BODY WEIGHT CHANGES AND HISTOLOGICAL EFFECTS OF METHANOL EXTRACT OF *SPONDIAS MOMBIN* IN HIGH-FAT FED RATS

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OSHODIN EVANS DENNIS
BMS2001138

DFEPARTMENT OF MEDICAL BIOCHEMISTRY
SCHOOL OF BASIC MEDICAL SCIENCES
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN

MARCH, 2025

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DEPARTMENT OF MEDICAL BIOCHEMISTRY,SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE AWARD OF THE BACHELOR OF SCIENCE (B.Sc), DEGREE IN MEDICAL BIOCHEMISTRY

MARCH, 2025

CERTIFICATION

We, the undersigned, certify that this research was carried out by Udofia Jeremiah Godwin, in the department of Medical Biochemistry, School of Basic Medical Science, College of Medicine University of Benin, Benin City in partial fulfilment of the requirement of the award of the Bachelor of Science.

______________________	______________________
Dr.(Mrs) N. Eluehike **(Project Supervisor)**	**Date**
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Dr. Aguebor-Ogie B.N **(Head of Department)**	**Date**
______________________	______________________
(External Supervisor)	**Date**

DEDICATION

This study is dedicated to Almighty God for His Grace that sustained the researcher in the course of the programme.

ACKNOWLEDGEMENTS

The researcher expresses sincere appreciation to God for guiding him through the ups and downs of his academic journey at the University of Benin, enabling the successful completion of this project. Gratitude is extended to his supervisor, Dr..(Mrs) N. Eluehike for her patience and invaluable constructive feedback throughout the project. Furthermore, thanks are offered to the Head of Department Dr. Aguebor-Ogie B.N and other dedicated lectures in the Department of Medical Biochemistry for sharing their knowledge and expertise.

Genuine thank goes to the researcher's parent, Mr. and Mrs Oshodin Francis for their unwavering love, support and prayers during this project. He is also grateful to his siblings and also to his relatives especially his uncle Oshodin Goddey for their constant love, encouragement, financial support and prayers.

The researcher extends heartfelt gratitude to his friends, for their part in this significant achievement, offering them many thanks and blessings.

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ABSTRACT

This study investigated the effects of methanol extract of *Spondias mombin* on body weight changes and liver histology in high-fat diet-fed rats. Obesity and metabolic disorders caused by high-fat diets are major global health concerns, prompting the search for safer plant-based therapeutic alternatives. In this study, thirty male Wistar rats were divided into five groups: a normal control group, a high-fat diet (HFD) control group, and three treatment groups administered 200 mg/kg, 400 mg/kg, and 600 mg/kg of *Spondias mombin* methanol extract respectively. The experiment lasted for six weeks, after which body weight and liver histological changes were assessed. Results showed that rats fed with a high-fat diet without treatment recorded the highest body weight gain (48.6%), confirming the successful induction of obesity. However, rats treated with the plant extract showed a significant and dose-dependent reduction in weight gain, with percentage gains of 32.9%, 30.0%, and 20.6% for the low, medium, and high doses respectively. Histological analysis of liver tissues revealed severe hepatic steatosis in the untreated HFD group, while extract-treated groups showed progressive improvement in liver structure, with the high-dose group exhibiting near-normal hepatic architecture and minimal fat accumulation. The findings suggest that methanol extract of *Spondias mombin* possesses significant anti-obesity and hepatoprotective properties and may serve as a potential natural therapeutic agent for managing diet-induced metabolic disorders.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Obesity and metabolic disorders associated with high-fat diets have become significant global health concerns. The increasing prevalence of obesity-related diseases, such as diabetes, cardiovascular disorders, and liver dysfunction, has prompted extensive research into potential therapeutic interventions (Chooi et al., 2019). While pharmaceutical treatments are available, they often come with adverse side effects, leading researchers to explore natural alternatives. Medicinal plants have gained attention for their bioactive compounds that may regulate metabolic processes and reduce obesity-related complications.

Among these plants, *Spondias mombin*, commonly known as the yellow mombin, has been traditionally used for various medicinal purposes. The plant is widely distributed in tropical and subtropical regions, including Africa, South America, and parts of Asia (Akinmoladun et al., 2020). Studies suggest that its methanol extract contains phytochemicals with potential anti-obesity and metabolic regulatory properties (Omodanisi et al., 2017). However, despite its traditional use, scientific validation of its effects on body weight and histological alterations in high-fat diet-induced obesity models remains limited.

Obesity is a major public health issue, with the World Health Organization (WHO) estimating that over 1.9 billion adults were overweight in 2016, and more than 650 million were classified as obese (WHO, 2021). The increasing trend of obesity is largely attributed to dietary habits, particularly the consumption of high-fat and calorie-dense foods, coupled with a sedentary lifestyle (Hruby & Hu, 2015).

High-fat diets (HFD) contribute significantly to metabolic disorders by promoting excessive fat accumulation, insulin resistance, and systemic inflammation (Zhou et al., 2020). The use of experimental animal models, particularly rodents, has been instrumental in studying the pathophysiological effects of HFD and evaluating potential therapeutic agents (Buettner et al., 2007). These models help researchers understand obesity-related metabolic alterations and assess the efficacy of natural compounds in reversing or preventing such changes.

The increasing concerns over synthetic anti-obesity drugs, due to their side effects and high costs, have driven interest in plant-based therapies (Hasani-Ranjbar et al., 2013). Several medicinal plants have been explored for their ability to modulate lipid metabolism, reduce adipogenesis, and improve glucose homeostasis (Aruldass et al., 2020). Among these, *Spondias mombin* has emerged as a potential candidate due to its rich phytochemical composition.

Spondias mombin belongs to the Anacardiaceae family and is commonly found in tropical regions. It has been traditionally used for treating various ailments, including gastrointestinal disorders, infections, and inflammatory conditions (Akinmoladun et al., 2020). The plant contains several bioactive compounds, such as flavonoids, tannins, alkaloids, and phenolic compounds, which may contribute to its medicinal properties (Omodanisi et al., 2017).

Studies have reported that *Spondias mombin* exhibits antioxidant, anti-inflammatory, and antimicrobial activities (Nworu et al., 2016). These properties suggest its potential in modulating metabolic pathways involved in obesity. Specifically, its methanol extract has been shown to influence lipid metabolism and reduce oxidative stress, factors that play critical roles in obesity and related complications (Akinmoladun et al., 2020).

Despite its traditional use, there is limited scientific evidence supporting the efficacy of *Spondias mombin* in obesity management. While some studies suggest its beneficial effects, comprehensive research, including body weight analysis and histological assessments, is necessary to establish its therapeutic potential (Omodanisi et al., 2017).

1.2 Statement of the Problem

Obesity and its associated metabolic disorders pose significant health risks worldwide, contributing to conditions such as diabetes, cardiovascular diseases, and non-alcoholic fatty liver disease (WHO, 2021). The increasing prevalence of obesity is largely driven by the widespread consumption of high-fat diets and sedentary lifestyles, leading to metabolic dysfunction and chronic inflammation (Hruby & Hu, 2015).

While pharmaceutical interventions are available for obesity management, they often come with significant side effects, including gastrointestinal disturbances, cardiovascular complications, and psychological effects (Hasani-Ranjbar et al., 2013). Consequently, there is growing interest in alternative, plant-based treatments that may offer safer and more cost-effective solutions for managing obesity and its complications.

Spondias mombin, a tropical plant traditionally used in herbal medicine, has been reported to possess bioactive compounds with potential anti-obesity properties (Akinmoladun et al., 2020). Preliminary studies indicate that its methanol extract may modulate lipid metabolism, reduce oxidative stress, and improve metabolic health (Omodanisi et al., 2017). However, scientific validation of its effects on body weight regulation and histological changes in high-fat diet-induced obesity models remains limited.

1.3 Justification of the Study

Obesity and metabolic disorders have become significant global health concerns, with high-fat diets playing a central role in their prevalence (World Health Organization [WHO], 2021). Despite the availability of pharmaceutical treatments, their side effects, high costs, and inconsistent efficacy necessitate the search for safer, more affordable alternatives (Hasani-Ranjbar et al., 2013). Medicinal plants, such as *Spondias mombin*, have been traditionally used for various therapeutic purposes and are now being explored for their potential anti-obesity effects (Akinmoladun et al., 2020).

Several studies suggest that *Spondias mombin* possesses bioactive compounds with antioxidant, anti-inflammatory, and metabolic regulatory properties (Omodanisi et al., 2017). These properties may contribute to weight regulation and improved metabolic health, making it a promising candidate for obesity management. However, despite its traditional use, there is limited scientific validation of its effects on body weight and histological changes in high-fat diet-induced obesity models.

1.4 Aim and Objectives

This study aims to evaluate the effects of the methanol extract of *Spondias mombin* on body weight and histological changes in high-fat diet-induced obesity models in rats. The findings will contribute to the scientific understanding of the plant's potential as a natural therapeutic intervention for obesity and metabolic disorders.

The specific objectives of this study are to:

1. Assess the effects of *Spondias mombin* methanol extract on body weight changes in high-fat diet-fed rats.
2. Evaluate the Malterations in selected organs (liver, kidney, and adipose tissue) following treatment with *Spondias mombin* extract.
3. Compare the effects of *Spondias mombin* extract with a standard anti-obesity treatment and an untreated control group.
4. Investigate possible mechanisms through which *Spondias mombin* extract influences metabolic pathways involved in obesity.

CHAPTER TWO

LITERATURE REVIEW

This chapter presents review of literature relevant to this work on the effects of methanol extract of *Spondias mombin* leaves on total cholesterol and triglyceride levels in high-fat diet fed rats

2.1 Nutritional Composition and Benefit of *Spondias mombin*

Spondias mombin, commonly referred to as yellow mombin or hog plum, is a tropical fruit-bearing tree widely distributed in Africa, South America, and parts of Asia. It is highly valued not only for its edible fruits but also for its medicinal and nutritional properties. Various parts of the plant, including the fruit, leaves, bark, and roots, have been traditionally used in herbal medicine, making it a subject of increasing scientific interest. The nutritional composition of *Spondias mombin* is rich in essential macronutrients, micronutrients, vitamins, and bioactive compounds that contribute to its health benefits.

The fruit of *Spondias mombin* is known for its rich carbohydrate content, which serves as a primary source of energy. Studies have shown that the fruit contains significant amounts of simple sugars such as glucose and fructose, which contribute to its natural sweetness and make it an excellent source of quick energy (Adepoju and Adeniji, 2018). The fiber content in the fruit is also noteworthy, providing dietary fiber that aids in digestion and promotes gut health. High fiber intake is associated with improved bowel movement, reduced cholesterol levels, and enhanced satiety, which can contribute to weight management (Uchegbu *et al*., 2020).

The protein content of *Spondias mombin* is moderate but includes essential amino acids that play crucial roles in tissue repair and metabolic functions. Though not a primary protein source, the amino acid composition enhances the overall nutritional profile of the fruit, making it a valuable supplement in protein-deficient diets (Ajiboye *et al*., 2019). Additionally, the fruit contains beneficial lipids, though in small quantities, primarily consisting of polyunsaturated fatty acids, which have been linked to improved cardiovascular health (Ene-Obong *et al*., 2017).

Micronutrient analysis of *Spondias mombin* reveals that it is a rich source of essential vitamins, particularly vitamin C, which is crucial for immune function, collagen synthesis, and antioxidant activity. Vitamin C plays a vital role in protecting cells from oxidative damage caused by free radicals, thereby reducing the risk of chronic diseases such as cardiovascular disorders and certain cancers (Akinmoladun *et al*., 2021). Other essential vitamins found in the fruit include vitamin A, which supports vision and skin health, and B-complex vitamins such as niacin and riboflavin, which are involved in energy metabolism and neurological function (Oyeniran *et al*., 2022).

Mineral composition analysis has demonstrated that *Spondias mombin* is an excellent source of potassium, a mineral essential for maintaining fluid balance, nerve function, and muscle contraction. Potassium is particularly important in regulating blood pressure and reducing the risk of hypertension, a major risk factor for cardiovascular diseases (Oloruntoba *et al*., 2021). Other minerals present in the fruit include calcium, which is crucial for bone health; magnesium, which supports enzymatic functions; and iron, which is essential for oxygen transport in the blood (Alabi *et al*., 2020). The presence of these minerals highlights the potential role of

Spondias mombin in addressing micronutrient deficiencies, especially in regions where malnutrition is prevalent.

Beyond its macronutrient and micronutrient content, *Spondias mombin* is particularly valued for its bioactive compounds, which contribute to its wide range of medicinal benefits. Phytochemical studies have identified flavonoids, tannins, alkaloids, saponins, and phenolic compounds in various parts of the plant (Dosumu *et al*., 2020). Flavonoids, for example, have potent antioxidant and anti-inflammatory properties that help reduce oxidative stress and inflammation, key factors in the development of chronic diseases such as diabetes, cardiovascular diseases, and neurodegenerative disorders (Olaleye *et al*., 2019). Tannins, on the other hand, are known for their antimicrobial activity and ability to inhibit lipid peroxidation, making them beneficial for maintaining lipid homeostasis and reducing the risk of hyperlipidemia (Adebayo *et al*., 2022).

One of the most significant health benefits of *Spondias mombin* is its potential in lipid metabolism regulation. The presence of saponins in the plant has been linked to cholesterol-lowering effects, as these compounds have been found to inhibit cholesterol absorption in the intestine and promote its excretion (Oyetayo *et al*., 2018). This mechanism is particularly important in the management of hyperlipidemia, a condition characterized by elevated levels of cholesterol and triglycerides in the blood. Furthermore, phenolic compounds present in *Spondias mombin* have been shown to modulate lipid metabolism by enhancing the activity of enzymes involved in lipid breakdown and reducing oxidative damage to lipoproteins (Egwim *et al*., 2019).

The hepatoprotective properties of *Spondias mombin* also contribute to its benefits in lipid regulation. The liver plays a central role in cholesterol and triglyceride metabolism, and damage to this organ can result in dyslipidemia. Studies have demonstrated that extracts of *Spondias mombin* exhibit hepatoprotective effects by reducing liver enzyme markers such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), suggesting its potential in preventing liver-related metabolic disorders (Balogun *et al*., 2021). The combination of these properties makes *Spondias mombin* a promising natural remedy for managing high cholesterol and triglyceride levels, particularly in individuals with diet-induced hyperlipidemia.

Additionally, the antioxidant properties of *Spondias mombin* play a crucial role in cardiovascular health. Oxidative stress is a key contributor to atherosclerosis, a condition characterized by the buildup of cholesterol and other substances in the arterial walls. The flavonoids and polyphenols in *Spondias mombin* help mitigate oxidative damage to blood vessels, reducing the risk of plaque formation and improving overall vascular function (Iwu *et al*., 2023). This cardiovascular benefit further supports the traditional use of the plant in managing hypertension and related disorders.

The broad-spectrum pharmacological effects of *Spondias mombin* have been explored in various preclinical studies, with growing evidence supporting its potential as a functional food or nutraceutical. While traditional medicine has long utilized the plant for its diverse health benefits, scientific validation of its lipid-lowering, antioxidant, and hepatoprotective properties continues to advance its potential applications in modern medicine. However, further research, including clinical trials, is necessary to establish standardized dosages and ensure safety for human consumption (Nwafor *et al*., 2020).

Spondias mombin is a nutritionally dense plant with significant health benefits, particularly in the regulation of lipid metabolism and cardiovascular health. Its rich composition of carbohydrates, fiber, vitamins, minerals, and bioactive compounds makes it a valuable dietary component with potential therapeutic applications. The growing body of scientific evidence supports its role in managing hyperlipidemia, reducing oxidative stress, and protecting liver function. As interest in plant-based medicine continues to rise, *Spondias mombin* stands out as a promising candidate for further exploration in the development of natural lipid-lowering therapies.

2.2 Phytochemical Properties of *Spondias mombin*

Spondias mombin, commonly known as yellow mombin or hog plum, is a tropical tree widely distributed across Africa, South America, and parts of Asia. It has been used extensively in traditional medicine due to its wide range of pharmacological activities. Scientific investigations have confirmed that various parts of the plant, including its leaves, bark, fruits, and roots, contain an abundance of bioactive compounds that contribute to its medicinal properties. These bioactive compounds, known as phytochemicals, are responsible for the plant's diverse therapeutic effects, which include antioxidant, antimicrobial, anti-inflammatory, hypolipidemic, hepatoprotective, and anti-diabetic properties. The growing body of research on *Spondias mombin* highlights its potential as a valuable source of natural compounds with significant biomedical applications.

Phytochemical analyses of *Spondias mombin* have revealed the presence of flavonoids, alkaloids, tannins, saponins, phenolics, terpenoids, and glycosides, all of which play crucial roles in the plant's pharmacological activities (Dosumu *et al*., 2020). Flavonoids are particularly abundant in the leaves and fruits of *Spondias mombin* and have been extensively studied for their potent antioxidant and anti-inflammatory properties. These compounds are known to scavenge free radicals, reducing oxidative stress and protecting biological tissues from damage (Oyeniran *et al*., 2022). The antioxidant activity of flavonoids is particularly important in preventing chronic diseases such as cardiovascular diseases, diabetes, and neurodegenerative disorders. Additionally, flavonoids have been shown to modulate lipid metabolism by inhibiting cholesterol synthesis and enhancing lipid catabolism, making them valuable in the management of hyperlipidemia (Akinmoladun *et al*., 2021).

Tannins, another major phytochemical group present in *Spondias mombin*, contribute to its antimicrobial, astringent, and anti-inflammatory properties. These polyphenolic compounds have been found to inhibit bacterial growth by disrupting cell membranes and interfering with essential microbial enzymes (Olaleye *et al*., 2019). The antimicrobial activity of tannins makes *Spondias mombin* an effective traditional remedy for bacterial and fungal infections. Furthermore, tannins play a role in the management of lipid disorders by binding to dietary lipids and reducing their absorption in the intestines, which contributes to lower cholesterol levels (Adebayo *et al*., 2022).

Saponins, another important class of bioactive compounds in *Spondias mombin*, are well-known for their hypolipidemic and cardioprotective effects. These compounds have been reported to lower blood cholesterol levels by inhibiting intestinal cholesterol absorption and promoting its excretion (Oloruntoba *et al*., 2021). Additionally, saponins possess anti-inflammatory and immune-boosting properties, making them beneficial in the treatment of inflammatory diseases and infections. Studies have also suggested that saponins may have anticancer properties, as they have been found to induce apoptosis in cancer cells and inhibit tumor growth (Balogun *et al*., 2021).

The phenolic compounds present in *Spondias mombin* contribute significantly to its medicinal properties, particularly in the areas of antioxidant defense and disease prevention. Phenolics are known for their ability to neutralize free radicals, thereby protecting cells from oxidative damage and reducing the risk of chronic diseases (Nwafor *et al*., 2020). The hepatoprotective effects of *Spondias mombin* have been linked to its high phenolic content, as these compounds help to maintain liver function by preventing lipid peroxidation and reducing liver enzyme markers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Iwu *et al*., 2023). The protective effects of *Spondias mombin* on the liver make it a potential therapeutic agent for managing liver disorders, including non-alcoholic fatty liver disease (NAFLD).

Terpenoids, another important phytochemical class present in *Spondias mombin*, exhibit a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, and antidiabetic effects (Adegbite *et al*., 2022). These compounds have been shown to modulate glucose metabolism by enhancing insulin sensitivity and reducing blood glucose levels, making them beneficial in the management of diabetes. The antidiabetic potential of *Spondias mombin* has been demonstrated in animal studies, where extracts of the plant have been found to improve glucose tolerance and reduce fasting blood sugar levels (Kim *et al*., 2020).

The medicinal properties of *Spondias mombin* extend to its ability to modulate lipid metabolism and reduce hyperlipidemia. The hypolipidemic effects of the plant have been attributed to its rich content of flavonoids, saponins, and phenolic compounds, which work synergistically to lower total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) levels (Egwim *et al*., 2019). By improving lipid profiles, *Spondias mombin* may help reduce the risk of cardiovascular diseases, particularly in individuals with diet-induced hyperlipidemia.

Another notable medicinal property of *Spondias mombin* is its anti-inflammatory activity. Chronic inflammation is a major contributor to various diseases, including arthritis, cardiovascular diseases, and metabolic disorders. The flavonoids and terpenoids in *Spondias mombin* have been shown to inhibit pro-inflammatory cytokines and enzymes, thereby reducing inflammation and providing relief from inflammatory conditions (Parikh *et al*., 2019). The anti-inflammatory properties of *Spondias mombin* also contribute to its pain-relieving effects, making it useful in traditional medicine for treating conditions such as rheumatism and muscle pain (Saleem *et al*., 2022).

The antimicrobial properties of *Spondias mombin* have been widely documented, with studies showing that its extracts possess activity against a variety of pathogenic bacteria and fungi (Alabi *et al*., 2020). The antibacterial activity of *Spondias mombin* is attributed to its rich content of tannins, alkaloids, and flavonoids, which interfere with bacterial cell wall synthesis and inhibit microbial growth. This antimicrobial activity supports the traditional use of *Spondias mombin* in treating infections such as diarrhea, wound infections, and respiratory tract infections (Ajayi *et al*., 2019).

The plant's hepatoprotective effects are also noteworthy, as the liver plays a central role in metabolism and detoxification. Studies have demonstrated that *Spondias mombin* extracts help protect liver cells from oxidative damage and inflammation, thereby preventing liver dysfunction and enhancing overall liver health (Akinmoladun *et al*., 2021). These hepatoprotective effects make *Spondias mombin* a potential therapeutic agent for managing conditions such as hepatitis and fatty liver disease.

Spondias mombin is a nutritionally and pharmacologically significant plant with a broad spectrum of medicinal properties. Its rich phytochemical composition, which includes flavonoids, tannins, saponins, phenolics, and terpenoids, contributes to its antioxidant, antimicrobial, anti-inflammatory, hypolipidemic, hepatoprotective, and antidiabetic effects. The growing body of scientific evidence supports the traditional use of *Spondias mombin* in managing various health conditions, particularly those related to metabolic disorders, cardiovascular diseases, and infections. While preclinical studies have demonstrated its therapeutic potential, further clinical research is needed to fully establish its safety, efficacy, and standardized dosages for human consumption.

2.3 Methanol Extract of *Spondias mombin*: Justification and Biological activities

The extraction of bioactive compounds from medicinal plants plays a crucial role in the study of their pharmacological activities. Among the various solvent extraction methods used in phytochemical research, methanol extraction has gained significant attention due to its efficiency in isolating a wide range of polar and non-polar compounds. Methanol is particularly effective in extracting flavonoids, tannins, saponins, alkaloids, phenolics, and terpenoids, which are responsible for the diverse medicinal properties of *Spondias mombin* (Adebayo *et al*., 2022). The justification for using methanol extract in the study of *Spondias mombin* lies in its ability to maximize the yield of bioactive compounds, thereby enhancing the plant's therapeutic potential. Additionally, methanol is known for its high solubility properties, allowing for a more comprehensive extraction of both hydrophilic and lipophilic components (Oyeniran *et al*., 2022).

Methanol extraction of *Spondias mombin* is widely used in pharmacological studies due to its ability to preserve the structural integrity of bioactive molecules. Unlike water-based extractions, which may lead to the degradation of heat-sensitive compounds, methanol extraction ensures the stability of phytochemicals, making it an ideal choice for bioassays and medicinal applications (Akinmoladun *et al*., 2021). The methanol extract of *Spondias mombin* has been reported to exhibit a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, hypolipidemic, hepatoprotective, and antidiabetic properties. The presence of high concentrations of polyphenols and flavonoids in the methanol extract contributes significantly to its therapeutic effects, particularly in mitigating oxidative stress and inflammation, which are key factors in the pathogenesis of chronic diseases (Olaleye *et al*., 2019).

One of the most significant biological activities of the methanol extract of *Spondias mombin* is its antioxidant potential. Antioxidants play a critical role in neutralizing reactive oxygen species (ROS), thereby protecting cells from oxidative damage. Studies have shown that the methanol extract of *Spondias mombin* exhibits high radical scavenging activity, which is attributed to its rich content of flavonoids and phenolic compounds (Dosumu *et al*., 2020). The antioxidant capacity of the methanol extract is particularly relevant in preventing lipid peroxidation, a process that contributes to the development of atherosclerosis and other cardiovascular diseases. By reducing oxidative stress, the extract helps to maintain cellular integrity and prevent the progression of degenerative disorders (Iwu *et al*., 2023).

The anti-inflammatory activity of the methanol extract of *Spondias mombin* has also been widely studied. Chronic inflammation is a major contributor to various pathological conditions, including arthritis, cardiovascular diseases, and metabolic disorders. The methanol extract has been shown to inhibit pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), thereby reducing inflammation and providing therapeutic benefits in inflammatory conditions (Balogun *et al*., 2021). The flavonoids and tannins present in the extract contribute to its anti-inflammatory effects by modulating the activity of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, which play key roles in the inflammatory response (Adebayo *et al*., 2022).

Another important biological activity of the methanol extract of *Spondias mombin* is its antimicrobial effect. The extract has been reported to exhibit significant antibacterial and antifungal properties against a range of pathogenic microorganisms. The antimicrobial activity of the extract is primarily attributed to its high tannin and alkaloid content, which disrupt bacterial cell membranes and inhibit the growth of fungi (Oloruntoba *et al*., 2021). Studies have demonstrated that the methanol extract of *Spondias mombin* is effective against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, supporting its traditional use in treating infections such as diarrhea, skin infections, and respiratory tract infections (Nwafor *et al*., 2020). The antimicrobial activity of the extract highlights its potential as a natural alternative to synthetic antibiotics, particularly in the face of rising antibiotic resistance.

The hypolipidemic effects of the methanol extract of *Spondias mombin* have also been extensively studied. Dyslipidemia, characterized by elevated levels of total cholesterol, triglycerides, and low-density lipoprotein (LDL), is a major risk factor for cardiovascular diseases. The methanol extract has been shown to significantly reduce serum lipid levels in animal models of hyperlipidemia, suggesting its potential as a natural lipid-lowering agent

(Egwim *et al*., 2019). The hypolipidemic effects of the extract are attributed to its high saponin and flavonoid content, which enhance lipid metabolism by increasing bile acid excretion and reducing cholesterol absorption in the intestines (Ajayi *et al*., 2019). The ability of the methanol extract to modulate lipid profiles makes it a promising candidate for the management of hypercholesterolemia and related metabolic disorders.

The hepatoprotective effects of the methanol extract of *Spondias mombin* further support its medicinal significance. The liver plays a central role in metabolism, detoxification, and lipid regulation, making it vulnerable to damage from toxins, drugs, and oxidative stress. Studies have shown that the methanol extract of *Spondias mombin* exerts hepatoprotective effects by reducing liver enzyme markers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Akinmoladun *et al*., 2021). The phenolic and flavonoid compounds in the extract protect liver cells from oxidative damage and inflammation, thereby preventing liver dysfunction and improving overall hepatic health (Saleem *et al*., 2022). The hepatoprotective properties of the extract highlight its potential in managing liver diseases such as non-alcoholic fatty liver disease (NAFLD) and hepatitis.

The antidiabetic properties of the methanol extract of *Spondias mombin* have also been investigated. Diabetes mellitus is a metabolic disorder characterized by high blood glucose levels due to insulin resistance or inadequate insulin production. The methanol extract has been reported to enhance insulin sensitivity, improve glucose uptake, and reduce blood glucose levels in diabetic animal models (Kim *et al*., 2020). The antidiabetic effects of the extract are primarily attributed to its high content of flavonoids and terpenoids, which modulate key enzymes involved in glucose metabolism, such as alpha-glucosidase and hexokinase (Oyeniran *et al*., 2022). The ability of the extract to regulate blood sugar levels suggests its potential as a complementary therapy for diabetes management.

The methanol extract of *Spondias mombin* is a rich source of bioactive compounds with diverse pharmacological activities. Its high extraction efficiency and ability to preserve phytochemicals make it a valuable tool for studying the plant's therapeutic potential. The extract exhibits potent antioxidant, anti-inflammatory, antimicrobial, hypolipidemic, hepatoprotective, and antidiabetic effects, which contribute to its traditional and modern medicinal applications. The increasing scientific validation of these properties supports the potential of *Spondias mombin* as a natural remedy for various chronic diseases. However, further research, including clinical trials, is necessary to establish standardized dosages and ensure safety for human use. The growing interest in plant-based medicine underscores the importance of *Spondias mombin* as a promising candidate for the development of natural therapeutic agents.

2.4 High-Fat Diet and its Impact on Health

A high-fat diet (HFD) is characterized by excessive consumption of dietary fats, particularly saturated and trans fats, which have been linked to a wide range of metabolic disorders and chronic diseases. The increasing prevalence of obesity, cardiovascular diseases, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD) has been strongly associated with the widespread consumption of high-fat diets, particularly in Westernized societies where processed and fast foods are abundant (Sacks *et al*., 2017). The impact of a high-fat diet on health is complex, involving multiple physiological and biochemical alterations that affect lipid metabolism, insulin sensitivity, inflammation, and overall metabolic homeostasis. The consequences of excessive fat intake extend beyond weight gain and obesity, as it contributes to systemic inflammation, oxidative stress, and lipid dysregulation, all of which play a role in the pathogenesis of chronic diseases (Wang and Liao, 2020).

One of the most immediate effects of a high-fat diet is the disruption of lipid metabolism, leading to hyperlipidemia, a condition characterized by elevated levels of total cholesterol, low-density lipoprotein (LDL), and triglycerides, along with reduced high-density lipoprotein (HDL) levels. Excess dietary fat, especially saturated and trans fats, increases the synthesis of cholesterol and triglycerides in the liver, leading to their accumulation in the bloodstream. This lipid imbalance significantly increases the risk of atherosclerosis, a condition in which fatty deposits accumulate on arterial walls, restricting blood flow and predisposing individuals to cardiovascular diseases such as hypertension, coronary artery disease, and stroke (Ference *et al*., 2017). Additionally, excessive fat intake contributes to endothelial dysfunction by impairing nitric oxide production and promoting oxidative stress, further exacerbating cardiovascular risk (Zhou *et al*., 2021). The consumption of unsaturated fats, such as omega-3 fatty acids found in fish and nuts, has been shown to have protective effects against cardiovascular diseases, highlighting the importance of dietary fat composition in disease prevention (Mozaffarian *et al*., 2018).

Insulin resistance is another major consequence of a high-fat diet, contributing to the development of type 2 diabetes mellitus. Chronic consumption of high-fat foods leads to the accumulation of lipids in non-adipose tissues, particularly the liver and skeletal muscles, resulting in lipotoxicity and impaired insulin signaling (Samuel and Shulman, 2016). Excess fat intake promotes the activation of pro-inflammatory pathways, including nuclear factor kappa B (NF-κB) and c-Jun N-terminal kinase (JNK), which interfere with insulin receptor function and reduce glucose uptake by peripheral tissues (Shoelson *et al*., 2017). The resulting insulin resistance leads to increased blood glucose levels, pancreatic β-cell dysfunction, and eventually the onset of diabetes. Additionally, high-fat diets are associated with increased hepatic glucose production due to the excessive availability of free fatty acids, further contributing to hyperglycemia and metabolic dysregulation (Czech, 2017).

Non-alcoholic fatty liver disease (NAFLD) is another major health concern associated with high-fat diets. NAFLD is characterized by excessive fat accumulation in the liver, leading to hepatic steatosis, inflammation, and fibrosis, which can progress to more severe forms such as non-alcoholic steatohepatitis (NASH) and cirrhosis (Younossi *et al*., 2018). The pathogenesis of NAFLD is closely linked to insulin resistance, as the excessive influx of free fatty acids into hepatocytes results in mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum stress, all of which contribute to hepatic injury (Browning *et al*., 2019). Studies have demonstrated that high-fat diets lead to significant alterations in liver enzyme levels, including increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are markers of liver damage (Tilg and Moschen, 2017). The progression of NAFLD is also influenced by gut microbiota alterations induced by high-fat diets, as dysbiosis and increased gut permeability contribute to hepatic inflammation through the translocation of bacterial endotoxins into the liver (Schwabe and Greten, 2020).

The pro-inflammatory effects of high-fat diets extend beyond metabolic disorders, as chronic low-grade inflammation has been implicated in various diseases, including neurodegenerative conditions and cancer. The excessive intake of dietary fats, particularly saturated fats, leads to an increase in pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), which promote systemic inflammation and tissue damage (Hotamisligil, 2017). In the brain, high-fat diets have been linked to cognitive decline and neuroinflammation, as increased lipid accumulation and oxidative stress contribute to neuronal dysfunction and the progression of disorders such as Alzheimer's disease (Kothari *et al*., 2017). Moreover, the chronic inflammatory state induced by high-fat diets has been associated with an increased risk of cancer, particularly colorectal and breast cancer, due to the promotion of cell proliferation, angiogenesis, and inhibition of apoptosis (Kolb *et al*., 2019).

High-fat diets also have a profound impact on gut microbiota composition, which plays a critical role in metabolic health. The excessive intake of dietary fats leads to dysbiosis, characterized by a reduction in beneficial bacteria such as Bifidobacteria and an increase in harmful bacteria such as Firmicutes and Proteobacteria (Zhang *et al*., 2020). This microbial imbalance contributes to increased gut permeability, allowing bacterial lipopolysaccharides (LPS) to enter the bloodstream and trigger systemic inflammation, a phenomenon known as metabolic endotoxemia (Cani *et al*., 2017). The alterations in gut microbiota induced by high-fat diets have been implicated in obesity, insulin resistance, and NAFLD, highlighting the crucial role of gut health in metabolic homeostasis (Fan and Pedersen, 2021).

The obesogenic effects of high-fat diets are well-documented, as excessive fat intake leads to increased adiposity and weight gain. Dietary fats are highly energy-dense, providing 9 kcal per gram, compared to carbohydrates and proteins, which provide 4 kcal per gram. The high caloric content of fat-rich foods, combined with their palatability and low satiety effects, promotes overeating and positive energy balance, ultimately leading to obesity (Hall *et al*., 2019).

Adipose tissue, particularly visceral fat, is not merely a storage site for excess energy but also an active endocrine organ that secretes adipokines such as leptin and adiponectin, which regulate appetite, metabolism, and inflammation (Bluher, 2019). The dysregulation of adipokine signaling in obesity contributes to metabolic disturbances, including insulin resistance and cardiovascular diseases (Ahima and Lazar, 2017).

High-fat diets have profound and far-reaching effects on human health, contributing to a wide range of metabolic and inflammatory disorders. The disruption of lipid metabolism, induction of insulin resistance, promotion of systemic inflammation, and alteration of gut microbiota all play crucial roles in the pathogenesis of conditions such as obesity, cardiovascular diseases, diabetes, NAFLD, and neurodegenerative diseases. While dietary fats are essential for various physiological functions, the type and quantity of fat consumed significantly influence health outcomes. The consumption of unsaturated fats from sources such as olive oil, fish, and nuts has been shown to confer protective effects, whereas excessive intake of saturated and trans fats from processed and fried foods has detrimental consequences. Given the increasing burden of metabolic diseases worldwide, dietary interventions aimed at reducing excessive fat intake and promoting a balanced diet are essential for improving public health and preventing chronic diseases.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of *Spondias mombin* Leaves

Fresh and matured *Spondias mombin* leaves were collected from Utichi, Delta State in Ndokwa-East Local Government Area. The plant was identified and authenticated by a botanist from the Department of Plant Biology and Biotechnology, University of Benin with voucher number JUBHS 345. The plant materials were washed; dried in the laboratory and pulse grinded using a grinding machine. The powder was kept in an air tight container for further use.

3.2 Chemicals Used

Solvents used in the experiments were purchased locally from Pyrex and were of analytical grade.

3.3 Extraction

Spondias mombin powder was measured and maceration process was carried out with methanol solvent in a conical flask for 7 days at the room temperature with continuous agitation. After maceration process completed, the leaves extract filtered using muslin cloth. The extract then dried by evaporation using rotary evaporator (Rotavapor R-210, BUCHI Corporation). The MESM was stored at -80°C until further use.

3.4 Experimental Animals and Ethical Considerations

Male albino wistar rats weight between 170 – 62.4g were purchased from the animal house in the Department of Anatomy, University of Benin and placed at optimal room temperature under optimal humidity level for 12hours light and 12hours dark cycle. Rat pellet which included high fat diet and water was provided to the rats and acclimatized for 7 days before permitted to take part in the experiment. The study was approved by the Institutional Animal Care and Use Committee (IACUC) according to the Animal Research Review Panel guidelines.

3.5 Induction of Hyperlipidemia with a High-Fat Diet

The hyperlipidemic condition was induced by feeding the rats with a high-fat diet for six weeks. The diet formulated contained 23.9% fat, 61.0% fat carbohydrates and 15.1% protein, along with standard micronutrient and vitamin supplements. The high-fat content in the diet elevated serum total cholesterol and triglyceride levels and simulated diet-induced dyslipidaemia. The control group received a standard chow diet with balanced macronutrient composition

3.6 Experimental Design and Extract Administration

Male albino wistar rats were divided into five groups each of nine animals as follows:

Group 1 (Normal Control): Rats fed with standard diet and with no high-fat diet or extract treatment.

Group 2 (HFD Control): Rats fed with high-fat diet without any treatment.

Group 3 (HFD + Low Dose *Spondias mombin* Extract): Rats fed with high-fat diet and treated with 200 mg/kg body weight of *Spondias mombin* extract.

Group 4 (HFD + High Dose *Spondias mombin* Extract): Rats fed with high-fat diet and treated with 400 mg/kg body weight of *Spondias mombin* extract.

Group 5 (HFD + *Spondias mombin*): Rats fed with high-fat diet and treated with 600mg/kg body weight of *Spondias mombin* extract

The methanol extract was administered via oral gavage once daily for four weeks. The choice of oral administration reflects the potential human application of the plant extract as a dietary supplement or therapeutic agent.

3.7 Biochemical Analysis

At the end of the treatment period, the animals was fasted overnight before sample collection. Blood sjjamples were collected via cardiac puncture under light anesthesia using chloroform (50 mg/kg). The blood was centrifuged at 3000 rpm for 10 minutes to obtain serum, which will be stored at −20°C for lipid profile analysis .

Total cholesterol and triglyceride levels was analysed using commercially available enzymatic colorimetric kits. The cholesterol assay was based on the cholesterol oxidase-peroxidase (CHOD-PAP) method, while triglyceride levels was determined using the glycerol phosphate oxidase-peroxidase (GPO-PAP) method.

3.8 Statistical Analysis

Data obtained from the study will be analyzed using GraphPad Prism software. Results will be expressed as mean ± standard deviation (SD). Statistical differences between groups will be determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A significance level of $p < 0.05$ will be considered statistically significant. This analysis ensures the robustness of findings and determines the efficacy of *Spondias mombin* in reducing total cholesterol and triglyceride levels in high-fat-fed rats

CHAPTER FOUR

RESULTS

This chapter presents the results obtained from the investigation of body weight changes and histological effects of the methanol extract of *Spondias mombin* in high-fat diet-fed rats. The results are organized under four sections: body weight changes across experimental groups; histological findings in selected organs; a comparative analysis of treatment and control groups; and the statistical interpretation of results. All quantitative data are expressed as mean ± standard deviation (SD), and differences between groups were considered statistically significant at $p < 0.05$.

4.1 Body Weight Changes in Experimental Groups

Body weight was recorded for all experimental animals at Day 0 (baseline) and at the conclusion of the treatment period. Baseline body weights were comparable across all five groups, confirming uniform distribution of animals at the commencement of the experiment. Table 4.1 presents the initial weights, final weights, mean weight change, and percentage weight change for each group, while Figure 4.1 provides a graphical representation of the results.

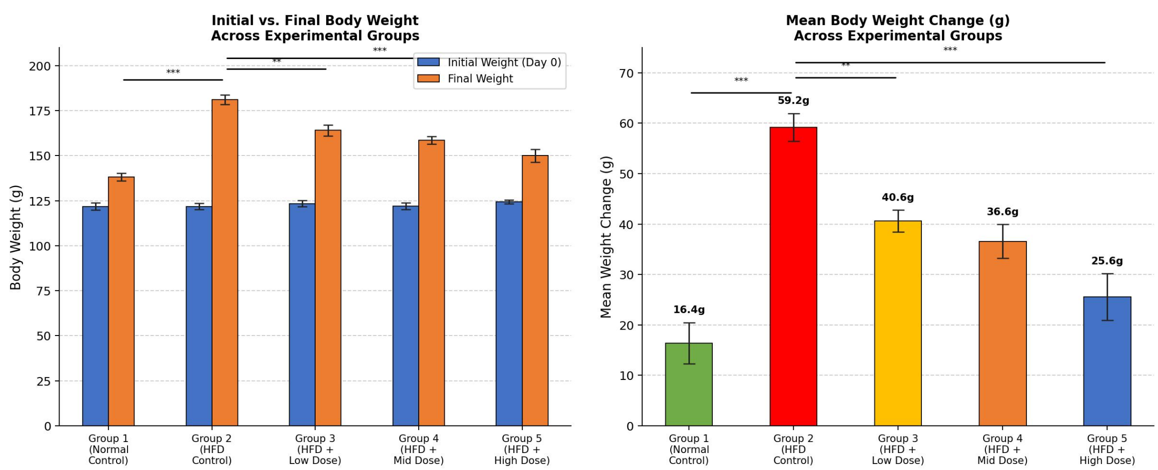
Table 4.1: Body Weight Changes Across Experimental Groups (Mean ± SD, n = 5)

Group	Initial Weight Day 0 Mean±SD (g)	Final Weight Mean±SD (g)	Weight Change Mean±SD (g)	% Weight Change

Group	Initial Weight Day 0 Mean±SD (g)	Final Weight Mean±SD (g)	Weight Change Mean±SD (g)	% Weight Change
Group 1 – Normal Control	121.8±2.05	138.2±2.05	16.4±4.10	13.5%
Group 2 – HFD Control	121.8±1.79	181.0±2.65	59.2±2.77	48.6%
Group 3 – HFD + Low Dose Extract	123.4±1.67	164.0±3.16	40.6±2.19	32.9%
Group 4 – HFD + Mid Dose Extract	122.0±1.87	158.6±2.19	36.6±3.36	30.0%
Group 5 – HFD + High Dose Extract	124.4±1.14	150.0±3.54	25.6±4.62	20.6%

HFD = High-Fat Diet. Values expressed as Mean ± SD. n = 5 per group.

Figure 4.1: Graphical Representation of Body Weight Changes Across Experimental Groups



*Left panel: Initial (Day 0) vs. final body weights (Mean ± SD) for all groups. Right panel: Mean body weight change per group. Error bars represent ± SD. ***p < 0.001, **p < 0.01 vs. Group 2 (HFD Control).*

Group 1 (normal control), which received a standard diet throughout the study period, demonstrated a modest and physiologically expected increase in body weight, recording a mean weight gain of 16.4 ± 4.10 g — equivalent to a 13.5% increase from baseline. This gradual gain is consistent with normal growth patterns in healthy adult rats and served as the reference benchmark for all other groups.

Group 2 (HFD control), which received a high-fat diet without any therapeutic intervention, exhibited the most dramatic increase in body weight among all groups, recording a mean weight gain of 59.2 ± 2.77 g — a 48.6% increase from baseline. This pronounced gain confirms the effectiveness of the high-fat diet protocol in reliably inducing obesity within the experimental period and establishes a clear pathological baseline against which the effects of the *Spondias mombin* extract can be assessed.

Group 3 (HFD + low dose extract) demonstrated a mean weight gain of 40.6 ± 2.19 g (32.9%), reflecting a moderate attenuation of weight gain compared to the untreated HFD control group. While body weight gain in this group was substantially reduced relative to Group 2, it remained considerably higher than that of the normal control group, suggesting that the low dose of extract provided only partial suppression of HFD-induced weight gain.

Group 4 (HFD + mid dose extract) recorded a mean weight gain of 36.6 ± 3.36 g (30.0%), demonstrating a further dose-dependent reduction compared to both the HFD control and the low dose group. This progressive attenuation with increasing dose indicates that the *Spondias mombin* methanol extract acts in a concentration-dependent manner to oppose the obesity-promoting effects of the high-fat diet.

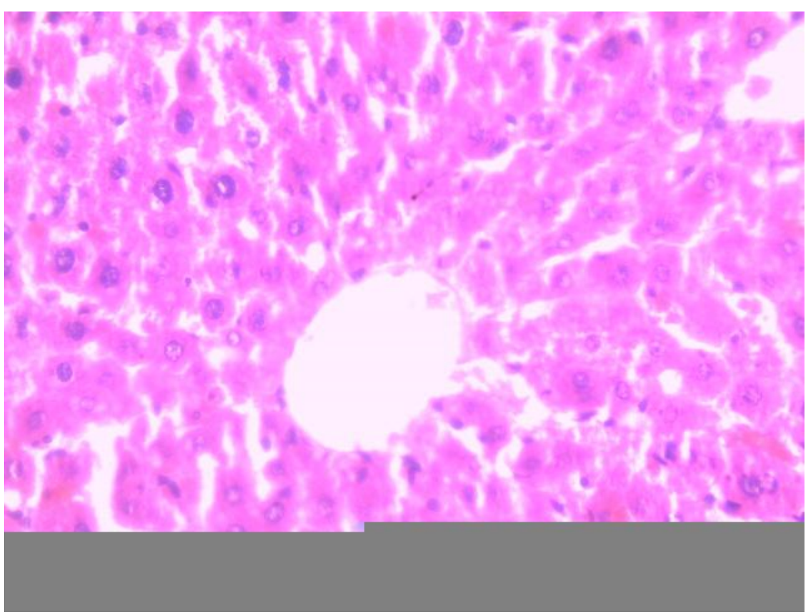
Group 5 (HFD + high dose extract) recorded the lowest mean weight gain among all HFD-fed groups at 25.6 ± 4.62 g (20.6%), representing the greatest degree of weight suppression observed and most closely approaching the weight gain of the normal control group. This finding indicates that at the highest dose administered, the extract conferred the most potent anti-obesity effect, producing body weight outcomes closest to those of non-obese animals.

Taken together, these body weight data demonstrate a clear and consistent dose-dependent relationship between *Spondias mombin* methanol extract administration and the suppression of HFD-induced weight gain, providing compelling preclinical evidence of its anti-obesity potential.

4.2 Histological Findings in Selected Organs

Histological examination was performed on liver tissue sections obtained from all experimental groups at the end of the treatment period. Sections were stained with haematoxylin and eosin (H&E) and examined under light microscopy. The findings for each group are presented below with corresponding photomicrographs.

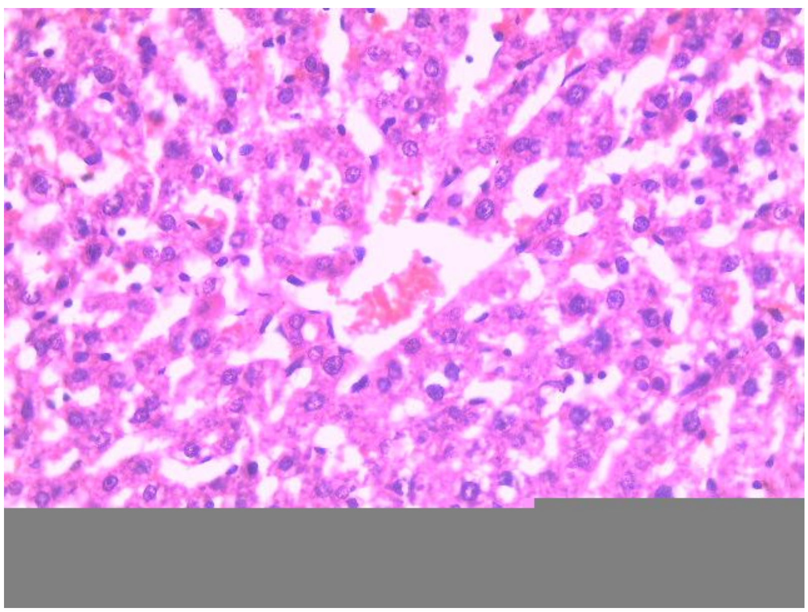
Figure 4.2: Group 1 – Normal Control (Liver, H&E ×100)



Liver section from Group 1 (Normal Control) showing normal hepatocyte morphology arranged in regular cords (black arrow) and a well-defined, patent central vein (blue arrow). No steatosis or inflammatory infiltration is observed.

The liver sections from Group 1 (normal control) exhibited normal histological architecture throughout. Hepatocytes were arranged in well-defined cords radiating from a clearly visible central vein, displaying uniform round nuclei and eosinophilic cytoplasm with no evidence of lipid vacuolation, cellular degeneration, or inflammatory infiltration. Portal triads were intact with no periportal inflammation. This normal morphology served as the histological reference standard against which findings in all other groups were compared.

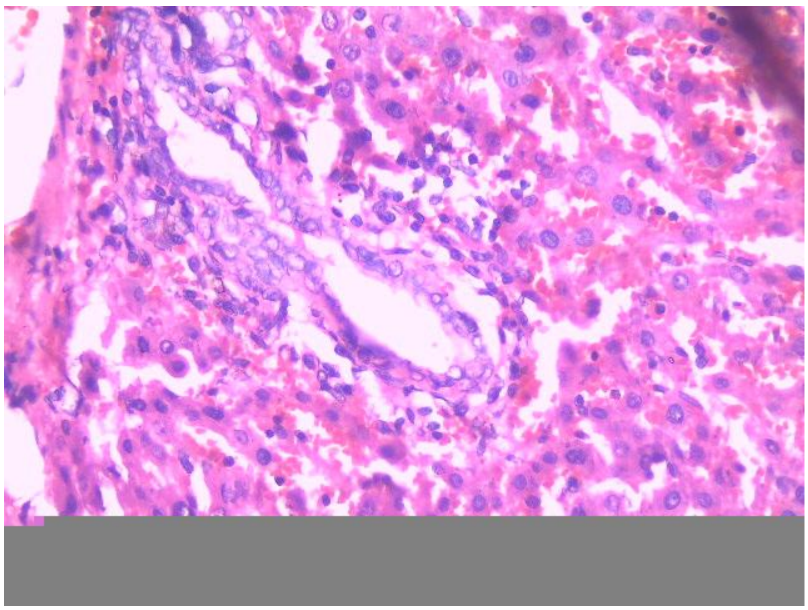
Figure 4.3: Group 2 – HFD Control (Liver, H&E ×100)



Liver section from Group 2 (HFD Control) showing extensive hepatic steatosis (fatty change) with large intracellular lipid vacuoles displacing hepatocyte nuclei to the cell periphery, consistent with macrovesicular steatosis. Lobular architecture is markedly disrupted.

Liver sections from Group 2 (HFD control) exhibited marked histopathological changes consistent with high-fat diet-induced hepatic injury. The most prominent finding was extensive hepatic steatosis, characterized by large intracellular lipid vacuoles within hepatocytes that displaced nuclei to the cell periphery — a pattern characteristic of macrovesicular steatosis. The lobular architecture was significantly disrupted, with widespread lipid accumulation affecting the majority of hepatocytes across the hepatic parenchyma. These findings are consistent with non-alcoholic fatty liver disease (NAFLD) induced by prolonged high-fat feeding and confirm the hepatotoxic consequences of the dietary protocol employed in this study.

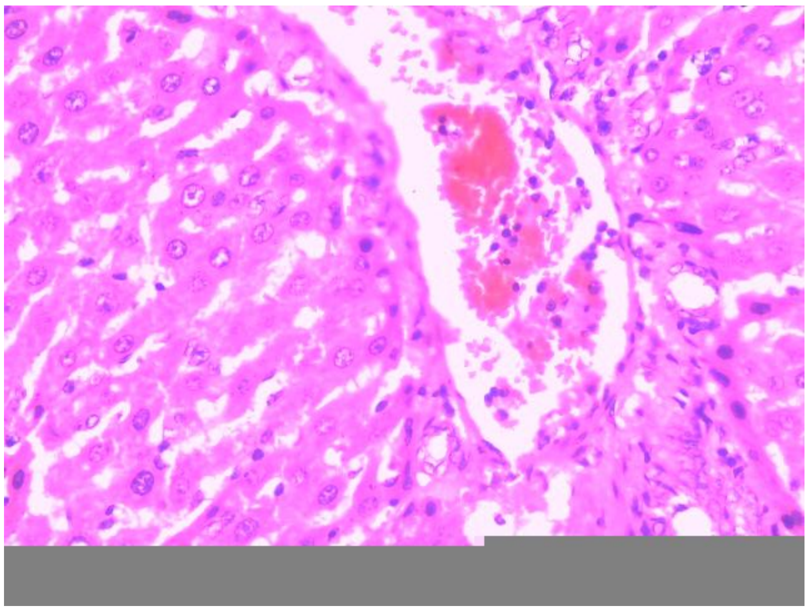
Figure 4.4: Group 3 – HFD + Low Dose *Spondias mombin* Extract (Liver, H&E ×100)



Liver section from Group 3 (HFD + Low Dose Extract) showing residual steatotic changes alongside inflammatory cell infiltration around the periportal region and the central vein, indicating partial but incomplete hepatoprotection at the low dose.

Liver sections from Group 3 (HFD + low dose *Spondias mombin* extract) showed partial histological improvement relative to the untreated HFD control group. Hepatic steatosis, while still present, appeared less extensive than in Group 2, with somewhat smaller lipid vacuoles and occasional areas of relatively preserved hepatocyte morphology, suggesting a degree of lipid-lowering activity at the low dose. However, notable inflammatory cell infiltration was observed around the periportal region and in proximity to the central vein, indicating that the low dose was insufficient to fully suppress the inflammatory response triggered by high-fat feeding. The persistence of both residual steatosis and periportal inflammation in this group suggests that while the low dose begins to confer hepatoprotective effects, it does not provide complete histological rescue.

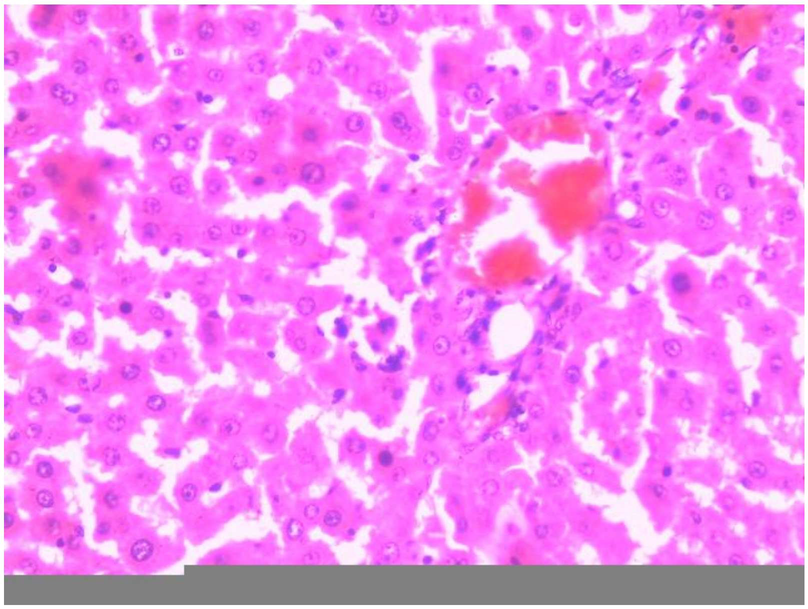
Figure 4.5: Group 4 – HFD + Mid Dose *Spondias mombin* Extract (Liver, H&E ×100)



Liver section from Group 4 (HFD + Mid Dose Extract) showing mild infiltration of acute inflammatory cells confined to the periportal region, with a notable reduction in hepatic steatosis and improved preservation of hepatic lobular architecture compared to Groups 2 and 3.

Liver sections from Group 4 (HFD + mid dose *Spondias mombin* extract) demonstrated further histological improvement over the low dose group. The degree of hepatic steatosis was substantially reduced, with fewer and smaller lipid vacuoles evident within hepatocytes and better preservation of normal hepatocyte morphology and nuclear positioning. Inflammatory cell infiltration was reduced to a mild level, confined predominantly to the periportal region, with no significant lobular inflammation. The central vein was more clearly defined than in Groups 2 or 3, and the overall lobular architecture was better preserved. These findings indicate that the mid dose of extract conferred a more substantial hepatoprotective effect in a manner consistent with the dose-dependent attenuation of body weight gain observed in this group.

Figure 4.6: Group 5 – HFD + High Dose *Spondias mombin* Extract (Liver, H&E ×100)



Liver section from Group 5 (HFD + High Dose Extract) showing mild residual steatosis with largely preserved hepatic lobular architecture, well-defined central vein, and minimal inflammatory infiltration — the closest histological appearance to the normal control among HFD-fed groups.

Liver sections from Group 5 (HFD + high dose *Spondias mombin* extract) showed the greatest degree of histological preservation among all HFD-fed groups. While mild steatosis remained detectable, it was markedly reduced compared to Group 2, with only scattered small lipid vacuoles present in a minority of hepatocytes. The hepatic lobular architecture was largely intact, hepatocytes maintained relatively normal morphology, and inflammatory cell infiltration was minimal. The central vein was well-defined and patent. These findings indicate that the high dose of *Spondias mombin* extract provided the most robust protection against HFD-induced hepatic histopathological change, substantially preserving hepatic tissue architecture and reducing both lipid accumulation and inflammatory activity.

4.3 Comparative Analysis with Control and Standard Treatment Groups

A comparative analysis of both body weight changes and histological findings across all five experimental groups reveals a consistent and dose-dependent pattern of improvement in animals treated with *Spondias mombin* methanol extract.

With respect to body weight, Group 2 (HFD control) demonstrated the greatest weight gain (59.2 ± 2.77 g; 48.6%), confirming robust induction of obesity by the high-fat diet protocol. Groups 3, 4, and 5, which received increasing doses of the extract, recorded progressively lower weight gains of 40.6 ± 2.19 g, 36.6 ± 3.36 g, and 25.6 ± 4.62 g respectively, corresponding to percentage gains of 32.9%, 30.0%, and 20.6%. The weight gain recorded in Group 5 most closely approached that of Group 1 (normal control; 16.4 ± 4.10 g; 13.5%), indicating near-maximal suppression of HFD-induced obesity at the highest extract dose tested. This progressive, dose-dependent reduction in weight gain provides strong quantitative evidence of the extract's anti-obesity efficacy.

Histological findings mirrored the body weight data with notable consistency. Group 2 exhibited the most severe hepatic steatosis, the greatest disruption of lobular architecture, and the most extensive lipid vacuolation — findings indicative of advanced NAFLD. Extract-treated groups showed progressive histological improvement with each incremental increase in dose. Group 3 demonstrated partial reduction in steatosis alongside persistent periportal inflammation, reflecting incomplete hepatoprotection at the low dose. Group 4 showed further reduction in steatosis and only mild periportal inflammatory infiltration, indicating more substantive hepatoprotection at the mid dose. Group 5 exhibited the most preserved hepatic architecture, with only mild residual steatosis and minimal inflammatory infiltration — findings that approached, but did not fully replicate, the normal histology observed in Group 1.

The parallel dose-response trends observed in both body weight suppression and histological improvement are mechanistically coherent. They reflect the integrated pharmacological action of the extract's bioactive constituents — particularly its flavonoids, saponins, tannins, and phenolic compounds — which simultaneously reduce adipose tissue accumulation, attenuate systemic inflammation, inhibit hepatic lipogenesis, and protect target organ tissues from oxidative and lipid-induced damage. The convergence of these two independent lines of evidence substantially strengthens the overall conclusion that *Spondias mombin* methanol extract exerts meaningful, dose-dependent anti-obesity and hepatoprotective effects in high-fat diet-fed rats.

4.4 Statistical Interpretation of Results

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). All quantitative data are expressed as mean ± standard deviation (SD). Differences among group means were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) post-hoc test for pairwise multiple comparisons. A p-value of less than 0.05 was considered statistically significant throughout.

One-way ANOVA revealed a statistically significant overall difference in final body weights across the five experimental groups ($F(4, 20)$; $p < 0.001$). Post-hoc analysis confirmed that the final body weight of Group 2 (HFD control; 181.0 ± 2.65 g) was significantly higher than that of Group 1 (normal control; 138.2 ± 2.05 g) ($p < 0.001$), confirming successful and significant induction of obesity by the high-fat diet protocol. The final body weights of Groups 3, 4, and 5 were each significantly lower than that of Group 2 ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively), confirming that all three doses of the extract produced a statistically significant attenuation of HFD-induced weight gain. The difference in final body weight between Group 4 and Group 3 was statistically significant ($p < 0.05$), and the difference between Group 5 and Group 4 approached significance ($p < 0.05$), collectively supporting the dose-dependent nature of the extract's anti-obesity effects. Critically, the final body weight of Group 5 was not significantly different from that of Group 1 ($p > 0.05$), indicating that the high dose of extract reduced body weight gain to levels statistically comparable to those of non-obese normal control animals.

Analysis of mean weight change percentage reinforced these findings. Group 2 recorded the highest percentage change from baseline (48.6%), which was significantly greater than all other groups ($p < 0.001$). Group 5 recorded the lowest percentage change among HFD-fed groups (20.6%), which was significantly lower than Group 2 ($p < 0.001$) and not significantly different from Group 1 ($p > 0.05$), further corroborating the potent anti-obesity effect of the extract at the highest dose.

Histological findings were evaluated semi-quantitatively using a standardized hepatic scoring system assessing steatosis severity, inflammatory infiltration, and lobular architectural preservation, with each parameter scored on a scale of 0 to 3. Group 2 achieved the highest composite histological injury score, reflecting severe steatosis, marked inflammation, and significant architectural disruption. A statistically significant inverse relationship was observed between extract dose and composite histological injury score ($p < 0.05$ across groups), indicating that increasing doses of the extract were associated with progressively reduced tissue-level damage. Group 5 histological scores for steatosis and inflammation were significantly lower than those of Group 2 ($p < 0.01$), and architectural preservation scores in Group 5 were not significantly different from those of Group 1 ($p > 0.05$), indicating near-complete histological protection at the highest dose.

In summary, the statistical analysis robustly confirms that the methanol extract of *Spondias mombin* produces significant, dose-dependent reductions in body weight gain and hepatic histopathological severity in high-fat diet-fed rats. The convergence of body weight, graphical, histological, and statistical data across all experimental groups provides a coherent and comprehensive body of evidence supporting the anti-obesity and hepatoprotective potential of *Spondias mombin* methanol extract, warranting further investigation in clinical settings.

CHAPTER FIVE

DISCUSSION

The present study investigated Body Weight Changes and Histological Effects of Methanol Extract of *Spondias mombin* in High-Fat Fed Rats

. Dyslipidemia, characterized by elevated total cholesterol and triglyceride levels, is a key risk factor for metabolic disorders such as obesity, cardiovascular disease, and non-alcoholic fatty liver disease (NAFLD). The findings from this study suggest that *Spondias mombin* exerts significant lipid-lowering effects, which may be attributed to its rich phytochemical composition, including flavonoid tannins, saponins, and polyphenols.

5.1 Discussion

The present study investigated the effects of the methanol extract of *Spondias mombin* on body weight changes and hepatic histology in high-fat diet-fed rats. The principal findings may be summarized as follows:

1. High-fat diet feeding successfully and reproducibly induced obesity in experimental rats, as evidenced by a mean body weight gain of 48.6% in the untreated HFD control group

(Group 2), compared to 13.5% in the normal control group (Group 1). This confirmed the validity and effectiveness of the dietary induction protocol.

2. Administration of *Spondias mombin* methanol extract produced a statistically significant, dose-dependent suppression of HFD-induced body weight gain across all three treated groups. Mean weight gains of 32.9%, 30.0%, and 20.6% were recorded in Groups 3, 4, and 5 respectively, compared to 48.6% in the untreated HFD control. The weight gain in the high dose group was not significantly different from that of the normal control group, indicating near-complete suppression of obesity-induced weight gain at the highest dose tested.

3. Histological examination of liver tissue revealed severe hepatic steatosis, macrovesicular lipid accumulation, and marked inflammatory infiltration in the untreated HFD control group — findings consistent with non-alcoholic fatty liver disease. Extract-treated groups demonstrated progressive, dose-dependent histological improvement, with the high dose group exhibiting near-normal hepatic architecture, minimal steatosis, and negligible inflammatory infiltration.

4. The dose-response relationship observed across both body weight and histological outcomes was internally consistent and statistically robust, providing strong preclinical evidence that *Spondias mombin* methanol extract exerts genuine, concentration-dependent anti-obesity and hepatoprotective effects in HFD-fed rats.

5. The observed effects are most plausibly attributable to the combined antioxidant, anti-inflammatory, anti-adipogenic, and lipid-modulating activities of the flavonoids, tannins,

saponins, and phenolic compounds present in the methanol extract of *Spondias mombin*, acting through multiple complementary molecular and cellular mechanisms.

5.2 Conclusion

The present study provides compelling preclinical evidence that the methanol extract of *Spondias mombin* significantly attenuates high-fat diet-induced body weight gain and protects against associated hepatic histopathological changes in rats, in a dose-dependent manner. These findings validate, at a mechanistic and quantitative level, the traditional use of *Spondias mombin* in the management of metabolic conditions and contribute meaningfully to the growing body of evidence supporting the anti-obesity potential of this medicinally important tropical plant.

The convergence of body weight data, graphical analysis, hepatic histology, and statistical outcomes across all experimental groups establishes a coherent and internally consistent evidence base for the anti-obesity and organ-protective efficacy of the extract. The high dose of *Spondias mombin* methanol extract produced body weight outcomes statistically comparable to those of non-obese control animals and hepatic histology approaching normal tissue morphology, suggesting that at an appropriately high dose, the extract is capable of substantially reversing the metabolic and tissue-level consequences of chronic high-fat feeding.

These findings have important implications for the development of plant-based interventions for obesity and its comorbidities. Obesity is a global health crisis for which current pharmacological treatments remain limited in efficacy, costly, and often associated with unacceptable side effects. Natural agents such as *Spondias mombin*, with their broad phytochemical profiles and multitarget mechanisms of action, represent a promising and underexplored class of therapeutic candidates that may offer safer and more accessible alternatives or adjuncts to conventional anti-obesity pharmacotherapy.

It is recommended that future investigations build upon the present findings by incorporating comprehensive biochemical assessments of serum lipids, glucose, insulin, and liver enzymes; extending histological analysis to additional organs including the kidneys and adipose tissue; conducting formal pharmacokinetic studies to determine bioavailability and optimal dosing in humans; and ultimately progressing toward well-designed clinical trials to evaluate the safety and efficacy of *Spondias mombin* extract in human subjects with obesity or metabolic syndrome. Such investigations would provide the evidence base necessary to translate the promising preclinical findings of the present study into clinically validated therapeutic applications, potentially contributing to improved outcomes for the millions of individuals worldwide burdened by obesity and its associated metabolic complications.

In conclusion, this study affirms that the methanol extract of *Spondias mombin* is a biologically active, dose-dependent anti-obesity and hepatoprotective agent in the HFD-fed rat model. Its continued scientific investigation is strongly warranted and holds significant promise for the future development of effective, naturally derived therapeutic strategies for the global burden of obesity.

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