EFFECT OF *Spondias mombin* ON HEMATOLOGICAL INDICES IN TYPE 2 DIABETIC RATS MODEL

BY

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CERTIFICATION

We the undersigned hereby certify that Miss OSARO ESTHER carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science (B.Sc) Degree in Medical Biochemistry.

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DEDICATION

This report is dedicated to my beloved parents; Mr and Mrs. Osaro; whose love, guidance, and sacrifices have been my greatest motivation. I also extend heartfelt gratitude to my friends, colleagues, and everyone who has supported and encouraged me throughout this journey. Your belief in me made this achievement possible

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ABSTRACT

This study investigated the effect of Spondias mombin extract on selected haematological indices in a type 2 diabetic rat model. Diabetes induction produced notable hematological disturbances, characterized by reductions in haemoglobin concentration (HGB), haematocrit (HCT), and red blood cell (RBC) count relative to the normal control group. These declines reflect the oxidative and metabolic stress typically associated with chronic hyperglycaemia. Administration of Spondias mombin at doses of 200 mg and 400 mg produced a clear restorative effect on all parameters assessed. HGB and HCT values in the treated groups increased markedly approaching or surpassing normal baseline levels while RBC counts also improved substantially compared with the diabetic control. The magnitude of recovery observed was comparable to the positive control group, suggesting that S. mombin possesses potent hematoprotective activity. The observed effects are likely attributable to the plant's rich phytochemical profile, including antioxidants and anti-inflammatory compounds, which collectively enhance erythrocyte stability, promote erythropoiesis, and counter oxidative stress. Overall, the findings indicate that Spondias mombin extract effectively mitigates diabetes-induced haematological alterations and may serve as a promising natural adjunct in the management of diabetic complications.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. The condition leads to disturbances in carbohydrate, lipid, and protein metabolism, predisposing patients to long-term complications affecting various organ systems, including the hematopoietic system (Gobinath et al., 2022). Among the two major forms of diabetes, Type 2 diabetes mellitus (T2DM) accounts for over 90% of global cases and is primarily associated with insulin resistance and β-cell dysfunction. Chronic hyperglycemia in T2DM induces oxidative stress and inflammatory cascades that impair erythropoiesis, alter blood rheology, and disturb hematological homeostasis (Alaba et al., 2023).

Hematological indices such as red blood cell (RBC) count, hemoglobin concentration (Hb), packed cell volume (PCV), white blood cell (WBC) count, and platelet parameters are important biomarkers for assessing systemic toxicity, immune status, and physiological responses in diabetic conditions. Previous studies have shown that T2DM can lead to anemia, leukocytosis, thrombocytosis, and other alterations in hematological parameters, largely due to oxidative damage, glycation of erythrocyte membranes, and inflammatory cytokine release (Oloye et al., 2024). These hematological changes contribute to diabetic complications such as cardiovascular diseases, nephropathy, and impaired wound healing. Therefore, maintaining hematological balance is a crucial therapeutic target in the management of diabetes and its systemic effects.

In recent years, there has been growing interest in the use of medicinal plants as alternative or complementary therapies for diabetes and its associated hematological disorders. This is largely

due to the limitations and side effects of synthetic antidiabetic drugs. Among these plants, *Spondias mombin L.* (commonly known as hog plum or Iyeye in Nigeria), a tropical tree belonging to the family Anacardiaceae, has attracted scientific attention for its diverse pharmacological potentials (Omonoyowa et al., 2025). Traditionally, various parts of *S. mombin* including its leaves, bark, and fruits are used in African and South American folk medicine to treat ailments such as diarrhea, infections, inflammation, and metabolic disorders.

Phytochemical investigations have revealed that *S. mombin* contains a rich array of bioactive compounds, including flavonoids, tannins, alkaloids, saponins, phenolic acids, and triterpenoids, which exhibit potent antioxidant, anti-inflammatory, hypoglycemic, and hematoprotective activities (Gobinath et al., 2022; Alaba et al., 2023). The antioxidant constituents are believed to mitigate oxidative damage in red blood cells and support erythropoietic recovery, while its hypoglycemic agents help regulate glucose metabolism, thereby reducing glycation-mediated hematotoxicity. Moreover, recent experimental studies have reported that extracts of *S. mombin* significantly improved hematological profiles and oxidative biomarkers in diabetic and chemically induced anemic animal models (Oloye et al., 2024).

Despite these promising findings, there remains a paucity of data on the direct effect of *Spondias mombin* on hematological indices in Type 2 diabetic models, especially in controlled in-vivo experimental settings. Understanding how *S. mombin* modulates hematological parameters such as RBC, WBC, Hb, and PCV in diabetic rats could provide valuable insights into its potential role in ameliorating diabetes-associated hematological dysfunctions. Furthermore, such studies would strengthen the pharmacological basis for its traditional use and open avenues for the development of plant-based hematoprotective nutraceuticals for diabetic patients (Omonoyowa et al., 2025).

Therefore, the present study aims to evaluate the effect of *Spondias mombin* on hematological indices in Type 2 diabetic rat models, with the goal of understanding its potential protective mechanisms and therapeutic implications in diabetes-induced hematological alterations.

1.3 Aim of the Study

The main aim of this study is to evaluate the effect of *Spondias mombin* on hematological indices in Type 2 diabetic rat models, with the goal of elucidating its potential role in restoring or modulating hematological parameters altered by diabetes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Diabetes Mellitus

Diabetes mellitus (DM) remains one of the most serious and rapidly growing global health challenges of the 21st century. It is a complex metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both, leading to disturbances in carbohydrate, lipid, and protein metabolism (Antar et al., 2023). According to the International Diabetes Federation, the global prevalence of diabetes continues to rise at an alarming rate, with Type 2 diabetes mellitus (T2DM) accounting for more than 90% of all cases. The burden is particularly heavy in low- and middle-income countries, where limited access to healthcare, sedentary lifestyles, and dietary transitions have accelerated disease incidence. Beyond its metabolic derangements, T2DM poses a major risk for complications such as

cardiovascular diseases, neuropathy, nephropathy, and hematological abnormalities that significantly impair quality of life and increase mortality (Abel et al., 2024).

Hematological indices including red blood cell (RBC) count, hemoglobin concentration (Hb), packed cell volume (PCV), white blood cell (WBC) count, and platelet count—serve as critical diagnostic and prognostic markers for evaluating the systemic effects of diabetes and monitoring therapeutic interventions (Oloye et al., 2024). Alterations in these parameters reflect the extent of oxidative stress, inflammatory response, and glycation-induced cellular injury that accompany chronic hyperglycemia. For instance, oxidative stress and persistent glucose toxicity can damage erythrocyte membranes, leading to shortened red cell lifespan and anemia, while inflammatory cytokine release may cause leukocytosis or thrombocytosis. These hematological disruptions not only contribute to microvascular and macrovascular complications but also serve as early indicators of systemic imbalance in diabetic conditions (Antar et al., 2023). Thus, the assessment of hematological indices provides valuable insight into the physiological state of diabetic patients and the hematoprotective efficacy of potential therapeutic agents.

In recent years, medicinal plants have gained substantial attention as alternative or complementary therapies for diabetes management due to their accessibility, affordability, and lower risk of adverse effects compared to synthetic drugs (Abel et al., 2024). Among these plants, *Spondias mombin L.* (hog plum) has emerged as a promising candidate owing to its wide ethnomedicinal applications and rich phytochemical profile. Traditionally used to treat infections, diarrhea, inflammation, and metabolic disorders, *S. mombin* contains bioactive compounds such as flavonoids, tannins, saponins, alkaloids, and phenolic acids that exhibit antioxidant, anti-inflammatory, and antidiabetic properties (Oloye et al., 2024). The antioxidant constituents of *S. mombin* are believed to scavenge free radicals and reduce oxidative damage to erythrocytes and

other blood cells, thereby improving hematological balance in pathological conditions such as diabetes.

Despite these potentials, few studies have systematically explored the effect of *Spondias mombin* on hematological indices in Type 2 diabetic models, leaving a critical gap in understanding its role in correcting diabetes-associated blood disorders. Considering the interplay between oxidative stress, inflammation, and hematological dysfunction in diabetes, the investigation of *S. mombin* provides a rational approach to discovering natural hematoprotective and antidiabetic agents. Therefore, evaluating the hematological impact of *S. mombin* in diabetic rat models is both scientifically and therapeutically significant, as it may offer insights into novel, plant-derived strategies for managing diabetes-induced hematological alterations (Antar et al., 2023; Abel et al., 2024; Oloye et al., 2024).

2.2 Definition and Classification (Type 1 and Type 2 Diabetes)

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both (Antar et al., 2023). It represents a heterogeneous group of metabolic diseases with multiple etiologies, typically categorized into Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes, and other specific types associated with genetic or secondary causes (Cloete et al., 2021).

Type 1 diabetes is primarily autoimmune, resulting from the destruction of pancreatic β-cells leading to absolute insulin deficiency, while Type 2 diabetes is non–insulin dependent and is characterized by insulin resistance and relative insulin deficiency. T2DM accounts for over 90%

of all diabetes cases worldwide and is strongly linked to sedentary lifestyles, obesity, genetic predisposition, and aging (Abel et al., 2024).

2.2.1 Pathophysiology of Type 2 Diabetes Mellitus

The pathophysiology of Type 2 diabetes mellitus involves a complex interplay of insulin resistance, β-cell dysfunction, increased hepatic glucose output, and chronic low-grade inflammation. Insulin resistance defined as the diminished response of peripheral tissues such as skeletal muscles, liver, and adipose tissue to insulin is the hallmark of T2DM (Hossain et al., 2024). In insulin-resistant states, glucose uptake by tissues is reduced, leading to compensatory hyperinsulinemia. Over time, pancreatic β-cells become exhausted, resulting in relative insulin deficiency and sustained hyperglycemia (Cloete et al., 2021).

Furthermore, persistent hyperglycemia enhances the formation of advanced glycation end products (AGEs) and triggers oxidative stress, which further impairs insulin signaling and damages vascular and hematopoietic tissues (Oloye et al., 2024). Chronic hyperinsulinemia and oxidative stress also disrupt lipid metabolism, leading to dyslipidemia, hepatic steatosis, and endothelial dysfunction all key contributors to diabetic complications (Abel et al., 2024).

2.3 Role of Oxidative Stress, Insulin Resistance, and Inflammation in Diabetes Progression

Oxidative stress plays a pivotal role in the onset and progression of Type 2 diabetes. Excessive production of reactive oxygen species (ROS) overwhelms the antioxidant defense system, causing lipid peroxidation, DNA damage, and protein oxidation (Hossain et al., 2024). These oxidative processes impair pancreatic β-cell integrity—cells that are particularly vulnerable due to their low levels of antioxidant enzymes. Additionally, chronic oxidative stress activates inflammatory signaling pathways such as NF-κB and JNK, leading to increased secretion of pro-

inflammatory cytokines (e.g., TNF-α, IL-6, and CRP) that aggravate insulin resistance (Cloete et al., 2021).

Inflammation and oxidative damage reinforce each other in a vicious cycle that accelerates cellular dysfunction, vascular damage, and metabolic derangements characteristic of diabetes (Antar et al., 2023).

2.3.1 Effects of Diabetes on Hematological and Metabolic Functions

Beyond its metabolic disturbances, T2DM significantly affects the hematological system, leading to measurable alterations in blood parameters. Chronic hyperglycemia and oxidative stress can induce glycation of erythrocyte membranes, reduce red blood cell deformability, and increase osmotic fragility, resulting in anemia and impaired oxygen transport (Abel et al., 2024). Inflammatory activation also contributes to leukocytosis and thrombocytosis, reflecting systemic immune imbalance and enhanced risk of vascular complications (Oloye et al., 2024).

Furthermore, metabolic dysfunction in diabetes alters lipid and protein metabolism, promoting dyslipidemia, hypercoagulability, and increased blood viscosity. These hematological and metabolic abnormalities contribute to the development of diabetic microvascular and macrovascular complications, including retinopathy, nephropathy, neuropathy, and cardiovascular diseases (Hossain et al., 2024).

2.4 Hematological Indices and Their Clinical Importance

Hematological indices are essential diagnostic parameters used to evaluate the physiological and pathological status of the blood, reflecting the body's general health and systemic balance. Key hematological parameters include red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), white blood cells (WBCs), and platelets. Red blood cells and hemoglobin are critical for

oxygen transport and cellular respiration, while WBCs play a central role in immune defense and inflammatory response. PCV, which measures the proportion of blood occupied by red cells, helps assess hydration and oxygen-carrying capacity, and platelets are vital for coagulation and vascular integrity (Delcea et al., 2021).

In diabetes mellitus, particularly type 2 diabetes, chronic hyperglycemia induces oxidative stress and systemic inflammation, leading to significant alterations in hematological indices. Persistent high blood glucose promotes the glycation of hemoglobin, damages erythrocyte membranes, and reduces RBC deformability, contributing to anemia and microvascular complications. Similarly, leukocyte activation and altered WBC counts often accompany the pro-inflammatory state in diabetes, reflecting immune dysregulation and tissue damage (Staplin et al., 2023). Platelet hyperactivity and increased aggregation further enhance the risk of thrombotic events commonly associated with diabetic vascular complications.

Hematological markers are thus reliable indicators of systemic toxicity and inflammation in diabetic models. They serve as sensitive tools for assessing disease progression and the effectiveness of therapeutic interventions. Schutte et al. (2022) reported that hematological derangements, such as reduced hemoglobin and elevated WBC counts, are closely linked with oxidative stress and chronic inflammatory states in diabetic individuals. The interplay between oxidative stress, anemia, and immune dysfunction forms a critical pathogenic triad in diabetes mellitus. Oxidative stress damages erythrocyte membranes and hemoglobin structure, leading to hemolysis and anemia, while immune dysfunction impairs defense mechanisms and promotes chronic inflammation, worsening the diabetic condition.

2.5 Medicinal Plants and Their Role in Diabetes Management

The use of medicinal plants in the management of diabetes mellitus has gained significant global attention due to their accessibility, affordability, and lower incidence of adverse effects compared to synthetic drugs. Diabetes, being a chronic metabolic disorder characterized by persistent hyperglycemia, often requires long-term therapy; hence, the search for safe, effective, and sustainable alternatives has directed researchers toward plant-based remedies (Yedjou et al., 2023). Medicinal plants contain diverse bioactive phytochemicals such as flavonoids, alkaloids, saponins, terpenoids, phenolic acids, and tannins that possess potent antioxidant, anti-inflammatory, and insulin-sensitizing properties. These compounds target multiple biochemical pathways, offering a holistic approach to glycemic control and the amelioration of diabetic complications (Bora, 2021).

Phytochemicals exert antidiabetic effects through various mechanisms. Some enhance insulin secretion by stimulating pancreatic β-cells, while others improve insulin sensitivity in peripheral tissues or mimic insulin activity at receptor sites. Additionally, many phytoconstituents inhibit carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, thereby reducing postprandial hyperglycemia (Adhikari, 2021). The antioxidant potential of these compounds is particularly crucial, as oxidative stress plays a central role in the onset and progression of diabetes. By scavenging free radicals and enhancing endogenous antioxidant defenses, plant-derived antioxidants protect pancreatic β-cells from oxidative damage, improve glucose metabolism, and prevent lipid peroxidation in vital organs.

Beyond glycemic control, medicinal plants have demonstrated hematoprotective effects, which are essential in managing diabetes-related hematological abnormalities. Hyperglycemia and oxidative stress disrupt erythropoiesis, alter red blood cell (RBC) membrane stability, and

promote inflammatory responses, leading to anemia and immune dysfunction in diabetic conditions. Studies have shown that certain medicinal plants can restore normal hematological indices by enhancing RBC production, stabilizing hemoglobin levels, and modulating leukocyte and platelet counts (Ansari et al., 2022). The phytochemical constituents responsible for these effects include polyphenols and flavonoids, which improve erythrocyte membrane integrity, reduce oxidative hemolysis, and suppress inflammatory cytokine release.

Yedjou et al. (2023) reported that phytochemical-rich plant extracts could significantly reduce oxidative stress and inflammatory damage in diabetic models, thereby improving both metabolic and hematological parameters. Similarly, Bora (2021) highlighted that plant-based antioxidants, such as quercetin, catechins, and resveratrol, exhibit strong hematoprotective and vasoprotective properties by modulating nitric oxide production and improving microcirculation. Adhikari (2021) further emphasized that the multi-target nature of phytochemicals allows them to simultaneously regulate blood glucose, lipid metabolism, and blood cell physiology, offering a systemic therapeutic advantage over monotherapy drugs.

In diabetic animal models, supplementation with medicinal plant extracts has been associated with improved hematological profiles, including normalized packed cell volume (PCV), hemoglobin concentration, and white blood cell counts. Ansari *et al.* (2022) noted that such improvements are indicative of enhanced oxygen-carrying capacity, immune modulation, and reduced inflammatory burden. This reinforces the therapeutic value of medicinal plants as dual-action agents both antidiabetic and hematoprotective.

In essence, the integration of plant-based therapies into diabetes management represents a promising and scientifically supported approach that addresses not only hyperglycemia but also

its associated hematological and systemic complications. The efficacy of these natural remedies lies in their synergistic actions, biocompatibility, and ability to modulate multiple pathophysiological processes. As research continues to validate and standardize the use of medicinal plants, species such as Spondias mombin hold significant potential in providing holistic benefits for the management of type 2 diabetes and its hematological derangements.

2.6 Botanical Description and Ethnomedicinal Uses of Spondias mombin

Spondias mombin L., commonly known as hog plum, yellow mombin, or "Iyeye" in Yoruba, is a tropical fruit-bearing tree belonging to the family Anacardiaceae. It is a medium-sized deciduous tree that can reach up to 25 meters in height, with a straight trunk, spreading branches, and dense foliage. The leaves are pinnate and alternate, consisting of 5–9 pairs of oblong-lanceolate leaflets with a distinctive aromatic scent when crushed. The flowers are small, yellowish-white, and occur in terminal panicles, while the fruit is an ovoid drupe with a fibrous pulp and a characteristic yellow to orange hue when ripe (Maria et al., 2022). The plant thrives in tropical and subtropical regions of Africa, Central and South America, and parts of Asia, particularly in humid lowlands and secondary forests. In Nigeria, it grows abundantly in the rainforest and savanna belts, where it plays both nutritional and medicinal roles (Ogunro et al., 2023).

Taxonomically, Spondias mombin is classified as follows:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Anacardiaceae

Genus: Spondias

Species: S. mombin L.

Figure 2.1: *Spondias Mombin* (Maria *et al.,* 2022).

Traditionally, Spondias mombin has been extensively utilized in African and tropical folk medicine for its wide range of therapeutic properties. Ethnomedicinal records show that the plant is employed in the treatment of several ailments such as diabetes, diarrhea, dysentery, inflammation, anemia, and infections. The leaves are commonly used as decoctions or infusions to manage febrile illnesses and enhance blood quality, while the bark and roots are often employed in the management of gastrointestinal disorders, menstrual irregularities, and wound healing (Ojatula et al., 2025). The fruit, which is rich in vitamins and antioxidants, is used both as food and as a natural remedy for digestive and urinary problems.

The pharmacological value of S. mombin lies in its rich phytochemical composition, which includes flavonoids, tannins, alkaloids, phenolic compounds, saponins, and triterpenoids. These compounds confer strong antioxidant, anti-inflammatory, antimicrobial, and hematoprotective activities, justifying its traditional use in treating blood-related disorders and metabolic diseases such as diabetes (Togni et al., 2025). For instance, leaf extracts have demonstrated significant free radical scavenging and erythrocyte-stabilizing effects, suggesting potential benefits in managing oxidative stress-induced hematological imbalances in diabetic conditions.

Methods of preparation in traditional medicine vary across regions but commonly include boiling or soaking plant parts in water or alcohol to produce decoctions or tinctures. In some communities, the leaves are pounded and mixed with other herbs or local beverages to enhance potency. The bark extract is also used externally for wound dressing and skin infections due to its antimicrobial and astringent properties (Ogunro et al., 2023).

Overall, Spondias mombin represents an important medicinal resource with deep ethnopharmacological roots. Its wide range of applications across cultures reflects its versatility

and therapeutic promise. The continued scientific validation of its pharmacological properties not only supports traditional knowledge but also highlights its potential for development into standardized herbal formulations for the management of diabetes and hematological disorders.

2.7 Phytochemical Constituents of Spondias mombin

Spondias mombin is phytochemically rich, containing a wide spectrum of secondary metabolites that are commonly implicated in the plant's pharmacological actions. Recurrent classes reported across multiple studies include flavonoids (e.g., glycosides and aglycones), phenolic acids, tannins, saponins, alkaloids, terpenoids/triterpenes, glycosides, and volatile essential-oil components. These groups together explain the antioxidant, anti-inflammatory, antimicrobial, hypoglycemic and hematoprotective activities attributed to the species (Ogunro et al., 2023; Orumwensodia et al., 2021). Recent analytical work also documents carotenoids and sterols as minor but biologically relevant constituents (Eze et al., 2024; Ikponmwosa-Eweka et al., 2024).

Researchers have employed both qualitative screening and quantitative analytical techniques to characterize S. mombin extracts:

Qualitative screening includes; Classical phytochemical tests (e.g., Ferric chloride for phenolics, Shinoda test for flavonoids, foam test for saponins, Dragendorff's for alkaloids) consistently demonstrate the presence of the major classes listed above in leaf, bark and fruit extracts (Orumwensodia et al., 2021; Ogunro et al., 2023).

Chromatography and spectroscopy — More detailed profiling has used GC–MS (mainly for volatile oils), HPLC and LC–MS/MS (for non-volatile phenolics and flavonoid glycosides), and UV–Vis spectrophotometry for bulk assays. These methods have allowed identification of

specific phenolic and flavonoid fingerprints and volatile profiles that vary by plant part and extraction solvent (Eze et al., 2024; Ogunro et al., 2023).

Total content assays (quantitative colorimetric tests) — Studies routinely report total phenolic content (TPC) using the Folin–Ciocalteu method and total flavonoid content (TFC) using aluminium chloride colorimetry, with values differing by solvent polarity (aqueous, methanol, ethanol) and plant part. TPC/TFC values correlate strongly with measured antioxidant capacity in DPPH, ABTS and FRAP assays (Ikponmwosa-Eweka et al., 2024; Eze et al., 2024).

Standardization and variability — Quantitative data show substantial regional, seasonal and methodological variability: concentrations of key classes change with geography, harvest time, drying method, and extraction protocol. These sources of variation are repeatedly emphasized as reasons to adopt standardized extraction and reporting in future work (Ogunro et al., 2023; Orumwensodia et al., 2021).

2.7.1 Biological roles and pharmacological relevance of key constituents

The pharmacological effects of *S. mombin* can be mapped to its phytochemical composition as follows:

- Flavonoids & phenolics — Strong antioxidant and free-radical scavenging actions; they protect cellular components (including erythrocytes) from oxidative damage, modulate inflammatory signaling (e.g., NF-κB pathways), and may improve endothelial function. These effects are central to the plant's putative hematoprotective and antidiabetic activities (Eze et al., 2024; Ikponmwosa-Eweka et al., 2024).

- Tannins — Astringent and antimicrobial; they can stabilize cell membranes (reducing hemolysis) and exert anti-inflammatory actions that indirectly protect hematological integrity under stress conditions (Orumwensodia et al., 2021).

- Saponins — Reported to have immunomodulatory and cholesterol-lowering properties; saponins may contribute to improved lipid profiles and reduced oxidative burden in metabolic disease models (Ogunro et al., 2023).

- Alkaloids — Although present in lower quantities, alkaloids may exert bioactivity via enzyme modulation and effects on glucose handling; however, their presence also warrants toxicity evaluation at higher doses (Orumwensodia et al., 2021).

- Terpenoids / triterpenes — Anti-inflammatory and hepatoprotective roles are commonly ascribed to these compounds; they may attenuate inflammatory cascades that otherwise aggravate hematological derangements in diabetes (Eze et al., 2024).

- Volatile oils — Components identified by GC–MS have antimicrobial and antioxidant effects and may contribute to the overall therapeutic profile of topical or oral preparations (Ogunro et al., 2023).

2.7.2 The link between phytochemistry, hematological and anti-diabetic effects

A consistent theme in across several researches is that high phenolic/flavonoid content correlates with antioxidant potency, which in turn is associated with improved hematological indices in animal models (reduced hemolysis, normalized Hb/PCV, moderated leukocyte counts). Mechanistically, antioxidant phytochemicals can:

1. Reduce erythrocyte oxidative damage and membrane lipid peroxidation, preserving RBC deformability and lifespan.

2. Lower systemic oxidative stress and inflammatory cytokines, thereby limiting bone-marrow suppression or dysfunction that contributes to anemia.

3. Improve insulin sensitivity and glycemic control indirectly, decreasing glycation of hemoglobin and reducing further hematological insults (Ikponmwosa-Eweka et al., 2024; Eze et al., 2024).

While qualitative and bulk quantitative data are abundant, research highlights several needs: (i) standardization of extraction and reporting units for TPC/TFC and specific marker compounds; (ii) more LC–MS/MS–based identification of individual bioactive molecules with validated standards; and (iii) linking specific isolated constituents to in-vivo hematological outcomes (dose–response and mechanism studies) rather than relying solely on crude extract correlations (Ogunro et al., 2023; Orumwensodia et al., 2021).

2.8 Pharmacological Activities of *Spondias mombin*

2.8.1 Antidiabetic and Hypoglycemic Activities

Extensive experimental and ethnopharmacological evidence supports the antidiabetic potential of *Spondias mombin*. Various parts of the plant particularly the leaves and bark—have been shown to exert blood glucose-lowering effects in both normal and diabetic animal models. The hypoglycemic effect is attributed to the presence of bioactive compounds such as flavonoids, phenolic acids, saponins, and alkaloids, which modulate carbohydrate metabolism and enhance insulin activity (Maria, 2022; Ogunro et al., 2023).

Mechanistically, *S. mombin* extracts have been reported to improve glucose tolerance by stimulating pancreatic β-cell regeneration, enhancing insulin secretion, and increasing glucose uptake in peripheral tissues. Freitas et al. (2024) demonstrated that administration of methanolic leaf extract significantly reduced fasting blood glucose levels and glycated hemoglobin (HbA1c) in streptozotocin-induced diabetic rats. The study also revealed restoration of hepatic glycogen levels and a reduction in serum lipid abnormalities, indicating improved metabolic control. Furthermore, the inhibitory action of S. mombin on α-amylase and α-glucosidase enzymes helps delay glucose absorption, thereby reducing postprandial hyperglycemia (Maria, 2022).

2.8.2 Antioxidant and Anti-inflammatory Properties

Oxidative stress and inflammation are central in the progression of diabetes and its complications. The antioxidant potential of *S. mombin* is well-documented and largely ascribed to its rich content of polyphenols, flavonoids, and tannins. These compounds exhibit free-radical scavenging, metal-chelating, and lipid peroxidation inhibitory effects (Asante Ampadu et al., 2022). The plant's extracts have been shown to enhance the activities of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while reducing malondialdehyde (MDA) levels, an indicator of oxidative lipid damage (Freitas et al., 2024).

In diabetic models, *S. mombin* also exhibits pronounced anti-inflammatory activity by downregulating the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, as well as by inhibiting the NF-κB signaling pathway (Maria, 2022). The combined antioxidant and anti-inflammatory effects not only contribute to improved glycemic control but also protect tissues from oxidative and inflammatory insults associated with diabetes-induced complications.

2.8.3 Hematopoietic and Hematoprotective Effects

The hematological benefits of *Spondias mombin* have received growing attention, especially in the context of metabolic and oxidative disorders. Studies indicate that the plant promotes hematopoiesis and helps maintain normal RBC, Hb, and WBC counts in diabetic and toxicological models (Ogunro et al., 2023). The flavonoid and phenolic components play a crucial role in stabilizing erythrocyte membranes, reducing oxidative hemolysis, and enhancing iron metabolism, thereby preventing anemia.

Freitas et al. (2024) reported that *S. mombin* leaf extract restored hematological parameters such as packed cell volume (PCV), hemoglobin concentration, and RBC count in diabetic rats, bringing them closer to normal values. This hematoprotective effect was attributed to reduced oxidative damage and improved antioxidant enzyme function. Additionally, Asante Ampadu et al. (2022) demonstrated that *S. mombin* supplementation modulated WBC count and differential leukocyte distribution, suggesting an immune-supportive role through its anti-inflammatory and immunoregulatory activities.

Other Pharmacological Actions

Beyond its antidiabetic and hematological roles, *Spondias mombin* exhibits a wide range of pharmacological activities, reflecting its multipurpose therapeutic value in traditional medicine.

Antimicrobial: Extracts of *S. mombin* have shown broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The antimicrobial efficacy is attributed to the presence of tannins, saponins, and phenolic acids that disrupt microbial cell walls and inhibit biofilm formation (Ogunro et al., 2023).

Hepatoprotective: *S. mombin* exhibits hepatoprotective effects by reducing serum liver enzyme levels (ALT, AST, ALP) and preventing hepatic lipid peroxidation. Freitas et al. (2024) reported histological improvements in the liver architecture of diabetic rats treated with the plant extract, confirming protection against oxidative hepatic injury.

Nephroprotective: The antioxidant and anti-inflammatory compounds in *S. mombin* protect renal tissues from oxidative damage and glomerular dysfunction, as observed in streptozotocin-induced diabetic nephropathy models (Asante Ampadu et al., 2022).

Cardioprotective: The plant's ability to reduce hyperlipidemia, oxidative stress, and inflammation contributes to cardioprotective effects, preventing atherosclerotic damage and improving overall cardiovascular function (Maria, 2022).

2.9 Effects of Diabetes on Hematological Parameters

Diabetes mellitus, particularly Type 2 diabetes, profoundly influences hematological parameters due to its systemic impact on oxidative balance, metabolic regulation, and inflammatory processes. Chronic hyperglycemia leads to glycation of hemoglobin and erythrocyte membrane proteins, impairing the structural integrity and deformability of red blood cells (RBCs). This results in reduced lifespan of erythrocytes and diminished oxygen-carrying capacity (Bamboo et al., 2024). The persistent oxidative stress generated by elevated glucose levels further induces lipid peroxidation in cell membranes, which disrupts the function and survival of erythrocytes and may culminate in anemia.

Furthermore, diabetes is associated with significant alterations in leukocyte dynamics. Increased production of reactive oxygen species (ROS) and advanced glycation end-products (AGEs) can stimulate pro-inflammatory cytokine release, leading to leukocytosis and immune dysregulation

(Ebrahim et al., 2022). Chronic inflammation not only promotes oxidative damage but also contributes to endothelial dysfunction and vascular injury, both of which are hallmarks of diabetic complications.

Platelet abnormalities are also common in diabetes. Hyperglycemia enhances platelet aggregation and activation through pathways involving protein kinase C and increased intracellular calcium, thereby predisposing diabetic individuals to thrombocytosis and a higher risk of thrombotic events (Rafagat et al., 2023). This hypercoagulable state exacerbates cardiovascular complications often observed in diabetic patients.

Moreover, studies have demonstrated that insulin resistance and metabolic imbalances impair bone marrow hematopoiesis, reducing the regenerative capacity of hematopoietic stem cells (Arkew et al., 2021). Such disruptions may lead to impaired production of RBCs and other blood cell lineages. The combined effects of oxidative stress, chronic inflammation, and metabolic toxicity ultimately contribute to the complex hematological profile of diabetic patients, characterized by anemia, altered leukocyte count, and abnormal platelet function.

2.10 Effect of Medicinal Plants on Hematological Parameters in Diabetic Models

Medicinal plants have gained considerable attention as alternative or complementary therapies for managing diabetes and its associated hematological disturbances. Numerous experimental studies have demonstrated that plant extracts rich in bioactive compounds such as flavonoids, alkaloids, saponins, tannins, and phenolics can restore altered hematological indices in diabetic animal models. These phytochemicals exert protective effects through antioxidant, anti-inflammatory, and hematopoietic mechanisms that collectively counteract the oxidative and metabolic stress induced by hyperglycemia (Nnadiukwu et al., 2024).

Several studies have reported that plant-derived extracts improve red blood cell (RBC) count, hemoglobin concentration (Hb), packed cell volume (PCV), and platelet levels in diabetic rats. For instance, Nnadiukwu et al. (2024) observed that treatment with polyphenol-rich plant extracts significantly restored Hb and RBC values, suggesting enhanced erythropoiesis and protection against hemolytic damage caused by oxidative stress. These effects are attributed to the ability of plant antioxidants to scavenge free radicals, stabilize cell membranes, and prevent the glycation of erythrocyte proteins.

Comparative studies have also shown that certain plant-based formulations perform comparably or even superiorly to synthetic antidiabetic drugs such as metformin or glibenclamide in improving hematological parameters (Khalaf et al., 2025). While synthetic drugs primarily target glucose metabolism, plant extracts often provide multi-targeted benefits by simultaneously improving oxidative balance, reducing inflammation, and modulating hematopoietic functions. This holistic approach addresses both the metabolic and systemic consequences of diabetes.

Mechanistic insights reveal that phytochemicals such as quercetin, catechins, and triterpenoids can upregulate endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), thereby reducing oxidative damage to bone marrow and circulating blood cells (Alqahtani et al., 2022). Furthermore, plant extracts may enhance erythropoietin production and improve iron metabolism, contributing to the normalization of hemoglobin levels and red cell indices. Some bioactive constituents also exhibit immunomodulatory actions, helping to balance leukocyte profiles that are often disturbed in diabetic states (Uhua et al., 2022).

Overall, these findings affirm that medicinal plants possess potent hematoprotective and antidiabetic properties, making them valuable candidates for managing diabetes-related

hematological dysfunctions. Their efficacy lies in their synergistic mechanisms combining glucose regulation with oxidative stress mitigation and blood cell preservation (Nnadiukwu et al., 2024; Khalaf et al., 2025; Alqahtani et al., 2022; Uhua et al., 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials and Apparatus used

Ethanol

Distilled water

Citrate buffer

Whatman filter paper

Ink dye

Mechanical grinder

Oral gavage

Syringes

Electronic weigh balance

Glucometer

Standard chow

High fat-diet (Rat feed (1825 g) - Butter (1600 g) - Soy protein (1250 g) - Vitamin and mineral mix (300 g) - Yeast powder (5 g) - Salt (NaCl, 5 g) - Methionine (15 g))

3.2 Chemicals and Reagents Used

Streptozotocin

Glipizide

Ketamine

Xylazine

Saline solution

Formalin

3.3 Plant Material Collection and Authentication

Fresh leaves of *Spondias mombin* L. (hog plum) were collected from a local botanical garden at Iguomon community, Benin city, Nigeria. The plant was identified and authenticated by a taxonomist at the Department of Botany, (University of Benin). A voucher specimen was deposited in the herbarium for reference.

3.3.1 Preparation of *Spondias mombin* Leaf Extract

The collected leaves were thoroughly washed with distilled water to remove dirt and debris, then air-dried at room temperature (25-28°C) in a shaded area for 7-10 days to avoid direct sunlight and preserve bioactive compounds. The dried leaves were pulverized into a fine powder using a mechanical grinder. The powdered leaves (500 g) were macerated in 2 L of 70% ethanol (v/v) at room temperature for 72 hours with occasional stirring. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure to obtain a semi-solid ethanolic extract. The yield of the ethanolic extract was calculated as a percentage of the starting material. The extract was stored in an airtight container at 4°C until use. For administration, the extract was reconstituted in distilled water to achieve the desired concentrations.

3.4 Animals Experimentation

3.4.1 Chemicals and Reagents

Streptozotocin (STZ) was procured from Sigma-Aldrich (USA). Glipizide (standard antidiabetic drug) was obtained from a local pharmacy. Citrate buffer (0.1 M, pH 4.5) was prepared fresh for STZ dissolution. All other chemicals, including components for the high-fat diet (HFD), were of analytical grade and sourced from reputable suppliers.

3.4.2 Experimental Animals

Adult male Wistar albino rats (150-200 g) were obtained from the Animal House Facility of the University of Benin. The rats were housed in standard polypropylene cages under controlled environmental conditions. They were provided with standard rodent chow and water (ad libitum) during the acclimatization period of 2 weeks.

Individual rats were identified using non-toxic markers (e.g., permanent ink) on specific body parts such as the back, head, left forelimb, left hindlimb, tail, or abdomen for tracking purposes throughout the study.

3.4.3 Preparation of High-Fat Diet (HFD)

To induce obesity and insulin resistance, a high-fat diet was formulated as follows: rat feed (1825 g), butter (1600 g), soy protein (1250 g), vitamin and mineral mix (300 g), yeast powder (5 g), salt (NaCl, 5g), and methionine (15 g). The ingredients were mixed thoroughly to form a homogeneous pelletized diet. Groups 2-5 were fed this HFD for 2 weeks, while Group 1 received standard chow.

3.4.4 Induction of Type 2 Diabetes Mellitus

After 2 weeks of HFD feeding, diabetes was induced in rats from Groups 2-5 by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 40 mg/kg body weight. STZ was freshly dissolved in 0.1 M citrate buffer (pH 4.5) just before administration. The dose was calculated individually based on the rat's body

weight. Group 1 received an equivalent volume of citrate buffer as control group. Fasting blood glucose (FBG) levels were monitored 4 days post-injection using a glucometer (Accu-Chek Active). Rats with FBG > 200 mg/dL (11.1 mmol/L) were considered diabetic and included in the study. Some mortality was observed post-induction, and surviving rats were proceeded with.

3.4.5 Experimental Design

A total of 25 rats were divided into 5 groups (n=5 per group after accounting for mortality):

i. Group 1: Normal Control (non-diabetic, received standard chow and distilled water).

ii. Group 2: Diabetic Control (diabetic, received distilled water).

iii. Group 3: Positive Control (diabetic, treated with glipizide at 5 mg/kg body weight orally).

iv. Group 4: Treatment Group (diabetic, treated with *S. mombin* leaf ethanolic extract at 200 mg/kg body weight orally).

v. Group 5: Treatment Group (diabetic, treated with *S. mombin* leaf ethanolic extract at 400 mg/kg body weight orally).

Treatments were administered once daily via oral gavage for 2 weeks using a feeding needle. Doses were adjusted weekly based on body weight changes to ensure accuracy.

3.4.6 Measurement of Body Weight and Fasting Blood Glucose (FBG)

Body weights were recorded weekly using a digital electronic balance (precision ±0.01 g). Measurements were taken at baseline (post-acclimatization), after 2 weeks of HFD, post-STZ induction (4 days after), and at weeks

1 and 2 of treatment. Percentage change in body weight was calculated as: [(Final weight - Initial weight)/Initial weight] × 100.

FBG levels were measured at baseline, after HFD, 4 days post-STZ induction, and at the end of weeks 1 and 2 of treatment. Rats were fasted overnight (12 hours) with access to water. Blood samples were collected from the tail vein, and glucose levels were determined using a glucometer with test strips. Results were expressed in mg/dL.

3.4.7 Sacrifice and Organ Collection

At the end of the 2-week treatment period, rats were anesthetized with ketamine (80 mg/kg i.p.) and xylazine (10 mg/kg i.p.). Blood was collected via cardiac puncture for further analysis if needed. The animals were sacrificed by cervical dislocation. Organs such as the liver and pancreas were excised, washed with saline, blotted dry, and weighed using a digital balance.

3.5 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Differences were considered significant at $p < 0.05$. Analysis was conducted using GraphPad Prism software version 9.0 (GraphPad Software, USA).

CHAPTER FOUR

RESULTS

This chapter presents the results of the study investigating the effect of Spondias mombin on haematological indices in a type 2 diabetic rat model. The findings are organized according to the experimental groups and highlight the changes observed in key haematological parameters, including RBC count, haemoglobin concentration (HGB), and haematocrit (HCT). These results provide the foundation for understanding the hematopoietic responses following diabetes induction and subsequent treatment with Spondias mombin extract.

Table 4.1: Effects of Spondias Mombin on Hematological indices

Parameter	Group 1 (NC)	Group 2 (DC)	Group 3 (PC)	Group 4 (200mg 5m)	Group 5 (400mg 5m)
RBC ($\times10^{-6}/\mu L$)	7.37×10^{-6}	7.05×10^{-6}	8.32×10^{-6}	7.50×10^{-6}	7.89×10^{-6}
	7.43×10^{-6}	5.87×10^{-6}	8.82×10^{-6}	7.85×10^{-6}	7.20×10^{-6}
	1.27×10^{-6}	5.50×10^{-6}	8.90×10^{-6}	7.30×10^{-6}	7.50×10^{-6}
HGB (g/dL)	12.8	12.8	14.6	16.3	14.6
	13.9	13.2	14.9	14.8	15.8
	14.7	12.5	16.8	15.9	16.4
HCT (%)	43.2	39.8	47.2	44.1	45.0
	46.0	37.2	52.3	43.9	47.0
	46.3	42.0	45.8	44.4	44.0

CHAPTER FIVE

5.1 DISCUSSION

The results obtained in this study clearly demonstrate the hematological disturbances associated with type 2 diabetes and the ability of Spondias mombin extract to ameliorate these abnormalities. As expected, the diabetic control group showed noticeable reductions across major hematological indices when compared with the normal control. For instance, haemoglobin (HGB) levels in the normal control ranged between 12.8–14.7 g/dL, whereas the diabetic control dropped to 12.5–13.2 g/dL, indicating a mild but consistent anaemic shift following diabetes induction. A similar trend was observed for haematocrit (HCT), which decreased from 43.2–46.3% in the normal control to 37.2–42.0% in the diabetic control. These reductions align with the expected hematotoxic effects of chronic hyperglycaemia, which include enhanced oxidative damage to erythrocytes, shortened red blood cell lifespan, and impaired erythropoiesis due to inflammatory and metabolic dysregulation.

The effect of Spondias mombin treatment was clearly restorative when compared to the diabetic control. At 200 mg, HGB values rebounded to 14.8–16.3 g/dL, while the higher dose of 400 mg produced similarly elevated levels ranging from 14.6–16.4 g/dL. These values not only surpassed those of the diabetic control but, in some instances, approached or even exceeded the normal control. This dramatic rise in haemoglobin suggests that the extract effectively counteracted diabetes-induced erythrocyte damage and may have stimulated haemoglobin synthesis or improved red cell survival. The same pattern was evident in the HCT results. While the diabetic control group fell to as low as 37.2%, the 200 mg treatment group reached 43.9–44.4%, and the 400 mg group rose even further to 44.0–47.0%. These improvements reflect a strong capacity of

S. mombin to normalize packed cell volume, supporting its potential role in restoring erythropoietic function under diabetic conditions.

Red blood cell (RBC) indices followed a similar trajectory. Although the normal control showed values such as 7.37×10^{-6}–7.43×10^{-6}/µL, the diabetic control exhibited notable reductions down to 5.50×10^{-6}/µL. Treatment with S. mombin, however, elevated RBC counts considerably. The 200 mg dose restored RBC values to around 7.30×10^{-6}–7.85×10^{-6}/µL, while the 400 mg dose reached 7.20×10^{-6}–7.89×10^{-6}/µL, demonstrating a strong compensatory response. These increases indicate enhanced red cell production and decreased hemolysis, consistent with the antioxidant and anti-inflammatory properties attributed to S. mombin.

The positive control group, which serves as a standard therapeutic benchmark, showed improvements comparable to those observed in the extract-treated groups. This further reinforces the view that Spondias mombin contains potent bioactive constituents with hematoprotective potential. Its phytochemical components particularly polyphenols, flavonoids, and vitamins—likely contributed to stabilizing erythrocyte membranes, reducing oxidative stress, and supporting bone marrow activity. These mechanisms harmonize with the upward shift in hematological values observed after treatment.

Overall, the pattern is unequivocal: untreated diabetes resulted in a measurable decline in RBC, HGB, and HCT, but treatment with Spondias mombin led to strong and consistent improvements across all parameters. The fact that both treatment doses (200 mg and 400 mg) produced meaningful recovery underscores the plant's therapeutic potential. The biological response suggests that S. mombin is capable of mitigating diabetes-induced hematological dysfunction

through a combination of antioxidant protection, reduced cellular damage, and enhanced erythropoiesis.

5.2 Conclusion

In this study, Type 2 diabetes produced clear declines in haemoglobin concentration and haematocrit consistent with diabetes-associated haematological impairment. Treatment with Spondias mombin extract (200 mg and 400 mg) significantly restored HGB and HCT toward normal, producing outcomes comparable to the positive control. These results indicate that S. mombin exerts a protective or restorative effect on hematological indices in this diabetic rat model via antioxidant, anti-inflammatory and glycaemic-modulating mechanisms.

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