potassium (Alobi *et al*., 2017).

Spondias mombin is frequently used as an herbal remedy for a variety of illnesses. Remarkably, almost every component of the tree, including its fruit, leaves, roots, seeds, stem bark, bark, and even flowers, possesses unique qualities that are thought to have therapeutic value. In Africa, the bark or leaf is used for fever, coughs, yaws, constipation, tapeworm, stomach problems, gonorrhea, and as a childbirth aid (Asprey & Thornton, 1955). The leaf is used as a laxative for cholera, thrush, gonorrhea, dizziness, cough, fever, diarrhea, aiding in childbirth, treating tapeworms, malignant tumors, and as an abortifacient, febrifuge, astringent, and diuretic in Nigeria(Banjo *et al*., 2006). The *S. mombin* tree's bark is typically used as an analgesic to treat rheumatism, arthritis, muscular soreness, injuries, and inflammation (Desmarchelier & Witting Schaus, 2000; Morton, 1987; Ogunro & Yakubu, 2021). In Peru, the roots are used for treatment of tuberculosis, diarrhea, as adjunctive with antibiotics, and as a childbirth aid (Duke, 2018). Herbal medicine makes use of *S. mombin's* many, tiny, aromatic blooms. The flowers' infusion is used to cure cataracts, eye infections, mouth sores, laryngitis, and sore throats (Egg, 1999). While the aqueous and ethanolic extracts of the leaf contained tannins, saponins, alkaloids, and anthraquinones, the methanolic extract of S. mombin's root bark was recently found to contain proportions of carbohydrates, reducing sugar, alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, steroids, oil, and terpenoids (Oludare, 2018).

Liver diseases are major causes of illness and death worldwide (Rehm *et al*., 2013; Byass, 2014; Mokdad *et al*., 2014; Wang *et al*., 2014) and constitute a public health challenge that requires the development of new therapeutic options. According to a health concerns forecast, Chronic Kidney Disease will be the sixth most common cause of death globally by 2040 (Jager *et al*., 2019). Investigating the protective effects of medicinal plants in laboratory animals is an important initial step in evaluating the safety of new biomolecules (Colark *et al*., 2013; Lee *et al*., 2015).

2.3 Phytochemical Constituents

Natural principles or bioactive components found in plants are known as phytochemicals (Batiha *et al*., 2020). Together with nutrition and dietary agents, these natural substances shield both people and animals against disease (Atanasov *et al*., 2015). The *S. mombin* root had been found to contain quantities of alkaloids (0.36–0.78 mg/100 g), flavonoids (0.16–0.32 mg/100 g), saponins (1.06–3.90 mg/100 g), tannins (0.17–0.36 mg/100 g) and phenols (0.09–0.10 mg/100 g)(Okwu & Okwu, 2004). Tannin, saponin and anthraquinone glycosides from *S. mombin* demonstrated antimicrobial effects, but, with no significant antifungal efficacy (Onwuka, 1992). Numerous pharmacological characteristics, such as abortifacient, uterine stimulant, antiepileptic, memory-enhancing, neuroprotective, antioxidant, gastroprotective, anti-inflammatory, antibacterial, and COX-inhibitory, are present in crude extracts, fractions, and isolated compounds from *S. mombin*.

Fig.2.1. Roots of *Spondias mombin* (Moduoeoluwa Atoyebi, 2025).

2.4 Organs of Study

2.4.1 Gross Anatomy of The Liver

The liver is located inferior to the diaphragm and takes up the majority of the abdomen's right upper quadrant (RUQ). It extends largely intraperitoneally, from the fifth intercostal gap at the midclavicular line to the right costal border. The superior posterior portion of the liver has a bare region where the diaphragm and inferior vena cava are located. The visceral peritoneum covers the rest of the liver and meets the diaphragm at the bare area's border to create the coronary ligament. The inferior liver is strongly related to the gallbladder and right kidney. The liver is anatomically divided into four lobes: right, left, caudate, and quadrate. The quadrate lobe is situated on the inferior surface of the right lobe. The caudate lobe is situated between the left and right lobes, anterior and superior. Another ligament worth mentioning is the Falciform ligament, which divides the liver into anatomic left and right along the front portion (Abdel-Misih & Bloomston, 2010; Alessandrino *et al*., 2019; Nota *et al*., 2019).

The lobule is the liver's functional unit. Liver lobules are hexagon-shaped groups of hepatocytes with a central vein at their core. A vascular gap with a thin fenestrated endothelium and a discontinuous membrane known as a sinusoid is located between the cords of hepatocytes that make up the lobules. Stellate cells, which are hepatic lipocytes, and Kupffer cells, the liver's resident macrophage, are found in these sinusoids. The portal triad, which consists of a hepatic artery branch, a portal vein branch, and a bile duct branch, is located at the hexagon's vertices (Tani *et al*., 2016).

The liver is the body's largest gland and is well-positioned to receive absorbed nutrients while also detoxifying ingested medications and other unpleasant compounds (Alessandrino *et al*., 2019). The liver protects the body from toxic substances absorbed from the gastrointestinal (GI) tract by processing and metabolism within the lobule. The cytochrome P-450 enzyme system catabolizes phase-I reactions, while phase-II reactions conjugate substances with substrates such as glucuronide, glutathione, and sulfate.

2.4.1.1 Blood Supply and Lymphatics

75% to 80% of the blood volume that reaches the liver comes via the portal vein, whereas 20% to 25% comes from the hepatic artery. The splenic and superior mesenteric veins, which are located behind the pancreatic neck, combine to produce the portal vein (Kawaguchi *et al*., 2013). The portal triad, which is encased in the hepatoduodenal ligament, is formed of the common bile duct, the proper hepatic artery, and a branch of the celiac trunk. In the portal triad and related branches, the bile exits the liver through the bile ducts as the hepatic artery and portal vein bring blood to the liver. This produces countercurrent flow, which facilitates the best possible exchange of bile acids, electrolytes, and other substances (Maema *et al*., 2002).

The left, right, and center hepatic veins are the main routes via which the liver drains venous. The left hepatic vein mostly drains the left lobe of the liver and makes for 20.7% of the venous drainage. The middle sections of the liver's left and right lobes are drained by the middle hepatic vein, which makes up 32.7% of hepatic drainage. The lateral part of the liver's right lobe is drained by the right hepatic vein, which makes up 39.6% of drainage. Most of the time, the liver's caudate lobe empties into the main hepatic vein. It occasionally empties straight into the inferior vena cava's retrohepatic segment (Ortale & Borges Keiralla, 2004; Tani *et al*., 2016).

2.4.1.2 Physiology of the Liver

The largest gland in the body, the liver is well situated to absorb nutrients and detoxify ingested medications and other harmful compounds (Brandes *et al*., 2019). It functions as an endocrine and exocrine organ (Hundt *et al*., 2022). The liver's primary exocrine functions include conjugating bilirubin and excreting it into the gut, as well as synthesizing and excreting bile salts into the common hepatic duct (Hundt *et al*., 2022). Insulin and glucagon are two of the liver's endocrine processes that contribute to glycemic control. Important proteins including fibrinogen, albumin, prothrombin, and other amino acids are synthesized by the liver, which also transforms proteins into enzymes and peptide hormones. The liver produces lipoproteins, cholesterol, and phospholipids in addition to taking role in the metabolism of fatty acids. It also plays a role in the metabolism of carbohydrates, including gluconeogenesis and glycogen storage. It also transforms ammonia into urea and plays a role in the metabolism of lactic acid. Iron and other vitamins and minerals are stored in the liver. In conclusion, the liver is a key mediator between the gut and the blood and is essential for the metabolism of hormones, blood plasma components, exocrine and endocrine chemicals, and macronutrients.

2.4.1.3 Development of the Liver

The caudal region of the foregut produces mesoderm from a ventral proliferation of endoderm, which gives birth to the liver, gallbladder, and biliary system. This protrusion, known as the liver bud or hepatic diverticulum, occurs during the fourth week of fetal development. The mesoderm of the liver bud develops into connective tissue and blood arteries, whilst the endoderm gives rise to hepatocytes. It is crucial to keep in mind that the liver plays a hematopoietic function throughout the fetal stage.

2.4.1.4 Nerve Supply

The liver is innervated by the hepatic plexus. Parasympathetic fibers from the anterior and posterior vagal trunks and sympathetic fibers from the celiac plexus make up this plexus. The portal triad's biliary ducts and arteries are joined by the hepatic plexus.

Gallbladder

Directly underneath the liver lies the gallbladder. At its broadest point, this pear-shaped organ with thin walls is up to 5 centimeters (2 inches) across and 7 to 10 centimeters (2.7 to 3.9 inches) long. Bile from the liver is stored and concentrated in the gallbladder. After that, the bile is discharged into the duodenum, the first segment of the small intestine, where it aids in the breakdown and absorption of dietary lipids.

Approximately 800 to 1,000 milliliters (roughly 27 to 34 fluid ounces) of bile are produced daily by the liver's cells. The yellow, brownish, or olive-green liquid known as bile aids in the body's fat digestion. Bile is secreted by the liver cells into tiny canals that eventually lead to the common bile duct. The gallbladder is reached via a smaller duct that splits off from there. The small intestine is where the common bile duct terminates.

Biliary System

The biliary system develops in the fourth week of embryonic life and is derived from the ventral foregut endoderm. The caudal part of the hepatic diverticulum lengthens between weeks 4 and 8, which leads to the development of the extrahepatic bile ducts. The intrahepatic bile ducts emerge from the cranial part of the ventral foregut endoderm about the eighth week of pregnancy. On the other hand, the extrahepatic biliary tree develops in the caudal region (Roskams & Desmet, 2008). A ventral outpouching of the bile ducts gives rise to the gallbladder and cystic duct.

The production, storage, and secretion of bile through the liver, gallbladder, and bile ducts are together referred to as the biliary system. There are two types of bile ducts: extrahepatic and intrahepatic. The common hepatic duct (CHD) is produced by the union of the left and right hepatic ducts, which are considered intrahepatic bile ducts. On the other hand, the common bile duct (CBD), which is formed by the CHD and cystic duct, is considered an extrahepatic bile duct. Bile passes via the ampulla of Vater, which is formed by the convergence of the pancreatic duct and the central business district, before entering the second part of the duodenum through the sphincter of Oddi (Hundt *et al*., 2025). The epicholedochal and paracholedochal venous plexuses are the two venous plexuses that drain the biliary system. The epicholedochal venous plexus drains into the paracholedochal venous plexus (PVP) and is located on the bile duct wall. The gastrocolic trunk, right gastric vein, superior mesenteric vein inferiorly, intrahepatic portal vein branches superiorly, and posterior superior pancreaticoduodenal vein are all related to the PVP (Ramesh Babu & Sharma, 2014).

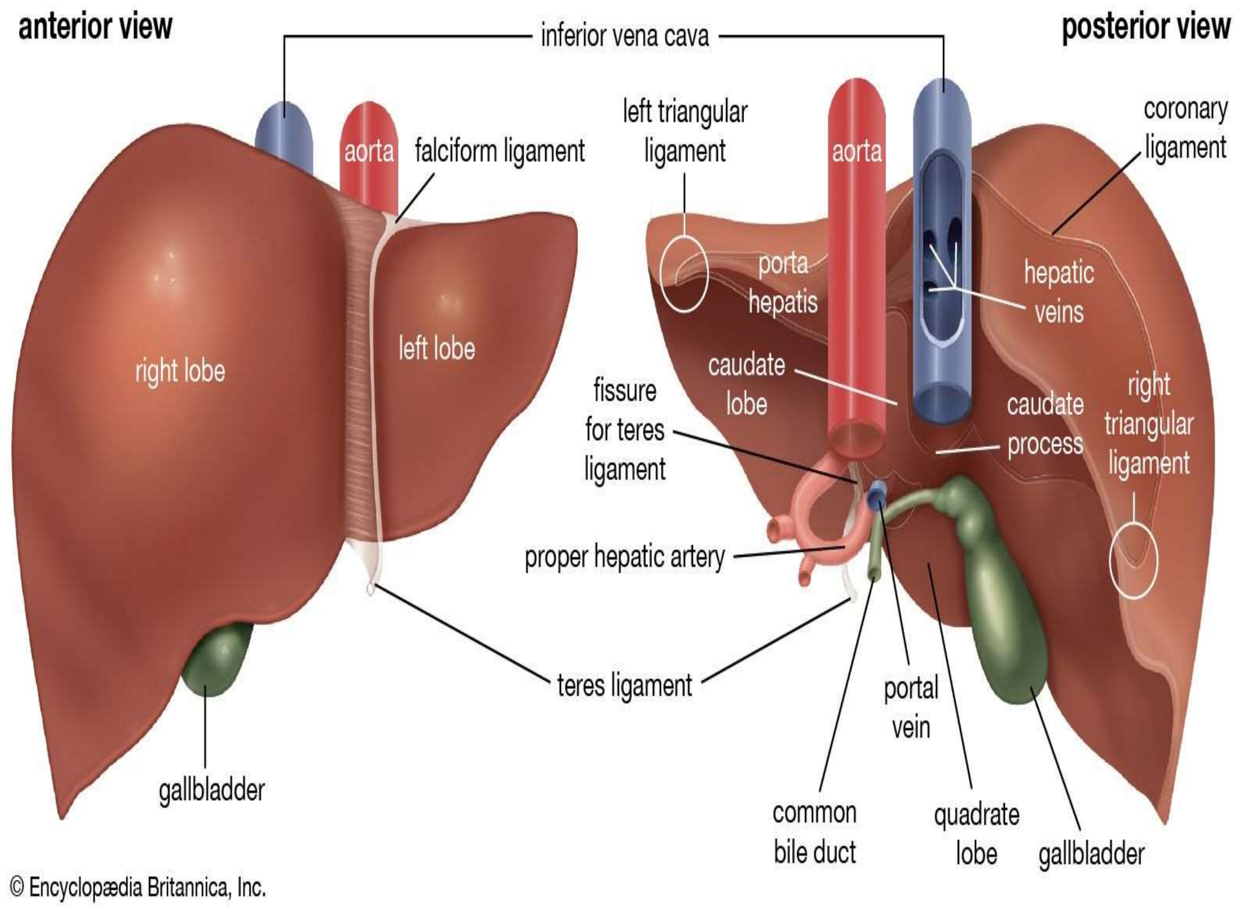


Figure 2.2: Anterior and posterior view of the right and left lobe of the liver (Hundt *et al*., 2022)

2.4.2 Gross Anatomy of the Kidney

The kidneys are bean-shaped organs that weigh between 150 and 200 g in males and around 120 to 135 g in females (Laguipo & Thomas, 2018). They have medial concavity and lateral convexity. Typically, the measurements are 10 to 12 cm in length, 5 to 7 cm in breadth, and 3 to 5 cm in thickness. A closed fist is roughly the size of each kidney. They are situated between the transverse processes of T12 and L3 on the posterior abdominal wall, retroperitoneally. In relation to the lower poles, both upper poles are often placed somewhat medially and posteriorly. A superior pole renal mass or a horseshoe-shaped kidney may be indicated if the upper renal poles are positioned laterally. Furthermore, due to the liver, the right kidney often occupies a somewhat lower position than the left (El-Reshaid & Abdul-Fattah, 2014). Each kidney is covered by a two-layered capsule and is surrounded by perinephric fat, Gerota's fascia, Zuckerkandl fascia, and paranephric fat. The entire area immediately involving the kidneys is considered the retroperitoneum (Tirkes *et al*., 2012; Coffin *et al*., 2015).

The kidneys' positions in relation to the surrounding organs are as follows: The suprarenal glands (adrenal glands) are located superiorly on top of each kidney and are divided by renal fascia. The left crescentic suprarenal gland is directed more medially on the left kidney, while the right pyramidal suprarenal gland is positioned apically on the right kidney. The hepatorenal recess divides the right kidney from the liver, the second portion of the duodenum medially, and the ascending colon. The left kidney is situated behind the descending colon, with its renal hilum next to the pancreatic tail, superomedial aspect next to the stomach's greater curvature, and left upper pole next to the spleen. These are all joined by splenorenal ligaments. The 12th rib passes posteriorly over the top pole, and the diaphragm rests over the upper portion of each kidney. The kidneys are typically situated above the lateral side of the quadratus lumborum and the medial portion of the psoas muscle. On their route to the bony pelvis, the proximal ureters usually cross the psoas muscle (Tirkes *et al*., 2012; Coffin *et al*., 2015; Megha *et al*., 2025).

The kidney is composed of two regions: the cortex and medulla. The cortex is composed of renal corpuscles, convoluted tubules, straight tubules, collecting tubules, collecting ducts, and vasculature. Medullary rays, comprised of straight tubules and collecting ducts, extend into the cortex from the medulla. The medulla also contains the vasa recta, a network of capillaries integral to the countercurrent exchange system. Pyramids are conical structures formed by the collecting of tubules in the medulla, oriented with the base towards the cortex and apices towards the hilum. The papillae at the apices of the pyramids extend into minor calyces and drain via the collecting ducts at their tips, the area cribrosa. As a lobule, a collecting duct drains a collection of nephrons (McMahon *et al*., 2025).

The kidney's functioning units are called nephrons. Each adult kidney has about 2 million nephrons. A double-layered epithelium called Bowman's capsule envelops the glomerulus, a network of capillary loops supplied by an afferent arteriole, to produce a renal corpuscle. The vasa recta, which feeds the renal tubules, is formed by an efferent arteriole that drains the glomerulus. The following are located in order distal to Bowman's capsule: the collecting tubule, cortical collecting duct, medullary collecting duct, papillary duct, minor calyx, major calyx, renal pelvis, ureter, proximal convoluted tubule, proximal straight tubule or thick descending limb of the loop of Henle, thin descending limb of the loop of Henle, thin ascending limb of the loop of Henle, distal straight tubule or think ascending limb of the loop of Henle, distal convoluted tubule, and renal pelvis. The tubules start in the brain, rise into the cortex close to their original renal corpuscle, descend into the medulla, and make a hairpin bend in the loop of Henle's thin limb (Scott & Quaggin, 2015; Madrazo-Ibarra & Vaitla, 2025).

2.4.2.1 Development of Kidney

The intermediate mesoderm gives rise to the kidney in mammals. The pronephros, mesonephros, and metanephros are the three stages of kidney formation (nephrogenesis). By the conclusion of the fourth week of development, the vestigial excretory units (nephrotomes) that make up the pronephros, which is situated in the cervical area, have regressed. The intermediate mesoderm from the upper thoracic to upper lumbar segments is where the mesonephros originates while the pronephros regresses. In order to construct a renal corpuscle (glomerulus encircled by Bowman's capsule), it is made up of excretory units that extend, form a loop, and grow capillaries. A collecting duct known as the mesonephric or Wolffian duct receives the excretory tubule. By the sixth week or thereabouts, two bilateral organs are present (Rehman & Ahmed, 2025). Comprising excretory units produced from metanephric mesoderm, the metanephros, the primordia of the permanent kidney, emerges in the fifth week. The combination of its tyrosine kinase inhibitor RET with glial-derived neurotrophic factor (GDNF) and its tyrosine kinase inhibitor MET with hepatocyte growth factor (HGF) stimulates the evagination of the Wolffian duct to generate the ureteric bud. WT1, a transcription factor expressed by the metanephric mesenchyme, controls the synthesis of GDNF and HGF (Dressler, 2006; Rehman & Ahmed, 2025). The calyces that are induced to divide into tubules at seven weeks later give rise to buds, which are then absorbed by growing tubules in the periphery to become minor calyces. Renal pyramids are formed when successive generations of collecting tubules continue to grow and converge. The proximal glomerulus grows into a surrounding Bowman's capsule, whereas the distal glomerulus joins the connecting tubules. The loop of Henle, distal convoluted tubule, and proximal convoluted tubule are the three lengthenings of the tubule. The functional excretory unit (nephron) is made up of these tubules and renal corpuscles. The nephrons continue to develop until there are around one million per kidney at birth, and by the twelfth week, they are operational and generating urine (Dressler, 2006; Upadhyay & Silverstein, 2014; Rehman & Ahmed, 2025).

2.4.2.2 Blood Supply

The kidneys get around 20% of the total cardiac output. The renal arteries, which emerge from the aorta inferior to the superior mesenteric artery and reach the kidney's hilum at L2, supply these highly vascularized organs. The inferior vena cava (IVC) is passed posteriorly by the lengthier right renal artery. Near the renal hilum, the two renal arteries split, producing five segmental arteries. The posterior region of the kidney is supplied by the first branch, the posterior segmental artery. The superior segmental artery, anterosuperior segmental artery, anteroinferior segmental artery, and inferior segmental artery are the four other major segmental arteries that emerge from the anterior branch of the renal artery and are called for the section of the kidney they nourish. Accessory renal arteries can emerge from the aorta or renal artery and typically reach the poles. They are left over embryologically in around 25% of persons because of fail. Anteriorly, the renal veins trace the course of the renal arteries. Because the left renal vein must cross the midline from the left side in order to reach the inferior vena cava at the level of L2 or L3, it is noteworthy that it is a few millimeters longer than the right vein. Because its vein is longer, the left kidney is typically used as a donor for transplants. The left gonadal vein often empties inferiorly into the left renal vein. The left suprarenal and left inferior phrenic veins are similarly received by the left renal vein. In 75% of individuals, branches of the lumbar or hemiazygos vein connect to the left renal vein.re of vascular degeneration (Jamkar *et al*., 2017; Lung & Lui, 2025; Wright & Burns, 2025). In order to reach the inferior vena cava, the left renal vein travels anterior to the aorta and posterior to the superior mesenteric artery. This puts the vein at danger of compression between the two arteries, which might lead to renal vein entrapment syndrome. In contrast, the gonadal and renal veins on the right side often empty into the inferior vena cava independently. Lastly, lymphatics drain the kidneys; occasionally, these lymphatics are joined by those draining the proximal ureters, which enter the right lateral inferior caval lymph nodes on the right and the left aortic lymph nodes on the left (Russell *et al*., 2019; Bowdino *et al*., 2025; Dalal *et al*., 2025; Lescay *et al*., 2025).

2.4.2.3 Nerve Supply

The renal nerve plexus, which is made up of sympathetic and parasympathetic fibers, transmits innervation to the kidneys, as well as to a section of the proximal ureters and suprarenal glands (Megha *et al*., 2025; Lescay *et al*., 2025). Fibers from the abdominopelvic splanchnic nerves supply this plexus. All of the nephrons and renal vasculature are innervated by sympathetic efferents, which are mostly found in the distal convoluted tubules, thick ascending limbs, and afferent arterioles. Primarily located in the renal pelvis, the sensory renal afferent neurons go down the renal artery or proximal ureter to the pelvic wall. They are crucial for controlling blood pressure and sympathetic outflow.

Some are orientated circumferentially, whereas others move parallel to the ureter or pelvis. They provide innervation to the renal vein, renal artery, and pelvic wall. There are very few sensory fibers visible in the brain and none in the medulla (Kopp, 2015; Frame *et al*., 2016). The spinal ganglia and cord segments T11-L2 get pain sensations from visceral afferent fibers via sympathetic fibers. Since the corresponding dermatome is frequently where pain is felt, flank discomfort may represent a referral of pain from the corresponding kidney.

2.4.2.4 Physiology of the Kidney

The elimination of waste materials like urea and ammonia, electrolyte control, and acid-base balance are just a few of the vital tasks carried out by the kidneys. Through the renin-angiotensin-aldosterone pathway, they are essential for maintaining intravascular volume and controlling blood pressure. In addition to secreting the hormones calcitriol and erythropoietin, they are in charge of the reabsorption of amino acids, electrolytes, calcium, phosphate, water, and glucose (Madrazo-Ibarra & Vaitla, 2025; McMahon *et al*., 2025; Scott & Quaggin, 2015).

2.4.2.5 Microscopic features of the kidney

Each renal lobule is made up of numerous epithelial tubules called uriniferous tubules that collectively form the parenchyma of the kidney. Each uriniferous tubule can be divided intp a nephron and a collecting duct. Each of these units has a different embryologic origin.

Nephron

The nephron is the minute or microscopic structural and functional unit of the kidney. It is composed of a renal corpuscle and a renal tubule. The renal corpuscle consists of a tuft of capillaries called a glomerulus and a cup-shaped structure called Bowman's capsule. The renal tubule extends from the capsule. Three layers filter blood: the capillary wall's endothelial cells, the basement membrane, and the space between the podocyte foot processes of the capsule lining. The tubule's ascending and descending sections are separated by nearby peritubular capillaries (Kumaran & Hanukoglu, 2024). The fluid from the capsule is processed by the tubule's lining epithelial cells as it descends into the tubule: Substances are exchanged (some are added, some are withdrawn) and water is reabsorbed; this occurs first with the interstitial fluid outside the tubules and then through the endothelial cells lining the neighboring peritubular capillaries into the plasma.

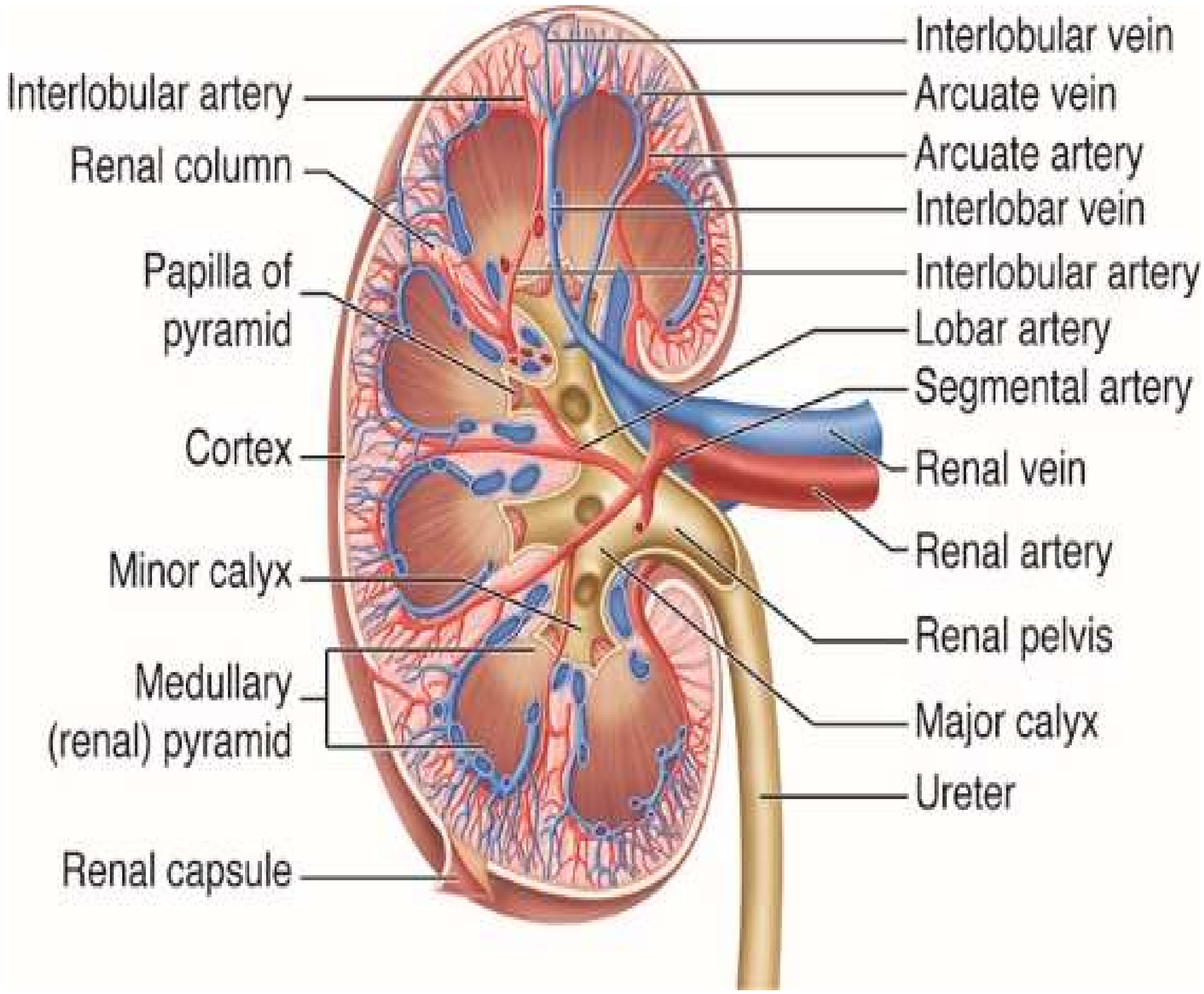


Figure. 2.3: Gross anatomy of the kidney, OpenStax. (2013). Anatomy and Physiology

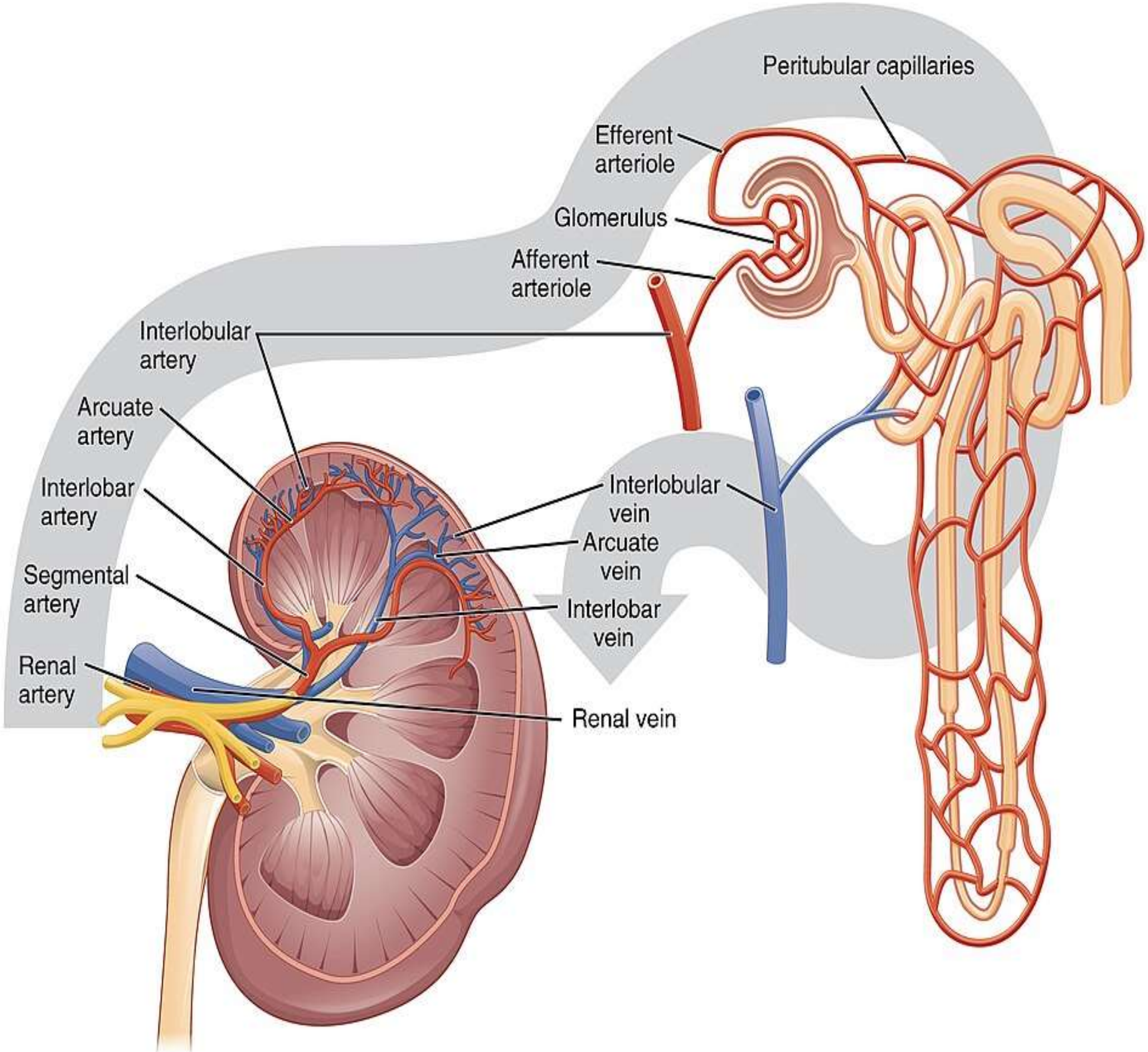


Figure. 2.4: Gross anatomy of the Kidney, OpenStax. (2013). Anatomy and Physiology

2.5 Experimental Model: Albino Wistar Rat

Albino wistar rats are a strain of the Norway rat species that is used as an animal model in a variety of fields, including neurology, pharmacology, and toxicology. These rats are particularly beneficial owing to their high level of genetic homogeneity, simplicity of handling, and consistent findings, which have maintained them on the list of must-haves for researchers looking to reduce extraneous factors in their investigations (Krubaa & Yogitha, 2024). Wistar albino rats will be used as an experimental model to assess the histomorphological effects of *S. mombin* root extract on renal and hepatic tissue. Various dosages of *S. mombin* root extract will be given to Wistar rats, and the effects on liver and kidney tissue will be assessed utilizing biochemical markers and histological examination.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and Duration of the Study

This study was undertaken at the Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. The research environment was in adherence to required ethical standards and safety measures. The entire study lasted from June 30^{th}, 2025 to August 10^{th}, 2025.

3.2 Reagents and Chemicals

Hematoxylin dye, Eosin dye, 1% acid-alcohol, xylene, ethanol, disperse plasticizer, ethanol, 10% neutral buffered formalin, distilled water and normal saline (Note: all reagents were distilled prior to use).

3.3. Equipment and Apparatus

Dissecting Board, Dissecting Set, Cotton Wool, Gauze, Husks, Measuring Cylinder, Conical Flask, Cover Slip, Slides, Universal Containers, 5ml Syringes. Automatic Tissue Processor (Hestion ATP7000 Tissue Processor, Germany), Embedding Machine (Hestion E500, Germany), LeukhartMoulds, Digital Rotary Microtome (Hestion ERM 4000, Germany), Water Bath (Gallenkamp), Hot Plate, Muslin Cloth, Staining Rack, Electric grinder, Forceps, Sieving Clothes, Microscope (Olympus England), Digital Electronic Balance (Gilbertini, Italy; Sensitivity = 0.001g), Analytical Weighing Balance, and Plastic Cages.

3.4 Plant Materials

Samples of fresh *Spondias mombin* tree roots were obtained from Itanla, Ondo East Local government, Ondo state.

3.4.1 Identification and authentication.

They were identified and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University Of Benin, By Prof. Henry A. Akinnibosun. After which, a sample plant was deposited at the departmental herbarium, and a voucher number UBH-S345 was assigned for referral and cross-referencing of possible changes to previous research.

3.4.2. Preparation of Plant Materials.

The roots were cut into smaller particles and air-dried at room temperature for about 2 weeks. After proper drying, the roots were pulverized into fine powdered form using electric grinding machine. The powdered particles were soaked with absolute ethanol at the ratio of 1g : 10ml for about 72 hours. Within this period of soaking, it was stirred with stirring rod from time to time. After 72 hours, it was filtered with sieving clothes. Thereafter, the residues were discarded and filtrates were concentrated using water baths and preserved with refridgerator when it came out in a paste form.

3.4.3. Storage.

To prevent degradation of the extract, it was stored in an airtight container in refridgerator.

3.5 Animal Housing.

Twenty-four (24) albino rats, weighing between 150g and 200g each were procured from the animal house at Anatomy department, University of Benin. The experimental rats were separated into 6 groups (1 to 6) and housed in plastic cages with wire gauge for sufficient ventilation, as well as provisions for meal and water available at all times and ensuring a sterile environment for the animals by cleaning the cages daily. Prior to the start of the study, the animal were allowed to acclimatize to the room's temperature (25^{o}c - 30^{o}c) and relative humidity for a period of two weeks.

3.6 Ethics

The procedure carried out during the process of the study was conducted in alignment with the international guidelines for handling experimental animals as given by the National Committee for Research Ethics in Science and Technology (NENT), 2018. Ethical approval for this study was sought from the Ethics and Research Committee and an ethical number was given by the Ministry of Agriculture, Edo State, Nigeria. (MAFSAEC: 025-08/13/0042)

3.7 Methodology

3.7.1 Experimental Design

The rats were divided into six (6) groups, with each group comprising of four (4) rats each. The groups are as follows:

Group A (n=4)

Control Rats- In the control group which received standard feed and water only. No *Spondias mombin* root extract or other treatment was given. This group served as the baseline.

Group B (n=4)

This group received 200mg/kg body weight of *Spondias mombin* root extract dissolved in distilled water via oral gavage daily for 4 weeks (28 days).

Group C (n=4)

This group received 400mg/kg body weight of *Spondias mombin* root extract dissolved in distilled water via oral gavage daily for 4 weeks (28 days).

Group D (n=4)

This group received 600mg/kg body weight of *Spondias mombin* root extract dissolved in distilled water via oral gavage daily for 4 weeks (28 days).

Group E (n=4)

This group received 800mg/kg body weight of *Spondias mombin* root extract dissolved in distilled water via oral gavage daily for 4 weeks (28 days).

Group F (n=4)

This group received 1000mg/kg body weight of *Spondias mombin* root extract dissolved in distilled water via oral gavage daily for 4 weeks (28 days).

After the four (4) weeks treatment period, the animals were anesthesized with chloroform and sacrificed by fenestration 24 hours after the last day of administration. Using a sterile surgical blade, the liver and kidney were removed from all experimental groups, including the control group. The harvested organs were immediately placed in fixative (10% neutral buffered formalin) to prevent degradation.

The estimated acute toxicity (LD50) : >2,000mg was extrapolated from work done by Offor, C. E., *et al*. (2019).

3.8 Enzymes Biochemistry Tests

Electrolytes, Urea, and Creatinine. E/U/Cr

Serum were obtained from blood samples and assayed for key kidney function indicators, including electrolytes (such as sodium, potassium, chloride, and bicarbonate), urea, and creatinine. The concentration of each electrolyte in the serum sample was determined using ion-selective electrodes. The samples were carefully prepared to minimize contamination or evaporation, ensuring precise results. Urea levels were measured using a colorimetric method. 0.2 ml of serum was combined with a specific reagent (urease) that reacts with urea to produce a color change, proportional to the concentration of urea in the sample. The mixture were then incubated at 37°C, allowing the reaction to develop fully. Creatinine was also measured using a colorimetric assay. 0.2 ml of serum was mixed with picrate solution, which reacts with creatinine to produce a color change. This reaction mixture was incubated under controlled conditions, allowing the color to stabilize. Both the urea and creatinine assays were read spectrophotometrically at a wavelength specific to the color developed, typically in the range of 500–600 nm, with a blank sample used as a control to standardize readings. The electrolytes (Sodium, Potassium, Chloride, and Bicarbonate), Urea, and Creatinine (EUC) test provides critical information about the body's hydration status, acid-base balance, and kidney filtering capacity.

SOP FOR ASPARTATE AMINOTRANSFERASE (AST)

Introduction:

Aspartate Aminotransferase is an enzyme present in cells in the body including various red blood cells, liver, e.t.c. Its estimation is used in the diagnosis of liver diseases. Though not specific for the diagnosis but when used in conjunction with Alanine aminotransferase, could be useful.

Principle of the Test: AST incubated at 370C with buffer containing α-oxo-glutarate and aspartate catalyzes the transfer of amino group from aspartate to oxoglutarate to produce glutamate and oxaloacetate which turns 2,4-dinitrophenylhydrazine forming 2,4-dinitrophenylhydrazone which turns red-brown in alkaline medium. The concentration of hydrazone formed is related with the activity of AST.

Sample: serum, plasma (heparinised)

REAGENT:

1. Buffer-	(a) Phosphate buffer	100mmol/L, pH 7.4
	(b) L-aspartate	100mmol/L
	(c) α-oxoglutarate	2mmol/L
2. 2,4-dinitrophenylhydrazine		2mmol/L
3. Sodium hydroxide		0.4mol/L

PROCEDURE:

Pipette into test tubes

	Sample Blank	Sample
Reagent 1(ml)	0.25	0.25
Sample (ml)	–	0.05
Incubate for exactly 30mins at 37^0C		
Reagent 2(ml)	0.25	0.25
Sample (ml)	0.05	–

Mix and allow to stand for exactly 20mins at room temperature

Sodium hydroxide (ml)	2.5	2.5

Mix, read the absorbance of the sample against the sample blank after 5mins, the colour is stable for 1 hour. Wavelength: 546nm

SOP FOR ALANINE AMINOTRANSFERASE (ALT)

Introduction: Alanine aminotransferase (ALT) is an enzyme present majorly in the liver cells and minute amounts in some other cells. This makes it more specific for liver disease diagnosis.

PRINCIPLE: ALT incubated at 37^0C with buffer containing α-oxoglutarate and alanine to produce glutamate and pyruvate. Pyruvate then react with 2,4-dinitrophenylhydrazine to produce 2,4-dinitrophenylhydrazone which turns red-brown in alkaline medium.The concentration of hydrazone formed is related to the amount of pyruvate produced which is related to the activity of ALT.

SAMPLE: Serum, plasma (heparinised)

REAGENT COMPOSITION:

1. Buffer

(a)Phosphate buffer	100mmol/L, pH 7.4
(b) L-alanine	200mmol/L
(c) α-oxoglutarate	2mmol/L

2. 2,4-dinitrophenylhydrazine 2mmol/L
3. Sodium hydroxide 0.4mol/L

TEST PROCEDURE:

Pipette into test tubes

	Sample Blank	Sample
Reagent 1(ml)	0.25	0.25
Sample (ml)	–	0.05
Incubate for exactly 30mins at 37^{0}C		
Reagent 2(ml)	0.25	0.25
Sample (ml)	0.05	–
Mix and allow to stand for exactly 20mins at room temperature		
Sodium hydroxide (ml)	2.5	2.5

Mix, read the absorbance of the sample against the sample blank after 5mins, the colour is stable for 1 hour. Wavelength: 546nm.

SOP FOR ALKALINE PHOSPHATASE (TECO COLORIMTRIC)

Introduction: Serum Alkaline Phosphatase is distributed in almost every tissue of the body. Most of the ALP in normal adult serum is from the liver or biliary tract. The measurement of ALP levels is mainly performed to investigate hepatobiliary disorder and bone disease. Normal ALP levels are age-dependent and levels are elevated during periods of active bone growth and also in the third trimester of pregnancy. Moderate elevations of ALP (not including liver or bone) may be attributed to Hodgins' Disease, congestive heart failure and abdominal; bacterial infections.

PRINCIPLE:

The alkaline phosphatase acts upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent (colour developer- 0.1M sodium hydroxide, 0.1M Sodium bicarbonate) stops enzyme activity and simultaneously develops a blue chromogen which is measured photometrically

TEST PROCEDURE:

PIPETTE INTO TEST TUBES

	Blank	Standard	Test
Alkaline phosphatase substrate (µl)	250	250	250

Equilibrate to 37^0C for 3 minutes

Standard (µl)	–	25	–
Sample (µl)	–	–	25
Deionised water (µl)	25	–	–

Mix and incubate for exactly 10 mins at 37^0C

Alkaline phosphatase Color Developer (ml)	1.2	1.2	1.2

Mix well and read against reagent blank at 590nm (580-630).

CALCULATION:

$$\text{Concentration of ALP in the sample in U/L} = \frac{T}{S} \times \text{conc. Of standard (50U/L)}$$

NOTE: (1) If the activity is greater than 100 U/l, repeat the assay with test specimen diluted with normal saline and multiply the test results by the dilution factor

(2) The final colored product is stable for 60 minutes at controlled room temperature (15^0C – 30^0C).

3.8.1 Processing of Histology Sample

The tissue was fixed for 24 hours to ensure proper fixation, grossed and then placed in the tissue cassettes to begin processing.

Dehydration

The tissues were dehydrated using ascending grades of alcohol i.e. 70%, 90% and absolute alcohol (100%), with each dehydration taking 1 hour.

Clearing:

Two changes of xylene was used to clear the tissues, with each change spanning for a period of 90 minutes.

Impregnation with wax:

The tissues were impregnated with molten paraffin wax in an automatic tissue processor. To ensure proper impregnation in tissue, two changes of impregnation was carried out, with each change spanning for a duration of 1 hours.

Embedding:

The embedding was carried out with the aid of an embedding machine. The impregnated tissue was placed in a suitable mold and then molten paraffin wax was dispensed into the mold. After which the mold is placed on the cold plate of the embedding machine to cool and solidify.

Microtomy:

After the blocks were completely solidified, 3-5 micron tissue sections were cut using a digital (hertz) rotary microtome create serial ribbons which were picked up unto a microscopic slide for staining.

Staining:

The sections were stained using hematoxylin and eosin (H&E) staining procedure.

Hematoxylin is a basic dye, and due to its basicity, it has an affinity for the acidic part of the cellular component (e.g. Nucleus). Therefore, the nucleus stains blue, which is the color of hematoxylin. Eosin, on the other hand is an acidic dye, hence, having an affinity for the basic component of the cells such as cytoplasm, which therefore it stains the color of the dye i.e. Pink. This staining procedure was facilitated with a mordant that linked the stain to the tissue and a differentiator (acid alcohol) that differentiated the nuclear stain from cytoplasmic stain.

3.8.2 Protocol for the Hematoxylin and Eosin (H&E) Method

- The section was dewaxed in two changes of xylene
- The section was hydrated by taking them through descending grades of alcohol. From absolute alcohol for 1 minute to 90% alcohol for 1 minute, 70% alcohol for 1 minute. It was then taken to water.
- The section was stained with Cole's hematoxylin (10 minutes).
- The section was rinsed with distilled water for few seconds.
- The section was differentiated in 1% acid alcohol briefly.
- The section was blued in Scott's tap water for 5 minutes.
- The section was counterstained with 1% eosin for 5 minutes.
- The section was washed in running tap water until excess eosin had been removed.
- The section was dehydrated in ascending grades of alcohol (70%, 90%, and absolute alcohol).
- The section was cleared in xylene and mounted in DPX (Omorodion *et al*., 2017).

3.9 Microscopy and Photomicrography

The sections were examined using a Olympus binocular microscope with an in-built lighting system at magnifications of ×40 and ×100. A microscope linked to a digital camera was used to acquire photomicrographs for the stained sections.

3.10 Statistical Analysis

The IBM Statistical Package for Social Sciences (SPSS) version 20.0 was used to carry out statistical analysis of the data. The statistical significance of the results was assessed using the analysis of variance (ANOVA). The mean standard deviation was employed to express all weight data. Pairwise comparisons between groups were compared using the Duncan post hoc test, with significance set at $P<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Histopathological Changes

The following are the histological findings observed in the course of investigating the kidney and liver tissue after administering different doses of *Spondias mombin* root extract (Group A= control, Group B= 200mg/kg body weight of *Spondias mombin* root extract, Group C= 400mg/kg body weight of *Spondias mombin* root extract, Group D= 600mg/kg body weight of *Spondias mombin* root extract, Group E=800mg/kg body weight of *Spondias mombin* root extract, Group F= 1000mg/kg body weight of *Spondias mombin* root extract):

4.1.1 Liver

Sections of the liver from control rats revealed a well-preserved hepatic architecture, with clearly visible lobular organization. Hepatocytes appeared polygonal, arranged in cords radiating from the central vein, and separated by narrow sinusoidal spaces. The cytoplasm of the hepatocytes was uniformly eosinophilic, and nuclei were centrally located, normochromic, and round, with indistinct but regular nucleoli, consistent with healthy hepatocellular morphology. The sinusoidal lining cells appeared intact, and no evidence of vacuolar degeneration, fatty change, necrosis, or inflammatory cell infiltration was observed. Portal tracts were normal in appearance, with preserved bile ducts and blood vessels, and no signs of fibrosis or congestion (Plate 4.1A).-Give an interpretation similar to the above-Section of the liver from the control rats showed hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli **(Plate 4.1A).**

Sections of the liver from rats administered *Spondias mombin* root extract at a dose of 200 mg/kg revealed hepatocytes (arrow) with well-preserved eosinophilic cytoplasm surrounding centrally placed normochromic nuclei, some with indistinct nucleoli. The hepatic cords appeared intact and radiated normally from the central vein, while sinusoidal spaces remained open and patent. No evidence of vacuolation, degeneration, inflammatory infiltrates, or fibrosis was observed **(Plate 4.1B).**

Sections of the liver from rats administered *Spondias mombin* root extract at a dose of 400 mg/kg revealed hepatocytes (arrow) with preserved eosinophilic cytoplasm surrounding centrally placed normochromic nuclei, some exhibiting indistinct nucleoli. The hepatic cords were orderly arranged and radiated normally from the central vein, while sinusoidal spaces appeared patent without evidence of compression. No cytoplasmic vacuolation, ballooning degeneration, inflammatory infiltrates, or fibrosis were observed **(Plate 4.1C).**

Sections of the liver from rats administered *Spondias mombin* root extract at a dose of 600 mg/kg revealed hepatocytes (arrow) with preserved eosinophilic cytoplasm surrounding centrally placed normochromic nuclei, some displaying indistinct nucleoli. The hepatic cords remained intact and radiated normally from the central vein, while sinusoidal spaces were widely patent. No evidence of cytoplasmic vacuolation, ballooning degeneration, inflammatory cell infiltration, or fibrosis was observed **(Plate 4.1D).**

Sections of the liver from rats administered *Spondias mombin* root extract at a dose of 800 mg/kg revealed hepatocytes with abundant eosinophilic cytoplasm containing multiple fine microvacuoles, consistent with ballooning degeneration. The vacuolated cytoplasm surrounded centrally placed normochromic nuclei, although occasional nuclear displacement was observed due to cytoplasmic expansion. Hepatic cords showed focal disruption, with hepatocytes losing their uniform arrangement compared to controls. Sinusoidal spaces remained patent with mild compression. No appreciable inflammatory cell infiltrates or fibrotic changes were observed at this dose. **(Plate 4.1E).**

Sections of the liver from rats administered *Spondias mombin* root extract at a dose of 1000 mg/kg revealed hepatocytes (arrow) with eosinophilic cytoplasm containing multiple microvacuoles, indicative of ballooning degeneration. The vacuolated cytoplasm surrounded centrally placed normochromic nuclei, with occasional displacement due to cytoplasmic expansion. Hepatic cords appeared focally disrupted compared to controls, while sinusoidal spaces remained patent with mild compression. No significant inflammatory infiltrates or fibrotic changes were evident at this dose **(Plate 4.1F).**

4.1.2 Kidney

Sections of the kidney from control rats revealed normal glomeruli (thick arrow) with intact mesangial cells, well-preserved capillary loops, and a normal epithelial lining. The renal tubules (thin arrow) appeared oval-shaped and were lined by cuboidal epithelium, with some tubules containing pale eosinophilic material within their lumina. The renal interstitium showed no evidence of edema, inflammation, or fibrosis **(Plate 4.2 A).**

Sections of the kidney from rats administered *Spondias mombin* root extract at a dose of 200 mg/kg revealed normal glomeruli (thick arrow) with intact mesangium, well-preserved blood vessels, and a normal epithelial lining. The renal tubules (thin arrow) were oval in shape and lined by cuboidal epithelium, with some tubules containing pale eosinophilic material in their lumina. No evidence of tubular degeneration, necrosis, inflammation, or interstitial changes was observed **(Plate 4.2B).**

Sections of the kidney from rats administered *Spondias mombin* root extract at a dose of 400 mg/kg revealed normal glomeruli (thick arrow) with intact mesangium, preserved blood vessels, and normal epithelial lining. The renal tubules (thin arrow) appeared oval in shape and were lined by cuboidal epithelium, with some tubules containing pale eosinophilic material within their lumina. No evidence of tubular degeneration, necrosis, inflammation, or interstitial pathology was observed **(Plate 4.2C).**

Sections of the kidney from rats administered *Spondias mombin* root extract at a dose of 600 mg/kg revealed normal glomeruli (thick arrow) with intact mesangium, well-preserved blood vessels, and a normal epithelial lining. The renal tubules (thin arrow) were oval in shape and lined by cuboidal epithelium, with some tubules containing pale eosinophilic material in their lumina. No signs of tubular degeneration, necrosis, inflammatory infiltrates, or interstitial alterations were observed **(Plate 4.2D).**

Sections of the kidney from rats administered *Spondias mombin* root extract at a dose of 800 mg/kg revealed normal glomeruli (thick arrow) with intact mesangium, preserved blood vessels, and normal epithelial lining. The renal tubules (thin arrow) appeared oval in shape and were lined by cuboidal epithelium, with some tubules containing pale eosinophilic material in their lumina. No evidence of tubular injury, necrosis, inflammation, or interstitial changes was observed **(Plate 4.2E).**

Sections of the kidney from rats administered *Spondias mombin* root extract at a dose of 1000 mg/kg revealed normal glomeruli (thick arrow) with intact mesangium, preserved blood vessels, and a normal epithelial lining. The renal tubules (thin arrow) were oval in shape and lined by cuboidal epithelium, with some tubules containing pale eosinophilic material in their lumina. No histological evidence of tubular degeneration, necrosis, inflammatory infiltrates, or interstitial alterations was observed **(Plate 4.2F).**

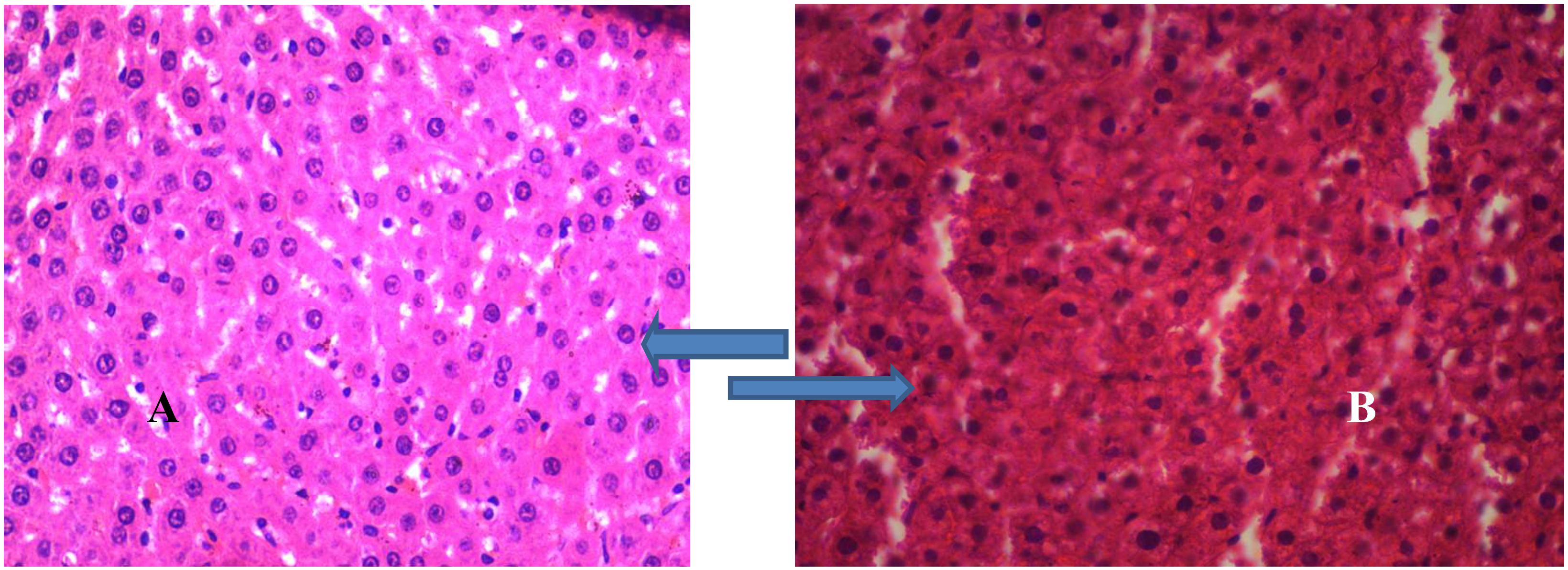
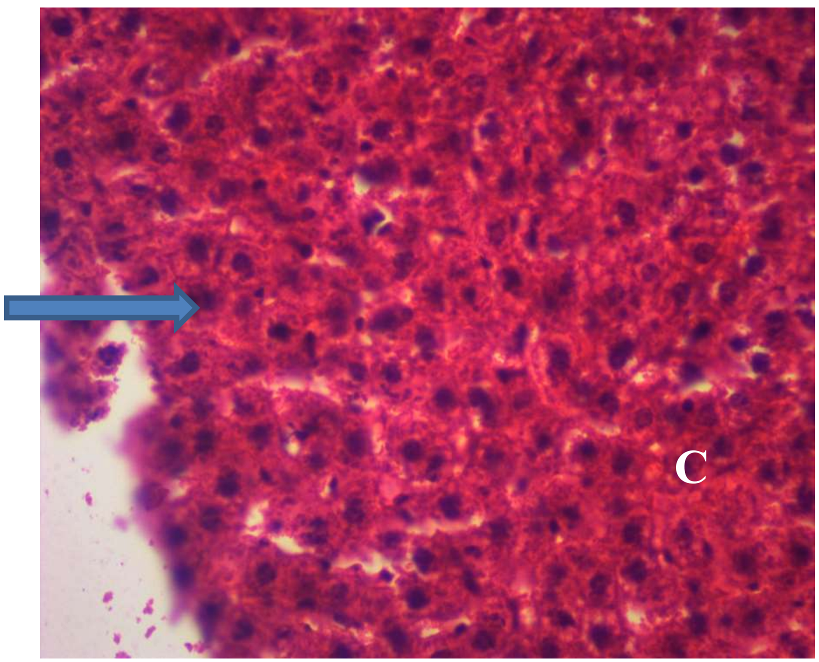


Plate 4.1A: Section of the liver from the control rats showed hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. Features In Keeping With Normal Hepatocytes H and E Mag x400.

Plate 4.1B: Section of the liver of rats administered 200mg/kg body weight of *Spondias mombin* root extract showed hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. Features In Keeping With Normal Hepatocytes H and E Mag x400.



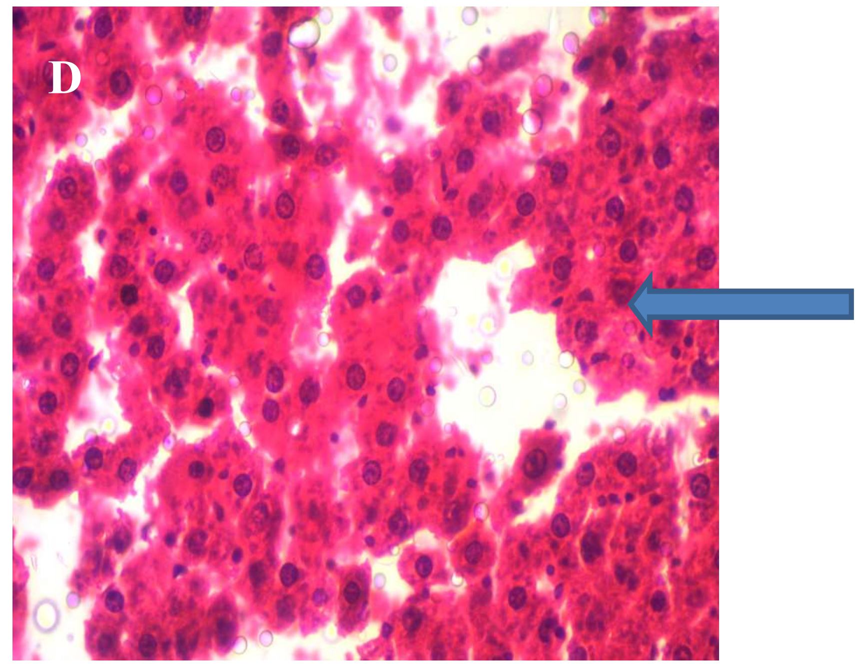
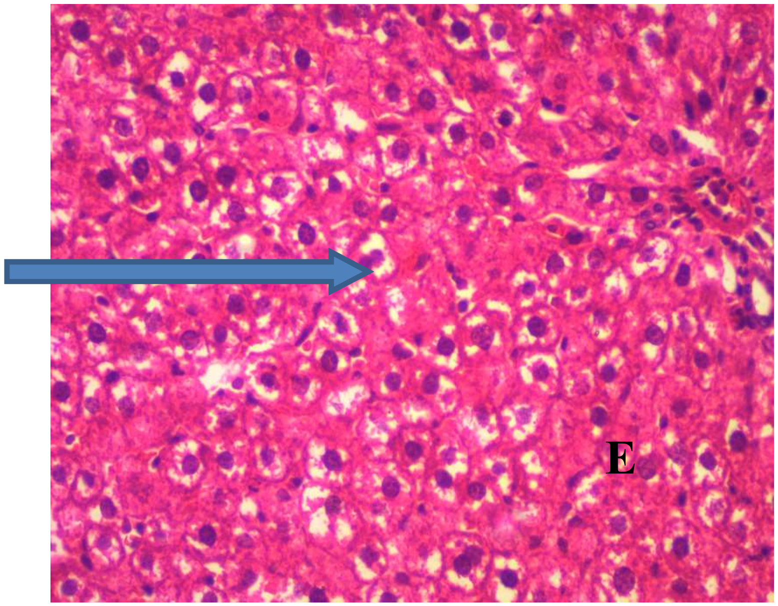


Plate 4.1C: Section of the liver of rats administered 400mg/kg body weight of *Spondias mombin* root extract showed hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. Features In Keeping With Normal Hepatocytes H and E   Mag x400.

Plate 4.1D: Section of the liver of rats administered 600mg/kg body weight of *Spondias mombin* root extract showed hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli.   Features In Keeping With Normal Hepatocytes H and E   Mag x400.



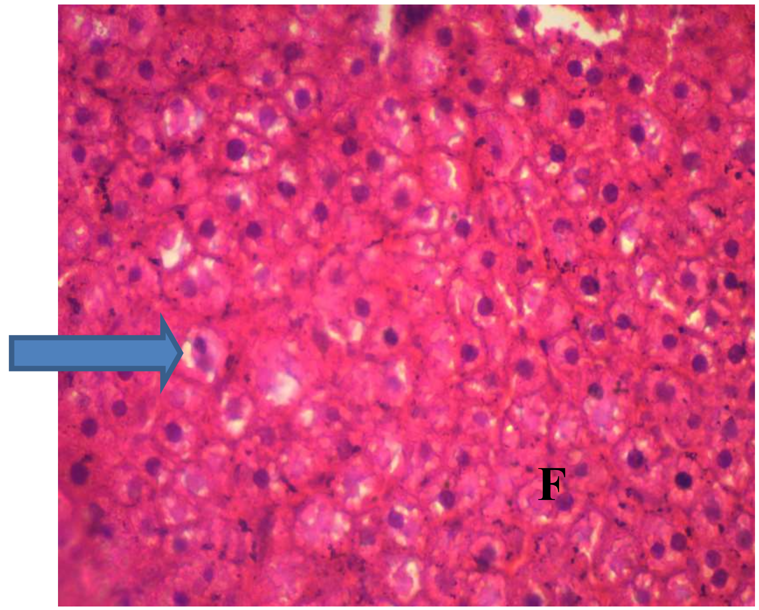


Plate 4.1E: Section of the liver of rats administered 800mg/kg body weight of *Spondias mombin* root extract showed hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei. Features In Keeping With Steatosis H and E Mag x400.

Plate 4.1F: Section of the liver of rats administered 1000mg/kg body weight of *Spondias mombin* root extract showed hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei. Features In Keeping With Steatosis H and E Mag x400.

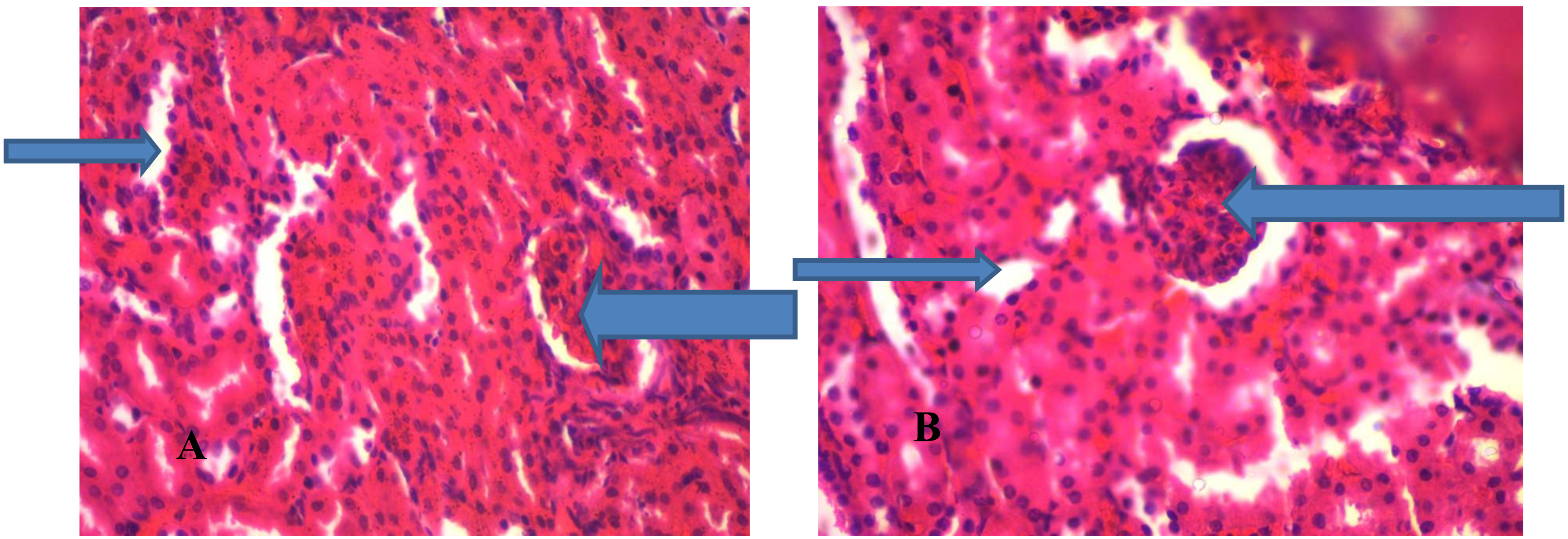


Plate 4.2A: Section of the kidney from the control rats showed normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with Normal Kidney H and E   Mag x400.

Plate 4.2B: Section of the kidney from rats administered *Spondias mombin* root extract at a dose of 200 mg/kg revealed 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

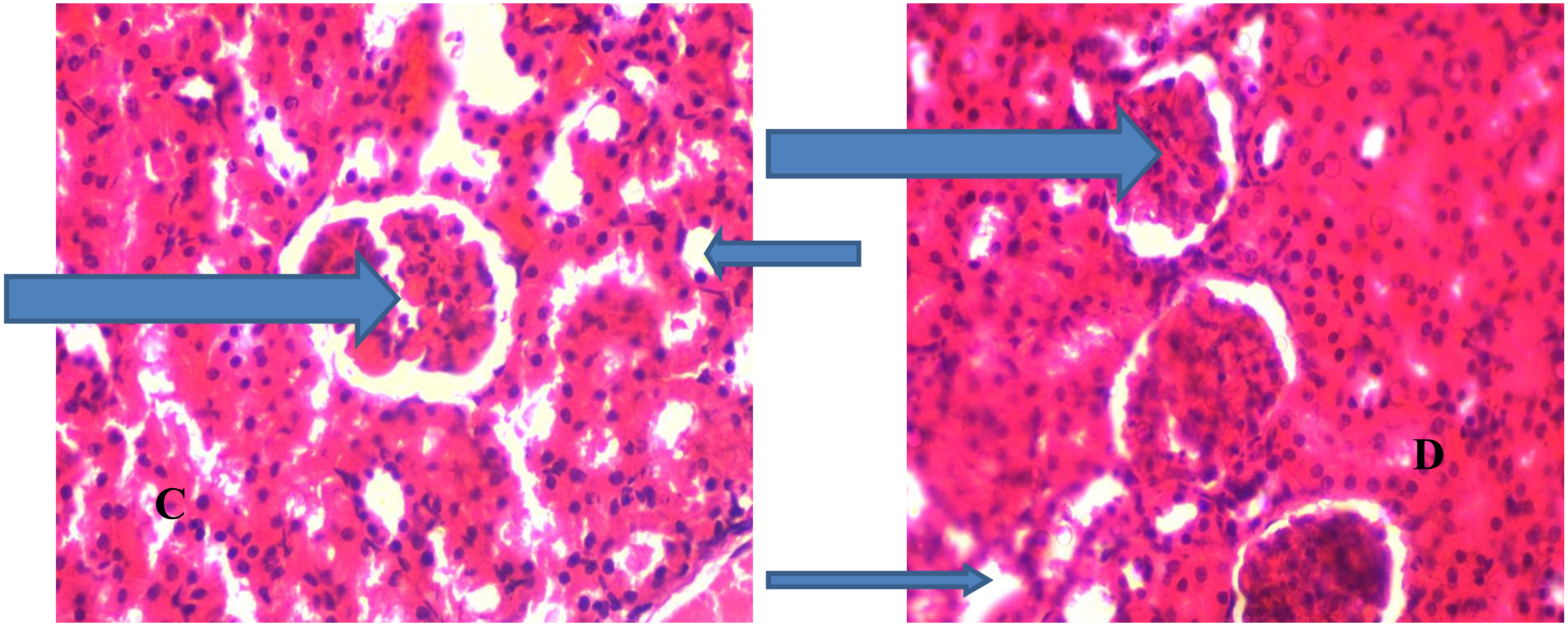
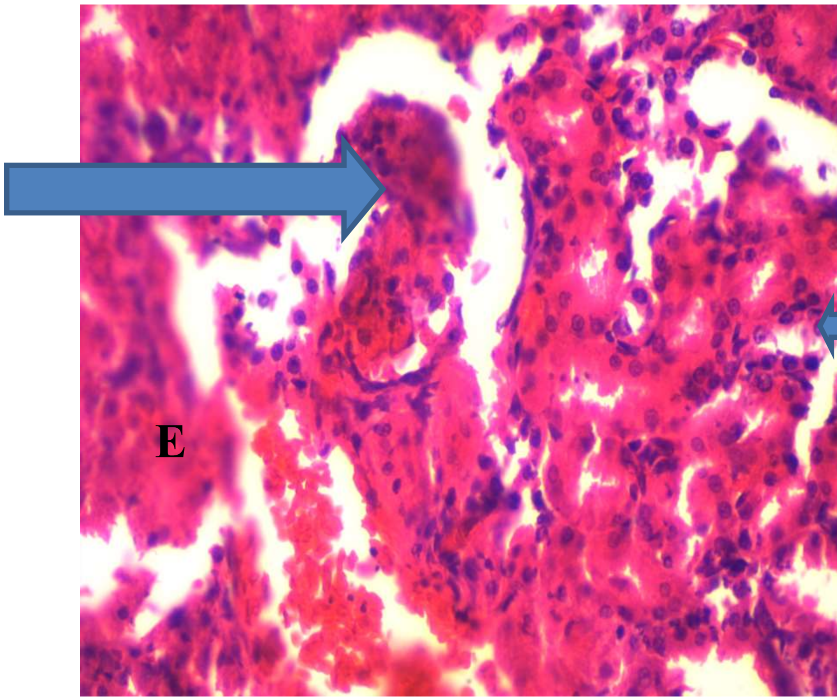


Plate 4.2C: Section of the liver of rats administered 400mg/kg body weight of *Spondias mombin* root extract showed hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. Features In Keeping With Normal Hepatocytes H and E Mag x400.

Plate 4.2D: Section of the kidney from rats administered *Spondias mombin* root extract at a dose of 600 mg/kg revealed 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 H and E Mag x400.



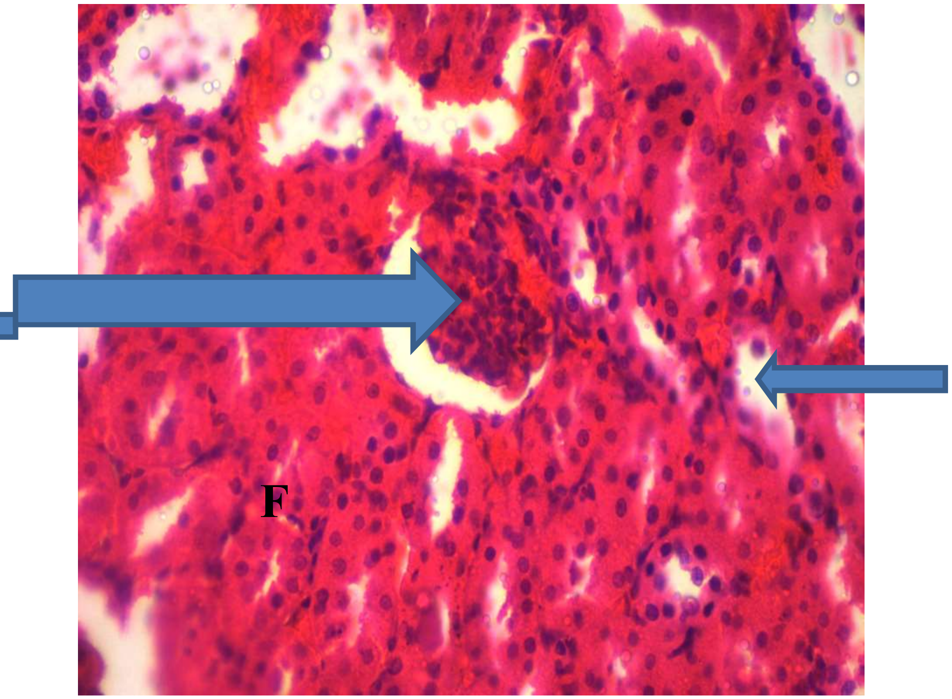


Plate 4.2E: Section of the kidney from rats administered *Spondias mombin* root extract at a dose of 800 mg/kg revealed normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with Normal Kidney H and E Mag x400.

Plate 4.2F: Section of the kidney from rats administered *Spondias mombin* root extract at a dose of 1000 mg/kg revealed 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 H and E Mag x400.

4.1.3 Statistical Analysis

The histomorphological effect of *Spondias mombin* root extract on the liver and kidney of adult Wistar rats was assessed using changes in body and organ weights, biochemical indices, and occult blood tests.

As shown in **Table 4.1**, the initial and final body weights of the rats varied significantly among the groups ($p = 0.004$ and $p < 0.001$, respectively). Group B recorded the lowest initial weight (116.33 ± 3.28 g) and also maintained the lowest final weight (134.33 ± 4.37 g). In contrast, Group E consistently had the highest values, with an initial mean weight of 156.00 ± 3.89 g and a final mean weight of 196.25 ± 6.43 g. Groups A, D, and F clustered closely with final weights ranging between 161–163 g, while Group C had an intermediate weight (151.50 ± 6.98 g). These results demonstrate that body weight changes were statistically significant across the groups, with Group B consistently showing the lowest weight gains.

With respect to organ weights, **Table 4.1** show that liver weights ranged from 7.23 ± 0.46 g (Group B) to 9.03 ± 0.70 g (Group D). However, these differences were not statistically significant ($p = 0.253$). Similarly, kidney weights ranged from 0.70 ± 0.06 g (Group B) to 1.20 ± 0.00 g (Group A), also without significant differences across the groups ($p = 0.080$). This indicates that although body weight changes were significant, the relative liver and kidney weights were not significantly affected.

The biochemical profile of the animals is summarized in **Table 4.2**. Serum urea values ranged between 24.50 ± 0.50 mg/dL (Group B) and 33.50 ± 0.50 mg/dL (Group C), with no significant difference among groups ($p = 0.153$). Creatinine levels, however, differed significantly ($p = 0.006$). The lowest mean creatinine concentration was recorded in Group F (127.50 ± 1.50 µmol/L), while the highest was seen in Group C (138.50 ± 0.50 μmol/L). Post hoc analysis confirmed that Group F was significantly different from the other groups, whereas Groups A–E were not significantly different from each other.

Total bilirubin levels ranged from 3.65 ± 0.25 mg/dL (Group A) to 4.40 ± 0.10 mg/dL (Group D), with no significant differences across the groups ($p = 0.309$). Conjugated bilirubin values varied between 0.30 ± 0.10 mg/dL (Group A) and 0.55 ± 0.05 mg/dL (Groups C and D), also without significant differences ($p = 0.227$).

The serum enzyme activities showed no significant variation among groups. AST activity ranged from 0.026 ± 0.024 U/L (Group E) to 0.100 ± 0.000 U/L (Groups C, D, and F), with $p = 0.126$. ALT values ranged between 0.0025 ± 0.000 U/L (Groups B, D, and E) and 0.050 ± 0.000 U/L (Group A), with $p = 0.212$. ALP activity ranged from 7.00 ± 0.00 U/L (Group F) to 9.00 ± 2.00 U/L (Group A), with $p = 0.762$. These findings indicate that aside from creatinine, other biochemical parameters did not differ significantly across the experimental groups.

Table 4.1: Initial and Final Body Weights, Liver and Kidney Weights of Adult Wistar Rats Exposed to Lead and Treated with *Spondias mombin* root Extract

Group	Initial Weight (g)	Final Weight (g)	Liver Weight (g)	Kidney Weight (g)
A	130.50 ± 4.50^{b}	162.00 ± 1.00^{b}	7.45 ± 1.65	1.20 ± 0.00
B	116.33 ± 3.28^{a}	134.33 ± 4.37^{a}	7.23 ± 0.46	0.70 ± 0.06
C	130.25 ± 7.88^{b}	151.50 ± 6.98^{ab}	8.38 ± 0.55	1.18 ± 0.11
D	129.00 ± 6.31^{b}	163.00 ± 4.32^{b}	9.03 ± 0.70	1.05 ± 0.09
E	156.00 ± 3.89^{c}	196.25 ± 6.43^{c}	7.40 ± 0.53	0.88 ± 0.19
F	138.00 ± 3.63^{b}	161.00 ± 6.87^{b}	7.28 ± 0.30	0.83 ± 0.09
p-value	0.004	<0.001	0.253 (ns)	0.080 (ns)

Values are presented as mean ± SEM (n = 2–4 per group). Superscript letters indicate statistical groupings from Tukey HSD post hoc test; groups sharing the same letter are ***not significantly different*** *at p<0.05.*

Table 4.2: Biochemical Parameters of Adult Wistar Rats Exposed to Lead and Treated with *Spondias mombin* Root Extract (Mean ± SEM, ANOVA p-values)

Parameter	A	B	C	D	E	F	p-value
Urea (mg/dL)	26.00 ± 4.00	24.50 ± 0.50	33.50 ± 0.50	28.50 ± 1.50	32.50 ± 0.50	30.50 ± 3.50	0.153
Creatinine (µmol/L)	130.50 ± 0.50^{b}	136.50 ± 0.50^{b}	138.50 ± 0.50^{b}	136.50 ± 2.50^{b}	136.00 ± 1.00^{b}	127.50 ± 1.50^{a}	0.006*
Total Bilirubin (mg/dL)	3.65 ± 0.25	4.00 ± 0.30	4.10 ± 0.20	4.40 ± 0.10	4.30 ± 0.30	3.90 ± 0.00	0.309
Conjugated Bilirubin (mg/dL)	0.30 ± 0.10	0.45 ± 0.05	0.55 ± 0.05	0.55 ± 0.05	0.50 ± 0.10	0.50 ± 0.00	0.227
AST (U/L)	0.075 ± 0.025	0.075 ± 0.025	0.100 ± 0.000	0.100 ± 0.000	0.026 ± 0.024	0.100 ± 0.000	0.126
ALT (U/L)	0.050 ± 0.000	0.0025 ± 0.000	0.026 ± 0.024	0.0025 ± 0.000	0.0025 ± 0.000	0.026 ± 0.024	0.212
ALP (U/L)	9.00 ± 2.00	8.50 ± 0.50	8.00 ± 1.00	8.00 ± 0.00	8.50 ± 0.50	7.00 ± 0.00	0.762

*Values are expressed as Mean ± SEM (n = 2 per group). Superscripts (^{a},^{b}) denote statistically significant differences between groups according to Tukey's post hoc test at $p < 0.05$, with groups sharing the same superscript letter not significantly different, while * indicates a significant ANOVA result at $p < 0.05$.*

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The present study investigated the histopathological and biochemical effects of graded doses of *Spondias mombin* root extract on the liver and kidney of Wistar rats. The results revealed that lower doses (200–600 mg/kg) preserved normal hepatic and renal histoarchitecture, while higher doses (800–1000 mg/kg) induced hepatocellular alterations characterized by ballooning degeneration and microvesicular steatosis. Interestingly, the kidneys remained histologically normal across all doses, even at 1000 mg/kg. Biochemical indices generally supported the histological findings, with most liver and kidney function markers within normal limits, except for a significant reduction in creatinine at 1000 mg/kg. The liver histology of control animals displayed well-organized hepatocytes with intact cords radiating from the central vein, normal sinusoids, and absence of degeneration, consistent with healthy liver architecture. This pattern was maintained in rats administered 200–600 mg/kg of the extract, suggesting hepatoprotection or at least an absence of hepatotoxicity at these doses.

At 800 and 1000 mg/kg, however, hepatocytes showed ballooning degeneration with microvacuolated cytoplasm, indicating early steatotic changes. Ballooning degeneration reflects cytoskeletal disruption and lipid accumulation within hepatocytes, which are classical hallmarks of steatosis and hepatocellular stress. Importantly, these changes occurred without overt necrosis, inflammatory infiltration, or fibrosis, suggesting a reversible stage of hepatocellular injury**.**

These findings are in agreement with earlier reports. Asuquo *et al*. found that *S. mombin* extracts were not hepatotoxic at moderate doses in rats (Asuquo *et al*., 2012). Similarly, Nwidu *et al*.

demonstrated that leaf and stem extracts of *S. mombin* provided hepatoprotection against carbon tetrachloride–induced liver injury, attributed to their antioxidant phytochemicals (Nwidu *et al*., 2018). In contrast, Gbogbo *et al*. reported that high-dose stem bark extracts induced mild hepatocellular alterations (Gbogbo *et al*., 2014), echoing the present findings of steatosis at 800–1000 mg/kg. Kidney histology across all groups revealed normal glomeruli with intact mesangium, preserved blood vessels, and tubules lined with cuboidal epithelium. No evidence of tubular degeneration, necrosis, interstitial edema, or inflammatory infiltration was found, even at the highest dose of 1000 mg/kg. These findings align with previous reports of nephroprotection by *S. mombin*. Marcellinus and Onitsha demonstrated that *S. mombin* ameliorated aluminum chloride–induced renal injury in female rats, preserving glomerular and tubular morphology (Marcellinus & Onitsha, 2022). Similarly, Akwu *et al*. reported that *S. mombin* co-administered with metformin mitigated streptozotocin-induced nephrotoxicity (Akwu *et al*., 2022). Biochemical assays showed no significant variations in serum urea, bilirubin, or liver enzyme activities (AST, ALT, ALP) across treatment groups. This biochemical stability mirrors the preserved hepatic histology at 200–600 mg/kg. The only significant change was reduced serum creatinine at 1000 mg/kg, a finding that is paradoxical since renal histology remained normal. Reduced creatinine may reflect enhanced clearance or altered muscle metabolism rather than renal dysfunction.

This is consistent with findings by Eluehike and Innih, who reported that *S. mombin* extracts did not significantly alter biochemical liver and kidney indices in diabetic rat models (Eluehike & Innih, 2022). The lack of correlation between steatotic changes at 800–1000 mg/kg and serum enzyme elevations suggests that histological injury was mild and not severe enough to cause leakage of hepatocellular enzymes into circulation. The ethnomedicinal use of *S. mombin* in West Africa for treating diarrhea, infections, and metabolic disorders is well-documented. Our findings support its safety at therapeutic ranges but caution against high-dose or prolonged use due to potential hepatic steatosis. This dose-dependent duality is common among medicinal plants, where phytochemicals act as antioxidants at low doses but may trigger oxidative stress at supratherapeutic levels (Ola-Davies *et al*., 2019).

5.2 Conclusion

The present study demonstrated that administration of *Spondias mombin* root extract to Wistar rats produced dose-dependent effects on hepatic architecture while preserving renal integrity across all tested doses. At lower to moderate doses (200–600 mg/kg), the extract maintained normal hepatocellular morphology, with no evidence of vacuolation, degeneration, or inflammation. However, higher doses (800–1000 mg/kg) induced ballooning degeneration and microvesicular steatosis, although these changes occurred without necrosis, fibrosis, or significant alterations in liver enzyme activity. Kidney histology remained unaffected at all doses**,** with glomeruli, tubules, and interstitium showing normal morphology. Biochemical analyses supported these histological findings, as serum urea, bilirubin, and liver enzyme activities remained within normal ranges, except for a significant reduction in creatinine at the highest dose, which did not correspond to structural renal damage.

5.3 Recommendations

From the findings of this study, it is recommended that *Spondias mombin* root extract should be administered within a safe range of 200–600 mg/kg, as these doses preserved hepatic and renal integrity without histological or biochemical evidence of toxicity. Higher doses (800–1000 mg/kg) should be avoided or used with caution, since they were associated with ballooning degeneration and early steatotic changes in the liver. In ethnomedicinal practice, emphasis should be placed on moderate dosing to ensure therapeutic benefit while minimizing potential adverse effects. Further research is necessary to evaluate the chronic toxicity profile of the extract, particularly to determine whether prolonged use may result in cumulative hepatic damage. Mechanistic studies are also recommended to identify the bioactive constituents responsible for both the hepatoprotective and hepatotoxic effects observed, while comparative studies using other plant parts such as the leaves, bark, and fruit will help clarify differences in phytochemical safety profiles. For clinical translation, it is essential to standardize dosing and conduct comprehensive toxicological profiling in order to establish therapeutic windows in humans. With such validation, *S. mombin* root extract may hold promise as a safe adjunct in liver and kidney protection when used at appropriate therapeutic doses.

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APPENDIX I

The instrument used for this research are as follows:

1. Animal House: during the time of feeding
   a. Feeding flat plate
   b. Feeding water bottles
   c. Feed
   d. ISOL disinfectant
   e. Digital thermometer
   f. Plastic cage

2. For sacrificing
   a. Hand gloves
   b. Sterile lancet
   c. Cotton wool
   d. Chloroform
   e. Plastic container sterile with a cover
   f. Dissecting set
   g. Sterile containers
   h. Formalin

3. Histology laboratory
   a. Scrape blade
   b. Spatula
   c. Tissue cassette
   d. Automatic tissue processor
   e. Molten wax
   f. Tissue basket
   g. Rotary type of microtome
   h. Water bath
   i. Hot plate
   j. Slides and cover slips
   k. Stain (Hematoxylin & Eosin)
   l. Binocular microscope
   m. Dibutylphthalate polysterene xylene (DPX)
   n. Xylene, alcohol and water

APPENDIX II

SERIAL NUMBER	SAMPLE ID	UREA	Na^+	K^+	HCO_3	Cl^-	CREAT	TOTAL BILIRUBIN	CONJUGATED BILIRUBIN	AST	ALT	ALP
1.	A_1	30	130	3.4	13	91	0.4	0.1	0.05	11	5	10
2.	A2	22	131	3.9	12	85	0.2	<0.1	0.05	7	6	7
3.	*B1*	25	136	3.7	21	100	0.5	<0.1	<0.05	8	8	10
4.	*B2*	24	137	4.3	19	103	0.4	0.1	<0.05	9	6	10
5.	C1	34	139	3.9	16	108	0.5	0.1	0.05	9	7	11
6.	C2	33	138	4.3	19	105	0.6	0.1	<0.05	7	7	12
7.	*D1*	30	139	4.5	21	104	0.5	0.1	<0.05	8	7	12
8.	*D2*	27	134	4.3	19	92	0.6	0.1	<0.05	8	5	11
9.	*E1*	32	137	4.6	21	99	0.6	<0.1	<0.05	8	7	13
10.	E3	33	135	4.0	21	94	0.4	<0.05	<0.05	9	7	12
11.	*F1*	34	129	3.9	20	88	0.5	0.1	0.05	7	6	13
12.	*F4*	27	126	3.9	21	89	0.5	0.1	<0.05	7	5	12

Measure	Group A	Group B	Group CGroup	D	Group E	Group F
Initial Weight (g)	A1=135 A2=126	B1=118 B2=121 B3=110	C1=114 C2=147 C3=140 C4=120	D1=112 D2=127 D3=140 D4=137	E1=162 E2=156 E3=161 E4=145	F1=147 F2=130 F3=135 F4=140
Final Weight (g)	A1=161 A2=163	B1=131 B2=143 B3=129	C1=135 C2=168 C3=156 C4=147	D1=155 D2=163 D3=175 D4=159	E1=208 E2=198 E3=201 E4=178	F1=178 F2=155 F3=146 F4=165

WEIGHT OF ORGANS

A1= Liver-9.1, kidney-1.2

A2= Liver-5.8, kidney-1.2

B1= Liver-6.4, kidney-0.8

B2= Liver-7.3, kidney-0.6

B3= Liver-8.0, kidney-0.7

C1= Liver-7.4, kidney-0.9

C2= Liver-9.9 kidney-1.3

C3= Liver-7.8, kidney-1.1

C4= Liver-8.4, kidney-1.4

D1=Liver-9.6, kidney-1.1

D2= Liver-8.2, kidney-1.1

D3= Liver-7.6, kidney-0.8

D4= Liver-10.7, kidney-1.2

E1= Liver-8.6, kidney-0.9

E2= Liver-8.0, kidney-1.4

E3= Liver-6.5, kidney-0.6

E4= Liver-6.5, kidney-0.6

F1= Liver-7.2, kidney-1.0

F2= Liver-7.5, kidney-0.9

F3= Liver-7.9, kidney-0.8

F4= Liver-6.5, kidney-0.6

APPENDIX III

Plant verification certificate:

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)

Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City, Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name:*Spondias mombin* Linn.

Family: Anacardiaceae

Common Name: Hug Plum, Yellow mombin

Voucher Number: UBH-S345

Student Name: Atoyebi Modupeoluwa Folashade

Plant Identification and Voucher Number Issued:

______________________11/06/2025_

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, LMBOSON, MNES; Nigeria)

APPENDIX IV

Ethical approval:

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

EDO STATE

CERTIFICATE OF ETHICAL APPROVAL

This is to certify that

MODUPEOLUWA FOLASHADE ATOYEBI

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

INVESTIGATING THE HISTOMORPHOLOGICAL EFFECT OF *SPONDIAS MOMBIN* EXTRACT IN THE LIVER AND KIDNEY OF WISTAR RATS.

In accordance with the Animal Disease Control Act, 2022.

Dr L.I Adebudo
Chairman MAFSAEC

Approval No.
MAFSAEC: 025-08/13/0042

Date Of Approval
13th August, 2025

(This Approval is only valid for this study)


TISSUE EMBEDDING CENTER


TISSUE EMBEDDING CENTER
COOLING PLATE