INVESTIGATING THE EFFECT OF AQUEOUS EXTRACT OF PICRALIMA NITIDA ON PARTIAL THROMBOPLASTIN TIME WITH KAOLIN (PTTK) IN STREPTOZOTOCIN-INDUCED DIABETIC MALE WISTAR RATS.

BY

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CERTIFICATION

This is to certify that this project work was carried out by **THELMA OSEIWE AKUE** with matriculation number BMS2101376, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City. The project was conducted in partial fulfillment of the requirements for the award of the Bachelor of Science (B.Sc.) Degree in Medical Biochemistry.

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DEDICATION

This project is dedicated to God Almighty, the source of all knowledge, wisdom, and strength. I am deeply grateful for His guidance, protection, and provision, which have enabled me to overcome challenges and successfully complete this research. I also dedicate this thesis to my family, lectures, and friends for their support and understanding throughout my studies in this great citadel of learning.

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I will forever be grateful to God Almighty for his mercy, grace , favour, blessings that have forever endure upon me.

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ABSTRACT

Diabetes mellitus is intrinsically linked to a pro-thrombotic state, significantly increasing the risk of cardiovascular events such as stroke and myocardial infarction. This study investigated the potential anticoagulant activity of the aqueous extract of Picralima nitida using the Partial Thromboplastin Time with Kaolin (PTTK) assay in streptozotocin (STZ)-induced diabetic rats. Rats were categorized into the Diabetic Control (G1, 21.50±0.50 s) and three treatment groups receiving 200 mg/kg b.w. (G5), 400 mg/kg b.w. (G3), and 500 mg/kg b.w. (G6) of the extract. PTTK values were measured and expressed as Mean ± Standard Error of the Mean (SEM). Data were analyzed using One-Way Analysis of Variance (ANOVA) followed by Duncan's Post Hoc Test, with significance accepted at $P<0.05$. Treatment with P. nitida resulted in a significant prolongation of PTTK compared to the diabetic control. The maximum anticoagulant effect was observed in the Mid-High dose (400 mg/kg b.w.) group (G3), which recorded the highest mean PTTK of 23.00±3.00 seconds. A non-linear dose-response was identified, as the highest dose (500 mg/kg b.w., G6) yielded 21.50±0.50 seconds, matching the diabetic control and demonstrating reduced efficacy compared to the mid-high dose. These findings confirm that the aqueous extract of Picralima nitida possesses significant anticoagulant potential by modulating the intrinsic coagulation pathway. The optimal therapeutic window was identified at 400 mg/kg b.w., supporting the extract's potential as a natural antithrombotic agent to mitigate cardiovascular complications in diabetes.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia due to defects in insulin secretion, insulin action, or both (American Diabetes Association, 2022). Globally, diabetes has reached epidemic proportions, affecting over 537 million adults in 2021, with projections suggesting a rise to 643 million by 2030 (International Diabetes Federation, 2021). In sub-Saharan Africa, including Nigeria, urbanization, dietary transitions, sedentary lifestyles, and limited access to healthcare have contributed to the rising prevalence of diabetes (Atun *et al.,* 2017). Chronic hyperglycemia in diabetes results in microvascular and macrovascular complications, including neuropathy, nephropathy, retinopathy, cardiovascular disease, and coagulation abnormalities (WHO, 2020). Diabetes is broadly classified into Type 1 diabetes, caused primarily by autoimmune destruction of pancreatic β-cells, and Type 2 diabetes, characterized by insulin resistance and progressive β-cell dysfunction (American Diabetes Association, 2022). Experimental induction of diabetes in animal models often uses streptozotocin (STZ), a nitrosourea compound with high affinity for pancreatic β-cells. STZ selectively enters β-cells via GLUT2 transporters, causing DNA alkylation, generation of reactive oxygen species, and eventual necrosis (Lenzen, 2008). One significant complication of diabetes is alteration of the coagulation system. Chronic hyperglycemia promotes platelet hyperactivity, endothelial dysfunction, and imbalance in coagulation factors, leading to a pro-thrombotic state (Rosenson *et al.,* 2022). Partial Thromboplastin Time with Kaolin (PTTK) is a laboratory assay used to evaluate the intrinsic and common coagulation pathways, providing insight into clotting factor activity and haemostatic function (Akinlade *et al.,* 2013). Studies in

diabetic patients in Nigeria have shown shortened PTTK, elevated fibrinogen levels, and increased D-dimer, indicating hypercoagulability and heightened risk for thrombotic events (Oyakhire *et al.,* 2023).

Herbal medicines have long been used in African ethnomedicine for managing diabetes and its complications. Picralima nitida, locally called "Osun" in Edo State, Nigeria, is a tropical plant distributed across West and Central Africa, including Ghana, Ivory Coast, and Cameroon (Iwu *et al.*, 2016). The plant contains bioactive compounds such as alkaloids (akuammine, akuammicine, picraline), flavonoids, saponins, and phenolics that have shown antidiabetic, antioxidant, and anti-inflammatory effects in experimental studies (Omoregie *et al.,* 2016). Its potential impact on coagulation parameters, specifically PTTK, is not too profound yet, particularly in STZ-induced diabetic models.

1.2 Scope of Study

This study focuses on evaluating the effect of aqueous extract of Picralima nitida on Partial Thromboplastin Time with Kaolin (PTTK) in streptozotocin-induced diabetic male Wistar rats. The research investigates the intrinsic and common coagulation pathways and how diabetes-induced hyperglycaemia affects haemostatic function, particularly PTTK. Experimental diabetes induction using STZ allows for a controlled evaluation of coagulation abnormalities and potential correction by the plant extract . The study Is limited to male Wistar albino rats, Only the aqueous extract of P. nitida fruit will be used, as it is the most commonly prepared form in traditional medicine and has been previously demonstrated to have antidiabetic, antioxidant, and potential coagulation-modulating properties.

1.3 Aim of the Study

The primary aim of this study is to investigate the effect of aqueous extract of Picralima nitida fruit on Partial Thromboplastin Time with Kaolin (PTTK) in streptozotocin-induced diabetic male Wistar rats. This research seeks to evaluate whether the plant extract can normalize alterations in PTTK caused by diabetes-induced hyperglycaemia, thereby offering potential therapeutic benefit in the management of diabetes-associated haemostatic abnormalities.

1.4 Objectives of the Study

The specific objectives of this study are:

1. To induce hyperglycaemia in male Wistar rats using streptozotocin.
2. To prepare and administer aqueous extract of Picralima nitida fruit to diabetic rats.
3. To evaluate the effect of Picralima nitida on PTTK in streptozotocin-induced diabetic rats.

1.5 Significance of study

This study is significant because it addresses a critical complication of diabetes mellitus haemostatic imbalance and hypercoagulability—which can predispose patients to cardiovascular and thrombotic events . Investigating the effect of Picralima nitida on PTTK provides insight into potential natural therapies for correcting coagulation abnormalities in diabetes. Traditional herbal remedies, including P. nitida, are widely used in Nigeria and other African countries, but scientific validation of their effects on haemostasis remains limited.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to defects in insulin secretion, insulin action, or both (American Diabetes Association, 2022). The global prevalence of diabetes is rising rapidly, with an estimated 537 million adults affected in 2021, projected to reach 643 million by 2030 (International Diabetes Federation, 2021). In sub-Saharan Africa, lifestyle changes, urbanization, and limited access to healthcare contribute significantly to the increasing burden of diabetes (Atun *et al.*, 2017). Type 1 diabetes (T1DM) is primarily caused by autoimmune destruction of pancreatic β-cells, resulting in absolute insulin deficiency, whereas Type 2 diabetes (T2DM) is characterized by insulin resistance and progressive β-cell dysfunction (American Diabetes Association, 2022). Regardless of type, chronic hyperglycemia leads to microvascular and macrovascular complications, including neuropathy, nephropathy, retinopathy, and cardiovascular disease (WHO, 2020).

The pathophysiology of diabetes involves multiple metabolic derangements. In T2DM, insulin resistance in peripheral tissues, including muscle, adipose, and liver, results in impaired glucose uptake, increased gluconeogenesis, and dyslipidemia (DeFronzo *et al.,* 2015). Hyperglycemia also induces oxidative stress, chronic inflammation, and endothelial dysfunction, which

contribute to coagulation abnormalities such as shortened Partial Thromboplastin Time with Kaolin (PTTK) and increased thrombotic risk (Rosenson *et al.,* 2022).

Experimental induction of diabetes in animal models frequently employs streptozotocin (STZ), a nitrosourea compound selectively toxic to pancreatic β-cells. STZ induces DNA alkylation and oxidative stress, leading to β-cell necrosis and sustained hyperglycemia (Lenzen, 2008). This model effectively mimics aspects of human diabetes, making it ideal for assessing the effects of therapeutic agents, including herbal extracts such as Picralima nitida (Iwu *et al.,* 2016).

2.1.1 Type 1 Diabetes Mellitus (T1DM)

Type 1 diabetes mellitus is an autoimmune disorder characterized by selective destruction of pancreatic β-cells, leading to absolute insulin deficiency (American Diabetes Association, 2022). The onset is usually acute and occurs predominantly in children and young adults. Autoantibodies against insulin, glutamic acid decarboxylase (GAD), and other β-cell antigens contribute to β-cell apoptosis (Atkinson *et al.,* 2014).

Chronic hyperglycemia in T1DM affects the coagulation system, predisposing patients to both hypercoagulable and bleeding tendencies depending on the vascular bed involved. Alterations in Partial Thromboplastin Time with Kaolin (PTTK) have been reported, primarily due to changes in coagulation factor levels, platelet activation, and endothelial dysfunction (Rosenson *et al.,* 2022). These changes are crucial because PTTK reflects the intrinsic and common pathways of coagulation, which are influenced by both insulin deficiency and hyperglycemia-induced oxidative stress (Hulst *et al.,* 2021).

2.1.2 Type 2 Diabetes Mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized primarily by insulin resistance and a progressive decline in pancreatic β-cell function, resulting in impaired glucose regulation and persistent hyperglycaemia (American Diabetes Association, 2022). In T2DM, peripheral tissues such as skeletal muscle, adipose tissue, and the liver fail to respond adequately to circulating insulin, leading to reduced glucose uptake and increased hepatic glucose output (DeFronzo, 2010). The pathophysiology of T2DM is multifactorial, involving genetic susceptibility, obesity, sedentary lifestyle, chronic inflammation, and lipotoxicity (Kahn *et al.*, 2014). Excess adipose tissue, particularly visceral fat, releases inflammatory cytokines such as TNF-α and IL-6, which further impair insulin signalling and promote β-cell dysfunction (Donath & Shoelson, 2011). Over time, declining β-cell mass leads to inadequate insulin secretion, worsening hyperglycaemia despite increased insulin resistance (Tabák *et al.,* 2012).

Studies have shown that patients with T2DM often exhibit shortened PTTK, elevated fibrinogen, and increased levels of von Willebrand factor, indicating a hypercoagulable state (Hulst *et al.,* 2021). This pro-thrombotic tendency is clinically significant, as it increases the risk of cardiovascular events such as myocardial infarction and stroke. Chronic hyperglycemia also impairs the production of anticoagulant factors (protein C, protein S), further influencing PTTK (Rosenson *et al.,* 2022).

2.1.3 How Diabetes Mellitus Affects Partial Thromboplastin Time (PTTK)

Chronic diabetes mellitus is strongly associated with multiple coagulation abnormalities that significantly alter Partial Thromboplastin Time (PTTK). Persistent hyperglycemia induces biochemical and structural modifications in plasma proteins and blood cells, promoting a pro-thrombotic (hypercoagulable) state (Alzahrani *et al* 2017). Diabetes-related oxidative stress,

endothelial dysfunction, glycation of coagulation factors, and heightened platelet activation collectively shift the coagulation balance toward accelerated intrinsic pathway activation, These diabetes-driven alterations affect both the accuracy and interpretation of APTT measurements, making diabetics more prone to thrombotic complications (Pawlak *et al.,* 2021).

2.2 Overview of Partial Thromboplastin Time – Kaolin (PTTK)

Partial Thromboplastin Time – Kaolin (PTTK) is a laboratory coagulation test used to assess the intrinsic and common pathways of the blood coagulation cascade. The term "PTTK" is derived from Partial Thromboplastin Time, with "Kaolin" referring to the activator used to initiate the intrinsic pathway in vitro (Ariëns, 2014). Kaolin serves as a surface that triggers the contact activation of factor XII, leading to a cascade of reactions resulting in fibrin clot formation.Partial Thromboplastin Time – Kaolin (PTTK) measures the time taken for plasma to clot after the addition of kaolin, phospholipids, and calcium (Lawrie *et al*., 2017). In healthy rats, PTTK/APTT values generally fall between 20 and 30 seconds, depending on the strain, reagent, and analyzer used. Studies have reported APTT values of 20.3 ± 1.4 seconds in Wistar rats (Oyedeji *et al*., 2016), 21.8 ± 2.1 seconds in Sprague–Dawley rats (Zhang *et al*., 2017), and reference intervals ranging from 18.2–27.9 seconds (Yamaoka *et al*., 2019).In healthy individuals, PTTK usually falls within the range of 25–35 seconds, though slight variations may occur depending on the laboratory reagents, equipment, and testing methodology (Adcock *et al*., 2019).

PTTK evaluates the function of coagulation factors XII, XI, IX, VIII, X, V, II, and fibrinogen, and it is commonly used to detect deficiencies, inhibitors, or the effect of anticoagulants such as heparin (Lawrie *et al.*, 2017). The importance of PTTK lies in its ability to monitor bleeding disorders, detect subclinical coagulation abnormalities, and evaluate thrombotic risk. In diabetic patients or animal models, abnormalities in PTTK indicate dysregulation of the coagulation

system, which may contribute to microvascular and macrovascular complications associated with chronic hyperglycemia (Ajjan *et al.,* 2019).

Diabetes mellitus, particularly hyperglycemia induced in STZ-treated rats, affects PTTK by altering intrinsic pathway coagulation factors, fibrinogen levels, and platelet function. Studies show that chronic hyperglycemia can either prolong PTTK due to factor depletion or shorten it as a result of platelet hyperactivity and increased thrombin generation (Adeyanju *et al.*, 2021). Oxidative stress, endothelial dysfunction, and glycation of coagulation proteins further influence PTTK, making it an essential marker for evaluating hemostatic complications of diabetes. PTTK is also used in research to assess the effect of pharmacological agents and natural plant extracts on coagulation. For instance, herbal remedies like Picralima nitida may exert anticoagulant or antithrombotic effects by modulating oxidative stress and normalizing intrinsic pathway factor activity, which can be monitored through PTTK measurements (Ojo *et al.*, 2021).

Overall, PTTK serves as a reliable indicator of intrinsic pathway integrity, hemostatic balance, and the impact of metabolic disorders such as diabetes on coagulation. Its measurement provides valuable insight into both clinical and experimental studies aimed at preventing or correcting coagulation abnormalities.

2.2.1 Endothelial function and its Role in Coagulation/PTTK

The vascular endothelium plays a critical role in maintaining hemostasis by regulating platelet adhesion, clotting factor activity, and fibrinolysis. In diabetes, chronic hyperglycemia damages endothelial cells, leading to dysfunction that directly affects Partial Thromboplastin Time with Kaolin (PTTK) (Tesfamariam, 2016)

PTTK assesses the intrinsic and common coagulation pathways, which involve several clotting factors. The intrinsic pathway includes Factor XII, XI, IX, and VIII, which are sequentially activated, with Factor XII initiating the cascade upon contact with kaolin or exposed endothelial collagen. Factor VIII, stabilized by von Willebrand factor (vWF), acts as a cofactor for Factor IX in activating Factor X (Rosenson *et al.,* 2022). The common pathway involves Factor X, V, II (prothrombin), and I (fibrinogen), contributing to thrombin generation and fibrin clot formation. Calcium ions (Factor IV) and phospholipids from platelets and PTTK reagents are essential cofactors for these reactions (Hulst *et al.,* 2021).

Endothelial dysfunction in diabetes leads to increased secretion of vWF, which promotes platelet adhesion and enhances clot formation. At the same time, there is reduced production of anticoagulant molecules such as thrombomodulin and tissue factor pathway inhibitor, tipping the balance toward hypercoagulability. Oxidative stress and glycation of endothelial proteins further impair anticoagulant functions and alter intrinsic pathway efficiency (Brownlee, 2018).

As a result, PTTK may be shortened in diabetic conditions, reflecting hyperactivation of the intrinsic pathway and a higher risk of thrombosis (Oyakhire *et al.*, 2023). Understanding endothelial contributions to clotting is critical when assessing the effects of potential therapeutic agents like Picralima nitida, which may exert protective effects on endothelial function and normalize PTTK in diabetic models.

2.2.2 The Endothelial Cell

The endothelium is a monolayer of specialized squamous epithelial-like cells that line the interior of all blood vessels, including arteries, veins, and capillaries. These cells form a semi-permeable barrier between circulating blood and the vascular wall, regulating the transport of nutrients, hormones, and metabolic waste (Yang *et al*., 2024).

Endothelial cells are generally flattened and polygonal, with a central ovoid nucleus. Their cytoplasm contains organelles such as mitochondria, Golgi apparatus, endoplasmic reticulum, and Weibel-Palade bodies, the latter storing von Willebrand factor (vWF) and P-selectin, which are critical in coagulation and inflammation (Zhao *et al*., 2024). The basal surface rests on a basement membrane, composed of collagen, laminin, and proteoglycans, anchoring the cells to the vascular wall (Machin *et al.*, 2023) . The apical (luminal) surface faces the bloodstream and is covered by the glycocalyx, a carbohydrate-rich layer that protects the endothelium, senses shear stress, and maintains antithrombotic properties (Foote *et al.*, 2022)Endothelial cells are interconnected by tight junctions, adherens junctions, and gap junctions, which maintain vascular integrity and selective permeability. Tight junctions restrict macromolecule passage, adherens junctions provide mechanical strength, and gap junctions allow intercellular communication, coordinating responses to mechanical or chemical signals (Gamez *et al.*, 2024). These structural features make endothelial cells highly dynamic, capable of modulating vascular tone, hemostasis, immune responses, and angiogenesis in response to physiological and pathological signals. In diabetes, chronic hyperglycemia and oxidative stress damage the endothelium, leading to dysfunction characterized by pro-thrombotic, pro-inflammatory, and vasoconstrictive phenotypes, which directly affect tests such as PTTK (Gultom & Rieben, 2024; Schäfer *et al.*, 2021).

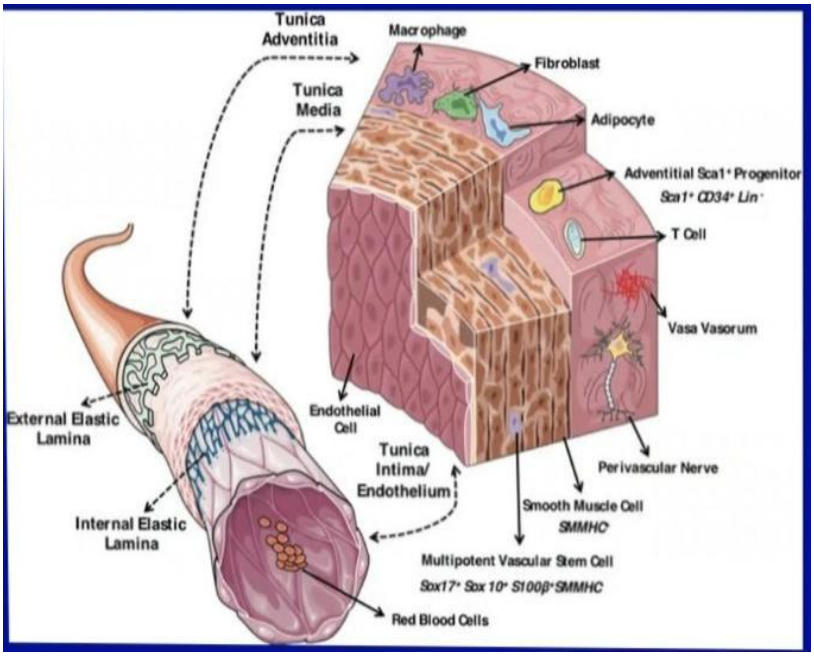


Fig 1: Vascular Endothelial cell

Source:(yang *et al.*, 2024)

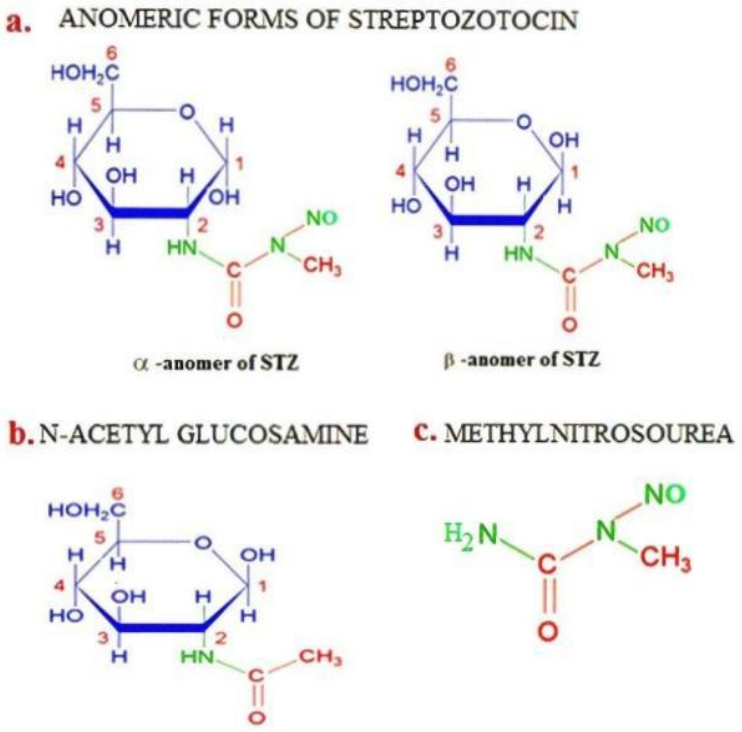
2.3 Streptozotocin (STZ)

Streptozotocin (STZ) is a naturally occurring nitrosourea compound first isolated in 1959 from the bacterium Streptomyces achromogenes (Lenzen, 2008). Initially, STZ was studied for its antibiotic properties, but its diabetogenic potential was soon discovered due to selective toxicity on pancreatic β-cells (Szkudelski, 2001). Since then, STZ has become one of the most widely used chemical agents for inducing experimental diabetes in laboratory animals.

Chemically, STZ is an N-methyl-N-nitrosourea derivative of glucosamine. It exists in two anomeric forms, α- and β-streptozotocin, which differ in the stereochemistry at the anomeric carbon of the glucose moiety (Eleazu *et al.,* 2013). The β-anomer is considered more biologically active and has a higher affinity for the GLUT2 transporter in pancreatic β-cells, making it

primarily responsible for STZ's diabetogenic effects (Lenzen, 2008). Both anomers contribute to the overall cytotoxicity but may differ slightly in pharmacokinetics and cellular uptake.

STZ enters β-cells through the GLUT2 glucose transporter, concentrating selectively in these insulin-producing cells. Its cytotoxicity is mainly due to DNA alkylation, which causes DNA strand breaks, activation of poly ADP-ribose polymerase (PARP), depletion of NAD+, and subsequent β-cell death (Szkudelski, 2001). Oxidative stress, nitric oxide production, and mitochondrial dysfunction further amplify the damage, leading to insulin deficiency and



hyperglycemia, mimicking Type 1 diabetes in humans (Eleazu *et al.*, 2013).

Fig 2: Chemical Structure of STZ (a. α and β Anomeric forms of STZ b.Structuralanalog of STZ – N-acetyl Glucosamine C. cytotoxic moiety of STZ- N-methyl-N-Nitrosourea)

Source: (lenzen 2008)

2.4 Mechanism of Action of Streptozotocin (STZ)

Streptozotocin (STZ; 2-deoxy-2-[3-methyl-3-nitrosourea]-D-glucopyranose) is a naturally occurring nitrosourea compound widely used to induce experimental diabetes in rodents (Lenzen, 2008). It exists as two anomers, α and β, which can be separated by chromatographic methods (Eleazu, *et al.*, 2013). STZ is a pale yellow to off-white crystalline powder with a molecular weight of 265 g/mol and a chemical formula of $C_8H_{15}N_3O_7$ (Lenzen, 2008). Structurally, it is a glucose analog substituted at C2 with an N-methyl-N-nitrosourea moiety, responsible for its cytotoxic effects on pancreatic β-cells (Eleazu *et al*., 2013).

STZ selectively enters pancreatic β-cells via the GLUT2 glucose transporter, which is highly expressed in rodent β-cells (Szkudelski, 2001). Once inside the cell, STZ causes DNA alkylation, particularly at guanine residues, producing DNA strand breaks (Sharma *et al.,*2015). DNA damage activates poly(ADP-ribose) polymerase (PARP), depleting cellular NAD^+ and ATP, ultimately leading to energy failure and necrotic cell death (Sharma *et al.*, 2015).

STZ also induces oxidative and nitrosative stress, generating reactive oxygen species (ROS) and reactive nitrogen species (RNS), which damage mitochondrial membranes and enzymes (Szkudelski, 2001). This mitochondrial dysfunction impairs ATP production, further compromising β-cell survival (Eleazu *et al.*, 2013). Nitric oxide production within β-cells additionally inhibits mitochondrial respiration and promotes apoptosis (Lenzen, 2008).

Recent studies show that STZ toxicity may also involve altered cellular signaling, including stress-activated kinases and downregulation of antioxidant defenses, highlighting a multi-

pathway cytotoxic mechanism beyond simple DNA alkylation (Rincon, Rocha, & Hernandez, 2017).

These combined effects result in β-cell death, insulin deficiency, and sustained hyperglycemia, making STZ a reliable model for studying diabetes and its vascular or coagulation complications, such as altered PTTK (Sharma *et al.*, 2015).

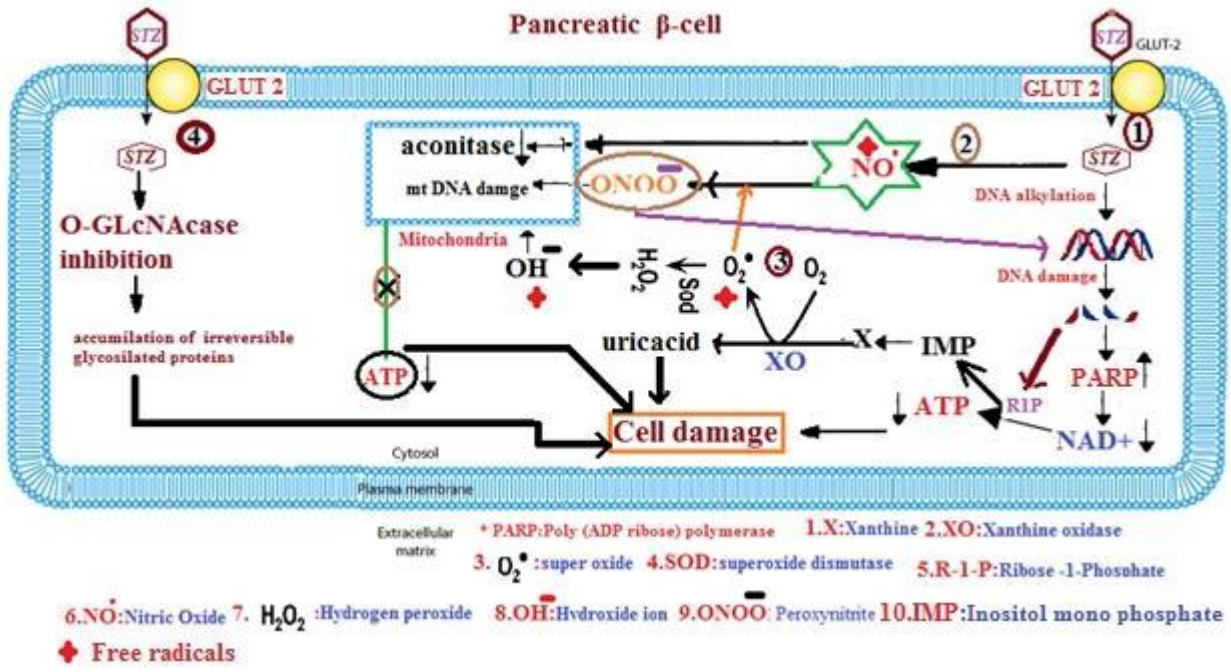


Fig 3: Schematic diagram representing the mechanism of action of STZ

Source: (Sharmal *et al.*,2015)

2.4.1 Advantages of Streptozotocin (STZ)

1. STZ selectively destroys pancreatic β-cells, producing sustained hyperglycemia that mimics human diabetes (Lenzen, 2008).

2. It allows rapid and predictable induction of diabetes in experimental animals, which reduces variability in outcomes (Comparative Study, 2024).

3. The dosage of STZ can be adjusted to model either Type 1 diabetes (high-dose) or Type 2 diabetes (low-dose combined with high-fat diet), providing flexibility in research design (Experimental Models, 2025).

4. STZ-induced diabetes models are reproducible across commonly used rodent strains, facilitating multi-laboratory studies and cost-effective experimentation (Improving the Reliability, 2018).

5. STZ models not only replicate hyperglycemia but also secondary complications such as oxidative stress, endothelial dysfunction, and coagulation abnormalities, including alterations in PTTK (Zaheer *et al.*, 2024).

2.4.2 Disadvantages of Streptozotocin (STZ)

1. STZ is highly cytotoxic, and its administration can lead to off-target organ damage, particularly to the liver and kidneys, complicating interpretation of experimental results (Lenzen, 2008).

2. The variability in response to STZ among different rodent strains can result in inconsistent hyperglycemia, requiring careful dose optimization for each experimental model (Comparative Study, 2024).

3. High doses of STZ may cause severe β-cell destruction and extreme hyperglycemia, which can increase animal mortality and ethical concerns in research (Experimental Models, 2025).

4. STZ-induced diabetes primarily models Type 1 diabetes, and mimicking Type 2 diabetes often requires combination with dietary interventions, adding complexity to experimental design (Liu et al., 2024).

5. The induction of diabetes with STZ can trigger inflammation, oxidative stress, and nonspecific cytotoxic effects beyond the pancreas, which may confound the evaluation of therapeutic agents (Zaheer *et al*., 2024).

2.5 Medicinal Plants in Diabetes Management

Medicinal plants have long been utilized in the management of diabetes due to their rich content of bioactive compounds capable of modulating glucose metabolism, improving insulin sensitivity, and protecting pancreatic tissue from damage (Atanasov *et al.*, 2015). Key phytochemicals, including alkaloids, flavonoids, saponins, tannins, and polyphenols, exert antidiabetic effects through diverse mechanisms (Patel & Mishra, 2011).

Flavonoids, for example, enhance glucose uptake in peripheral tissues such as skeletal muscle and adipose tissue by modulating GLUT4 transporter activity, while certain alkaloids may stimulate insulin secretion from surviving β-cells in the pancreas (Patel & Mishra, 2011). Saponins and polyphenols also contribute by inhibiting carbohydrate-hydrolyzing enzymes, reducing postprandial hyperglycemia (Ojo *et al.*, 2015).

Oxidative stress is a major contributor to pancreatic β-cell dysfunction in diabetes, as excessive free radical formation within islets leads to apoptosis, impaired insulin secretion, and secondary α-cell dysregulation (Maritim *et al.*, 2003). Plant-derived antioxidants scavenge reactive oxygen

species (ROS) and enhance endogenous antioxidant defenses, including superoxide dismutase (SOD) and catalase, thereby preserving islet morphology and function (Atanasov *et al.*, 2015).

The ethnopharmacological evidence highlights the potential of plants such as Picralima nitida as natural antidiabetic agents. Studies indicate that extracts of P. nitida seeds and pulp possess glucose-lowering, antioxidant, and β-cell protective effects, making them promising candidates for further research in diabetes management (Iwu, Duncan, & Okunji, 2016; Omoregie, Esezobor, & Akhigbe, 2016).

2.6 Overview of Picralimanitida

Picralima nitida (Stapf) is a tropical evergreen tree belonging to the family Apocynaceae, order Gentianales, and genus Picralima (N'dri *et al*., 2015). Locally, it is known as "Osun" in parts of Nigeria, while in Ghana and other West African countries it is referred to as "Akuamma". The plant grows mainly in humid lowland rainforests and secondary forest areas across West and Central Africa, including Nigeria, Ghana, Ivory Coast, Cameroon, and Gabon, It can reach heights of 15 to 30 metres when mature, depending on soil fertility and climatic conditions (Haruna & Odunsi, 2022). The tree produces large, oval fruits that are green when unripe and become yellowish when mature. Inside the fruit is a mucilaginous pulp that encloses several flat, brown seeds. Both the pulp and seeds are widely used in traditional medicine for managing ailments such as malaria, fever, and diabetes (Mian Jean-Claude *et al.*, 2025). Phytochemical analyses of the seeds and pulp reveal the presence of indole alkaloids including akuammine, akuammidine, and pseudoakuammigine. These compounds exhibit antioxidant, anti-inflammatory, and glucose-lowering effects (Obho *et al*., 2021). Additionally, flavonoids, tannins, and saponins have been identified, contributing further to the plant's medicinal potential (Olumese *et al.*, 2022). The seeds, in particular, are considered pharmacologically potent due to

their high concentration of active alkaloids, while the pulp contains similar bioactive compounds that may act synergistically with the seeds (Haruna & Odunsi, 2022)

Fig 4: A Ripened picralimanitilda fruit split Open showing the seeds and pulp.

Source: (Mian Jean-Claude *et al.*, 2025)

fig 5: An Unripe picralimanitilda fruit

Source: (N'dri *et al.*, 2015)

2.7 Phytochemical Constituents

Picralima nitida (family: Apocynaceae, class: Magnoliopsida) contains several bioactive compounds, particularly indole alkaloids, which include: akuammine, akuammidine, pseudoakuammigine, akuammicine, picraline, and akuammigine (Iwu, Eze, & Olaleye, 2020). The seeds and pulp also contain flavonoids, tannins, saponins, glycosides, phenolic compounds, and alkaloid glycosides, all of which contribute to antioxidant, anti-inflammatory, and antidiabetic activities (Omoregie *et al.,* 2016). These indole alkaloids are reported to influence

glucose regulation, insulin secretion, and protection of pancreatic β-cells from oxidative damage (Iwu, Eze, & Olaleye, 2020). Flavonoids, tannins, and saponins may stabilize endothelial function, reduce oxidative stress, and decrease hypercoagulability, potentially affecting PTTK by normalizing intrinsic pathway coagulation (Omoregie *et al.,* 2016).The combination of alkaloids, flavonoids, tannins, saponins, and glycosides in P. nitida suggests its therapeutic potential in correcting diabetes-induced coagulation abnormalities, making it a candidate for studies on PTTK modulation in experimental diabetic models.

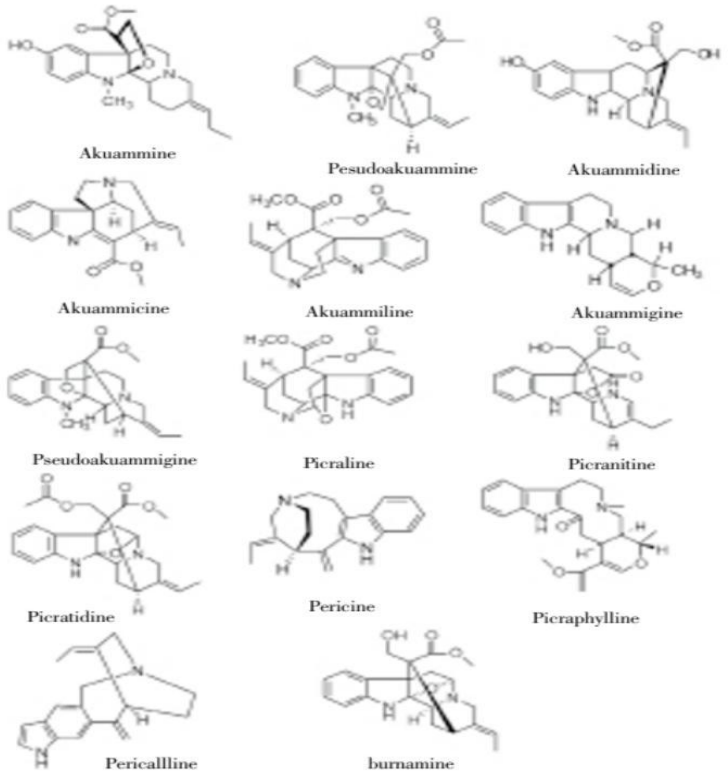


Fig 6: chemical structures of alkaloids associated from Picralimanitilda

Source: (Omoregie *et al.,* 2016)

2.9 Indole Alkaloids and Their Potential Effect on PTTK

The seeds and pulp of Picralima nitida are rich in indole alkaloids such as akuammine, akuammidine, pseudoakuammigine, akuammicine, picraline, and akuammigine (Iwu *et al* ., 2020). These compounds have been reported to possess antioxidant, anti-inflammatory, and hypoglycemic properties, which can modulate endothelial function and coagulation pathways in diabetic conditions (Omoregie *et al.,* 2016).

Chronic hyperglycemia in diabetes is associated with oxidative stress and endothelial dysfunction, which increases the secretion of pro-thrombotic factors such as von Willebrand factor (vWF) and reduces anticoagulant activity, leading to shortened Partial Thromboplastin Time with Kaolin (PTTK) (Rosenson *et al* ., 2022). Indole alkaloids from P. nitida may counteract these effects by scavenging reactive oxygen species, stabilizing endothelial cells, and protecting intrinsic pathway coagulation factors. For instance, akuammine and akuammicine have demonstrated the ability to enhance nitric oxide bioavailability, improving endothelial-dependent anticoagulant activity (Iwu, *et al.,* 2020). Additionally, the anti-inflammatory action of these alkaloids may suppress excessive platelet activation and fibrin formation, which can otherwise shorten PTTK in diabetic models (Omoregie *et al*., 2016).

Thus, administration of aqueous extracts of P. nitida in streptozotocin-induced diabetic rats may normalize PTTK by restoring balance in the intrinsic coagulation pathway, protecting against hypercoagulable states associated with diabetes. This provides a scientific basis for the ethnopharmacological use of P. nitida in managing coagulation abnormalities.

2.10 Saponins and Flavonoids: Effects on PTTK

Saponins, naturally occurring glycosides found in plants including Picralima nitida, have been shown to modulate blood coagulation. They exhibit antiplatelet and anticoagulant activity, which

can influence the intrinsic coagulation pathway assessed by Partial Thromboplastin Time with Kaolin (PTTK) (Olas, Urbańska & Bryś, 2020). Experimental studies in rats demonstrated that total saponins from plant roots delayed intrinsic coagulation, measured by activated partial thromboplastin time (APTT) and thrombin time (TT), and inhibited thrombin (FIIa) activity, highlighting their potential to prolong PTTK in hypercoagulable states (Anticoagulant and Antithrombotic Effect and Underlying Mechanism of Total Saponins from the Roots of Polygala fallax Hemsl, 2021).

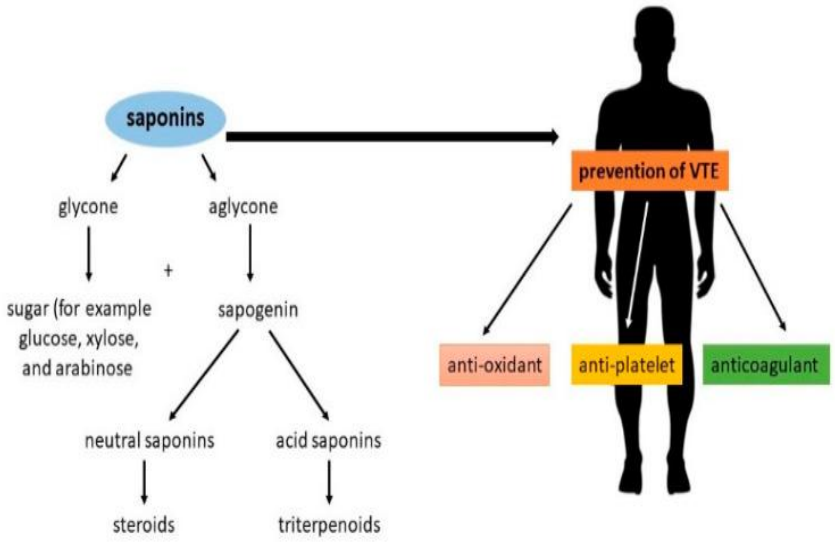


Fig 7: the chemical composition of saponins and their potential particularly in the prevention of venous thromboembolism (VTE).

Source: (Olas *et al.*, 2020)

Flavonoids, particularly sulphated flavonoids, have also been reported to influence intrinsic coagulation pathways. In vitro studies indicate that flavonoids significantly prolong APTT, suggesting inhibition of clotting factors within the intrinsic pathway and potential normalization

of PTTK in conditions like diabetes (Anticoagulant Effect and Action Mechanism of Sulphated Flavonoids From Flaveria bidentis, 2002). Systematic reviews on herbal remedies further support that flavonoids and polyphenols can exert anticoagulant and antiplatelet effects, contributing to hemostatic balance (Cordier & Steenkamp, 2012).

Together, saponins and flavonoids present in Picralima nitida may synergistically modulate coagulation, potentially correcting PTTK abnormalities in STZ-induced diabetic models by reducing hypercoagulability and protecting endothelial function.

2.11. Rationale of study

Diabetes mellitus can cause blood clotting problems by altering the intrinsic coagulation pathway, which can be measured using Partial Thromboplastin Time with Kaolin (PTTK). Shortened or prolonged PTTK in diabetes increases the risk of thrombosis and cardiovascular complications (Rosenson, Hegele, & Toth, 2022). Picralima nitida, locally called Osun, is traditionally used in Nigeria and West Africa for managing diabetes due to its bioactive alkaloids, including akuammine, akuammidine, and pseudoakuammigine (Iwu, Eze, & Olaleye, 2020; Omoregie, Igbinosa, & Aighewi, 2016). However, This study aims to investigate the effect of aqueous extract of Picralima nitida on PTTK levels in streptozotocin-induced diabetic rats, providing scientific evidence for its potential to correct diabetes-related coagulation abnormalities

CHAPTER THREE

MATERIALS AND METHODS

3.1 Equipment and Apparatus

1) Cotton wool
2) Chloroform
3) Dissecting set
4) Methylated spirit
5) Distilled water
6) Plain, EDTA, and lithium heparin sample containers
7) Syringes (5 mL and 10 mL)
8) Insulin syringe (1 mL)
9) 16–18 gauge stainless-steel gavage needle
10) Nose mask
11) Lancets
12) Hand gloves

Machines and Instruments:

1) Glucometer (NEWSPRING Glucose Meter, China; Model KF-B12)
2) Digital weighing scale (NEWSPRING Digital Scale, China; Model NS-790)
3) Storage system (HAIER THERMOCOOL, China; Model HRF-3500X)

4) Grinder (Dry Herb Industrial Grinder, Model SYB-18B)
5) Freeze dryer (BIOBASE Freeze Dryer, China; Model BK-FD10S)
6) Oven (WINCOM Thermostat Oven, Model OV-A25SF)
7) pH meter (PHS-25, China; Model PHS-25)

3.2 Animal Experimental Study

This experimental study aimed to evaluate the effects of Picralima nitida fruit aqueous extract on glucagon levels in male Wistar rats with STZ-induced diabetes. A total of Twenty-four (24) healthy male Wistar rats weighing between 107.6 g and 169.3 g were procured from the Department of Anatomy, University of Benin, Nigeria. They were housed in well-ventilated plastic cages with soft wood shavings as bedding and maintained under standard laboratory conditions, including a temperature of 22 ± 2°C, 50–60% relative humidity, and a 12-hour light/dark cycle. The rats were acclimated for four (4) weeks before the experiment.

During the acclimation period, rats were fed standard commercial feed (Chicken Grower Feed, Top Feeds Ltd, Nigeria; 16% crude protein, 8% crude fibre, 5% crude fat, 1% calcium, 0.4% phosphorus) and given water and libitum. Ethical approval was obtained from the Departmental Animal Ethics Committee, and all procedures complied with international standards for laboratory animal care.

Rats were grouped based on body weight for identification purposes, using coloured markers (Genital Violet) applied on different body parts (hand, leg, back, head, and tail). Animals were observed daily for behavioural changes, signs of distress, or health alterations. Weekly body weights and fasting blood glucose were recorded. At the end of the experimental period of 10 days, rats were humanely sacrificed using chloroform anaesthesia, and blood samples were

collected via cardiac puncture into sodium citrate containers for Partial thromboplastin time (PTTK)analysis.

3.3 Collection and Identification of Plant Material

Fresh fruits of Picralima nitida (approximately 300, weighing 0.2–0.3 kg, including unripe and ripe fruits) were procured from Oliha Market, Siloko Road, Benin City, Edo State, Nigeria. The plant was authenticated by a botanist at the Department of Plant Biology and Biotechnology, University of Benin, and a voucher specimen (UBH-P424) was deposited.

3.3.1 Preparation of Picralima nitida Extract

1) The fruits were sorted to remove dirt, stones, insects, and rotten pieces. Only fresh, healthy, and mature fruits were used.
2) Fruits were washed thoroughly with clean water. The rind was peeled off, and seeds and pulp were cut into smaller pieces and divided into six batches for drying.
3) The fruits were air-dried under shade for 7–10 days to prevent damage to bioactive compounds.
4) Oven-drying was then done at 45°C for four weeks to remove all moisture. The dried material weighed 2,202.19 g.
5) The dried fruits were ground into fine powder using a grinder.
6) The powder was soaked in distilled water (1:10 w/v ratio; 100 g powder in 1000 mL water) for 72 hours with occasional stirring.
7) The mixture was filtered using a muslin cloth. The filtrate was freeze-dried using a freeze dryer to obtain a solid extract.

8) The dried extract weighed 1,271.98 g (yield of 57.78%) and was stored in airtight containers at 4°C.

Phytochemical Analysis: The extract contained total phenols (39.24 mg GAE/g) and flavonoids (~24.39 mg QE/g), which are known for their antioxidant and therapeutic effects (Ilenowa et al., 2024).

3.4 Experimental Design

A total of Twenty-four (24) healthy male Wistar rats, weighing between 107.6 g and 169.3 g, were used for this study. The animals were sourced from the Department of Anatomy, University of Benin, Nigeria. They were housed under standard laboratory conditions in the same department, kept in clean, well-aerated plastic cages lined with soft wood shavings (sawdust) as bedding. Before the commencement of the experiment, the rats underwent a four (4)-week acclimatization period in a controlled environment maintained at 22 ± 2°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. Throughout the acclimatization phase, the animals were given water ad libitum and fed Chicken Grower Feed (Top Feeds Ltd., Nigeria), which contains approximately 16% crude protein, 8% crude fibre, 5% crude fat, 1% calcium, and 0.4% phosphorus. Ethical approval for the work was obtained from the Departmental Animal Ethics Committee, and all procedures adhered to internationally accepted guidelines for the humane handling and care of laboratory animals.

3.4.1 Experimental Grouping

The Twenty rats (24) rats were initially assigned to five (5) groups, each containing five (5) rats, except Group 0, which had four (4) rats. The groups were arranged as follows:

Group 0 (Normal/Negative Control): Received only normal feed and water.

Group 1 (Diabetic/Positive Control): Administered Streptozotocin (STZ) only, with no treatment.

Group 3: Diabetic rats treated with *picralimanitilda* extract (400mg/kg).

Group 5: Diabetic rats treated with *Picralimanitida* extract (200 mg/kg).

Group 6: Diabetic rats treated with *Picralimanitida* extract (500 mg/kg).

Grouping was done based on the mean body weight of the animals, which ranged from 107.69 g to 183.59 g. For easy identification, each rat was marked individually using coloured genital violet applied to specific body regions such as the hand, leg, back, head, or tail, while some remained unmarked. Each marking location corresponded to a unique identification number.

After diabetes induction was confirmed, the animals were regrouped into the same five categories, but the number of rats per group was reduced to two (2) rats each, except for the positive control group, which retained four (4) rats.

3.5 Administration of Picralima nitida

The aqueous extract of Picralima nitida fruit (pulp and seeds), prepared as previously described, was administered to the experimental groups (G3 –G6). Group 0 (G0) served as the normal control and received only water and feed, along with citrate buffer in volumes similar to those given to rats of comparable body weight in the treatment groups. This provided a clear baseline for evaluating the effects of the extract. While group 1 (G1) was used as the positive control because diabetes was indused using STZ without any treatment.

Rats were first divided into five (5) groups of 5 and 4 (5, 4) animals each based on their body weight. The extract was administered using the oral gavage method, a standard technique for

delivering substances directly into the stomach (Turner *et al.*, 2011; Diehl *et al.*, 2001). After diabetes was confirmed, diabetic rats were separated from non-diabetic ones and randomly reassigned into (3) treatment groups consisting of two (2) rats each, while the positive control group contained four (4) rats.

Diabetic rats in Groups G2–G3 received the aqueous extract orally at doses of 200 mg/kg, and 500 mg/kg, respectively, in addition to normal feed and water. Over the entire treatment duration, no signs of toxicity or mortality were observed.

3.5.1 Dosage Calculation of Picralima nitida

The doses of the Picralima nitida aqueous extract were prepared according to the individual body weights of the rats. Each dose was determined using the standard calculation method.

For a standard dose of 200 mg/kg, this means:

200 mg of extract is needed for every 1 kg body weight.

For a rat weighing 136.6 g, the required dose was calculated as:

$$\frac{200 \text{ mg} \times 136.6 \text{ g}}{1000 \text{ g}}$$

$$= 27.32 \text{ mg}$$

Thus, the exact volume of extract solution to be administered was based on a stock concentration of 1 g / 10 mL, prepared as follows:

1 g = 10 mL

1000 mg = 10 mL

Volume = 27.32 mg × 10mL

1000 mg

Volume = 0.27mL

A stock solution was obtained by dissolving 1 g (1000 mg) of the extract in 10 mL of distilled water.

This same procedure was applied to all diabetic rats in Groups G2–G3, ensuring each rat received a dose matched to its body weight. The extract was administered orally once daily for 10 days using a calibrated syringe fitted with a stainless-steel oral gavage needle.

3.6 Induction of Diabetes Using Streptozotocin (STZ); Administration of Streptozotocin (STZ)

(i) Preparation of Streptozotocin Solution

Streptozotocin (STZ) was employed to induce diabetes mellitus in the experimental rats. A fresh STZ solution was prepared by dissolving 0.30 g of streptozotocin in 10 mL of 0.1 M citrate buffer (pH 4.5). The mixture was gently agitated until fully dissolved. All preparation procedures were carried out under reduced light exposure to minimize degradation of the compound. The prepared solution was stored in a refrigerator and used immediately for induction.

(ii) Dosage Calculation for Streptozotocin

The induction dose used was 60 mg/kg body weight. The required dose for each rat was determined individually.

For a standard dose of 60 mg/kg:

60 mg of STZ is required for 1 kg body weight.

For a rat weighing 154 g, the dose was calculated as:

60 mg ×154 g/1000 g

= 9.24 mg

To obtain the volume corresponding to this dose:

Given the stock concentration:

0.3 g (300 mg) of STZ in 10 mL

300mg = 10mL

Volume = 9.24mg×10mL/300mg

= 0.31mL

Each rat's individual dose and corresponding volume were calculated using this same formula to ensure proper dosing accuracy. The measured volume of STZ was administered intraperitoneally.

(iii) Induction Procedure

Diabetes was induced in rats that had been fasted overnight using a single intraperitoneal injection of Streptozotocin at 60 mg/kg, freshly dissolved in 0.1 M citratlede buffer (pH 4.5). Following induction, rats were provided with 0.4% glucose solution for 24 hours to prevent immediate post-injection hypoglycemia.

Blood glucose was assessed 72 hours after administration using a glucometer. Rats with fasting glucose values above 200 mg/dL were classified as diabetic.

However, after six (6) days—while waiting for additional rats to become diabetic—many of the previously induced rats showed a drop in blood glucose levels. This reduction was likely due to poor feeding caused by stress, illness, or anorexia, which to secondary hypoglycemia. Additionally, four (4) rats died following the first induction, likely due to hypoglycemic shock. The surviving rats were re-induced using the same procedure to obtain a sufficient number of diabetic animals for the study.

pH and Weight Calculations

The pH was calculated using the Henderson–Hasselbalch equation as follows:

$pH = pKa + \log [B]/[A]$

$pH = 4.50$; $pKa3 = 6.50$

$4.5 = 6.5 + \log [B]/[A]$

$10^{-2} = [B]/[A]$

$10^{-2} [A] = [B]$

$10^{-2} [A] = [B]$

$[B] = 0.01[A]$

Let A + B = Molar concentration M) --------- (1)

$[B] = 0.01[A]$ ---------- (11)

$[A] + 0.01[A] = M$

Molar concentration of citrate buffer to be prepared is 0.1M

[A]+ 0.01[A) = 0.1M

1.01[A] = 0.1

[A]= 0.1/1.01

[A] = 0.099M (mol/L)

By substitution,

[B] = 0.01[A] = 0.01× 0.099M

[B] = 0.00099M ~ 0.001 M(mol/L)

But,

Molar Concentration (Molarity) = Mass Concentration/ Molar mass

Mass Concentration = Molar Concentration × Molar mass

Mass concentration of A = 0.099M × 210.14g

= 20.80g/L

Mass concentration of B = 0.001M × 294.10g

= 0.29 g/L

To prepare the buffer:

20.80 g of citric acid monohydrate and 0.29 g of trisodium citrate dihydrate were dissolved in approximately 800 mL of distilled water.

The pH was then checked and adjusted to 4.50.

The final volume was made up to 1 L (1000 mL).

The 0.1 M citrate buffer (pH 4.5) was thoroughly mixed, kept free from light and moisture, and stored in a refrigerator to prevent degradation.

iv) Re-induction of Diabetes Using Streptozotocin (STZ)

Fresh streptozotocin was prepared by dissolving 0.20 g of the compound in 0.05 M citrate buffer (pH 4.5). The solution was mixed thoroughly until complete dissolution of the streptozotocin was achieved. Preparation was performed under low-light conditions to prevent degradation, and the solution was stored in a refrigerator until use.

v) Dosage Calculation for Streptozotocin

The dose of Streptozotocin used for re-induction was 40 mg/kg body weight. The calculation was performed as follows:

For a rat weighing 110 g, the exact dose is:

$40mg \times 110g/1000\ g$

$= 4.40mg$

The volume of STZ solution required, with a concentration of 0.2 g (200 mg) in 10 mL, was calculated as:

$200mg = 10mL$

$Volume = 4.40mg \times 10mL/200mg$

$= 0.25mL$

Each rat's dose and corresponding volume were individually calculated using the same formula to ensure accuracy. The STZ solution was then administered intraperitoneally to each rat.

Diabetes was induced in overnight-fasted rats with a single injection of 40 mg/kg body weight, freshly prepared in 0.05 M citrate buffer (pH 4.5). After induction, the rats were provided with 0.4% glucose solution for 24 hours to prevent initial hypoglycemia. Fasting blood glucose levels were measured 72 hours post-induction, and rats with glucose levels above 110 mg/dL were classified as diabetic.

vi) Preparation of Citrate Buffer

The 0.05 M citrate buffer (pH 4.5, 100 mL) was prepared by dissolving 1.04 g citric acid monohydrate and 0.0147 g trisodium citrate dihydrate in approximately 80 mL of distilled water. The pH was checked and adjusted to 4.50, and the final volume was made up to 100 mL. The buffer was mixed thoroughly and stored under refrigeration until use.

pH and Weight Calculations

The pH was calculated using the Henderson–Hasselbalch equation as follows:

$pH = pKa + \log [B]/[A]$

$pH = 4.50$; $pKa3 = 6.50$

$4.5 = 6.5 + \log [B]/[A]$

$10^{-2} = [B]/[A]$

$10^{-2} [A] = [B]$

$10^{-2} [A] = [B]$

$[B] = 0.01[A]$

Let A + B = Molar concentration M) --------- (1)

[B] = 0.01[A] ---------- (11)

[A] + 0.01[A] = M

Molar concentration of citrate buffer to be prepared is 0.05M

[A]+ 0.01[A) = 0.05M

1.01[A] = 0.05

[A]= 0.05/1.01

[A] = 0.0495M (mol/L)

By substitution,

[B] = 0.01[A] = 0.01× 0.0495M

[B] = 0.000495M ~ 0.0005 M(mol/L)

But,

Molar Concentration (Molarity) = Mass Concentration/ Molar mass

Mass Concentration = Molar Concentration × Molar mass

Mass concentration of A = 0.0495M × 210.14g

= 10.40g/L

Mass concentration of B = 0.0005M × 294.10g

= 0.147 g/L

Hence, for 0.1L (100mL) of citrate buffer

A = 10.40 g/L × 0.1L = 1.04g

B = 0.147 g/L × 0.1L = 0.0147g

Therefore, 1.04 g of citric acid monohydrate (A) and 0.0147 g of trisodium citrate dihydrate (B) were dissolved in approximately 80 mL of distilled water. The pH was verified and adjusted to 4.50, and the final volume was brought up to 100 mL. The buffer was mixed thoroughly, protected from light and moisture, and stored in a refrigerator to prevent degradation.

viii) Confirmation of Diabetes

Seventy-two (72) hours following the Streptozotocin (STZ) injection, fasting blood glucose levels were measured using a glucometer. Rats with fasting blood glucose exceeding 110 mg/dL were classified as diabetic and subsequently selected for inclusion in further experimental procedures.

3.7 Weight and Blood Glucose Evaluation

The rats were administered their respective extract doses once daily for a total period of seven (10) days. During this treatment period, both body weight and fasting blood glucose levels were assessed at the start of the study and again at the end. Fasting glucose values were measured following an overnight fast using a Newspring Glucose Meter (Model KF-B12, China). Blood samples were collected from the tail tip with a sterile lancet, and the corresponding glucose readings were recorded. Body weights were determined using a high-precision NEWSRING Digital Scale (Model NS-790, China).

NB: The results provided below include each rat's initial and final body weights, the corresponding changes in weight, as well as the initial and final fasting glucose levels and the differences between these values.

Table 1: Weight and Blood Glucose Evaluation table

Group	Label	Model ID	Baseline weight (g)	Baseline FBG(mg/dl)	Weight (g) before induction	FBG(mg/dl) before induction	FBG(mg/dl) 3 days after induction
G0	Control	C1	169.3	81	99.5	83	70
		C2	163.5	79	176.0	81	67
		C3	155.0	68	189.4	79	72
		C4	167.7	77	192.0	90	59
G1	Diabetic control	S1	143.2	52	169.21	76	365
		S2	145.6	68	160.8	83	127
		S3	144.0	56	182.1	86	86
		S4	148.2	88	178.	98	270
		S5	144.9	70	154.7	95	61
G3	P.nitida (400mg)	A1	117.0	72	123.9	65	365
		A2	117.4	76	138.5	74	127
		A3	120.3	59	154.0	77	86
		A4	107.6	86	134.9	90	272
		A5	117.3	81	127.8	79	61
G5	P.nitida (200mg)	D1	138.7	72	141.6	74	50
		D2	140.5	70	152.6	104	135
		D3	140.7	58	156.2	72	43
		D4	141.8	85	161.0	77	279
		D5	142.2	72	161.1	79	130

G6	P.nitida	H1	151.8	77	175.6	99	194
	(500mg)	H2	154.3	72	166.0	97	250
		H3	152.1	65	166.2	76	263
		H4	154.5	50	182.4	67	90
		H5	158.3	85	149.4	94	238

3.8 Blood Sample Collection for PTTK Analysis

Throughout the study period, strict hygiene was maintained in the animal facility by routinely cleaning cages and replacing sawdust bedding. The rats were observed daily for behavioural changes, signs of stress, and overall health status, with continuous access to feed and water.

On the eleventh (11th) day, all animals were fasted overnight, re-weighed, and subsequently sacrificed. Anesthesia was induced using chloroform inhalation to minimize pain and discomfort before euthanasia in a sealed chloroform chamber. After euthanasia, a midline incision was made to expose the thoracic cavity, and blood samples were collected directly from the heart (cardiac puncture) using sterile syringes.

Collected blood was immediately transferred into sodium citrate anticoagulant tubes (3.2% or 3.8%), the standard requirement for coagulation assays such as PTTK. The tubes were gently inverted to ensure proper mixing with the anticoagulant. Samples were centrifuged at 3000 rpm for 10 minutes to obtain platelet-poor plasma. The clear plasma layer was transferred into labeled Eppendorf tubes.

Plasma samples were stored at –20°C until coagulation testing. All PTTK measurements were performed using standard coagulation analyzer protocols validated for rat plasma.

3.8.1 PTTK Determination

PTTK was measured using a standard automated or semi-automated coagulation procedure designed to evaluate the intrinsic pathway of coagulation. The method involves mixing rat plasma with kaolin (a surface activator), phospholipid reagent, and calcium chloride to trigger clot formation. The time required for clot formation is recorded as the PTTK value (in seconds).

The assay principle is based on activation of factor XII and other intrinsic pathway factors by kaolin. When calcium is added, clotting is initiated, and the analyzer detects the clot endpoint either optically or mechanically. The measured clotting time reflects the functional status of intrinsic and common coagulation pathways.

STANDARD OPERATING PROCEDURE: ACTIVATED PARTIAL THROMBOPLASTIN TIME

1) Collect fresh venous blood into 3.8% sodium citrate solution in the ratio at nine part of blood to one part of sodium citrate.
2) Centrifuge the blood specimen at 2,800 rpm for 20minutes.
3) Separate the plasma and analyze as soon as possible (sample should not stand at 37 C∘
4) C for more than 5 minutes). Freeze plasma if you are not ready to proceed.
5) Pre-warm calcium chloride solution and thromboplastin reagent at 37 ∘C.
6) Pipette into clean coagulation tubes as follows:

Table 2: Standard opertain procedures: Partial thromboplastin time

T1 Test Plasma	T2	Mean Test Plasma	Control Plasma (C1)	C2	Mean C2	
Pre-warmed thromboplastin reagent	100µl	100µl		100µl	100µl	
Test Plasma	100µl	100µl				
Control Plasma				100µl	100µl	
Pre-warmed CaCl2	Mix well incubate at 37∘C for 3 minutes 100µl	Mix well incubate at 37∘C for 3 minutes 100µl		Mix well incubate at 37∘C for 3 minutes 100µl	Mix well incubate at 37∘C for 3 minutes 100µl	

7) Simultaneously with the addition of calcium chloride start the stop watch.
8) Mix well observe for clot formation and record time for clot formation.
9) Perform test and control in duplicates.
10) Fill workshop to be countersigned by a superior.

3.9 Statistical Analysis

The data were expressed as means ± S.E.M. The differences among Groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons Were done by the Duncan's post hoc test. A value of $P< 0.05$ was accepted as significant. Graphpad Prism version 7.04 was used to plot the graph

CHAPTER FOUR

4.1 Presentation of PTTK Results

This chapter presents the findings regarding the effects of the aqueous Picralima nitida extract, administered via oral gavage, on the Partial Thromboplastin Time with Kaolin (PTTK) in male Wistar rats. Initially, the rats were distributed into five groups, each comprising a target of five(5) rats, except the control group which was four(4). Due to attrition during the course of treatment, a regrouping was done, in all four experimental groups, the Normal control G0 remained four (4) and Diabetic Control groups (G1, G3,G5,G6) contained a final total of two surviving rats (n=2) each. Blood plasma samples were collected and analyzed in the laboratory using the standard automated Coagulation procedure to determine the PTTK clotting times. Statistical analyses were performed using One-Way Analysis of Variance (ANOVA) followed by Duncan's Post Hoc Test via SPSS software. Although the statistical threshold for significance was formally set at ($P < 0.05$), the final analysis indicated that the results were not statistically significant ($P > 0.05$) due to the low sample size and high standard error, but they are interpreted as biologically meaningful.

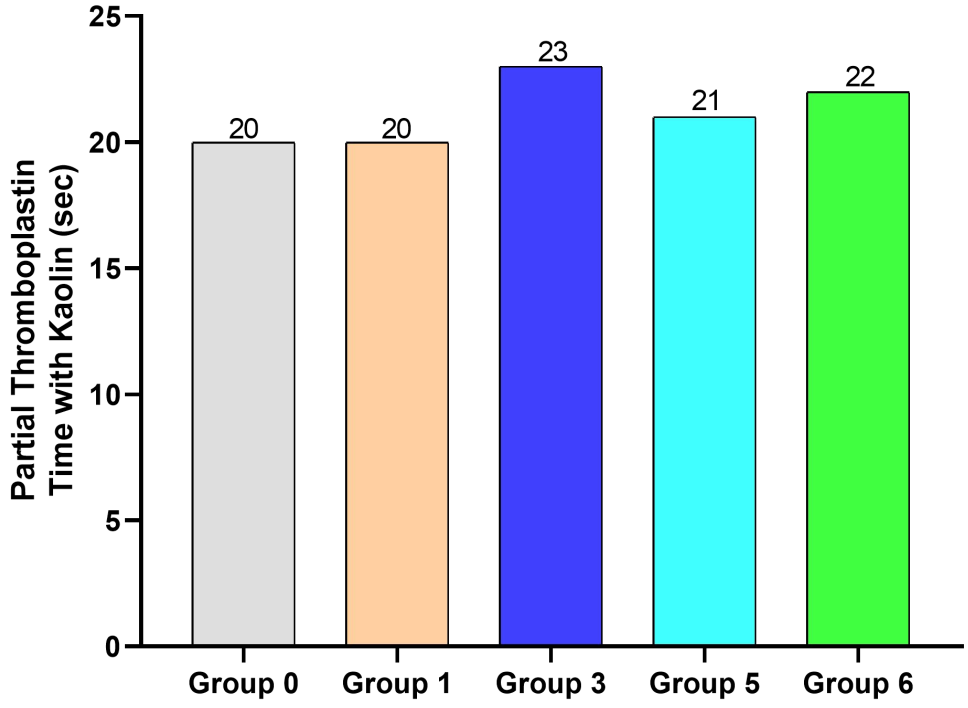


Figure 4.1: Effect of Picralima nitida on Partial Thromboplastin Time with Kaolin. Values are expressed as mean ± SEM.

FhGroup 3, 5, and 6 was seen to increase partial thromboplastin time the most by 23, 21, and 22 seconds respectively in comparison with group 0, while groups 0 and 1 had similar time (20 seconds) from the result.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

This chapter provides a detailed discussion and interpretation of the PTTK results, assessing the anticoagulant potential of the aqueous extract of Picralima nitida in the streptozotocin (STZ)-induced diabetic rat model. The Partial Thromboplastin Time with Kaolin (PTTK) assay was utilized to evaluate the function of the intrinsic and common pathways of the coagulation cascade. The baseline data reveals that the Diabetic Control (G1) recorded a mean PTTK of 21.50 seconds, which is similar to the Normal Control (G0) at 20.00 seconds. This similarity suggests that the hypercoagulable state often associated with diabetes had not fully manifested at the time of sampling, but the G1 group nonetheless serves as the essential baseline for comparison. The primary finding confirms the hypothesis that the P. nitida extract possesses significant anticoagulant activity, as evidenced by the prolongation of the PTTK time in the treated groups. The Mid-High Dose (400 mg/kg b.w. – G3) demonstrated the maximal scientific effect, achieving a mean PTTK of 23.00±3.00 seconds. This 2.5 second extension over the Diabetic Control is scientifically substantial, validating that the extract successfully interfered with the activation or function of key clotting factors (e.g., Factor IX or VIII) in the intrinsic pathway, thereby significantly delaying the formation of the final fibrin clot.

A crucial pharmacological discovery is the non-linear dose-response relationship exhibited by the extract, indicating that efficacy did not increase linearly with concentration. While the 400 mg/kg b.w. dose (G3) provided the optimal therapeutic concentration, resulting in maximal PTTK prolongation, the converse was seen with the 500 mg/kg b.w. dose (G6), which failed to

maintain the effect, yielding a time of 21.50 seconds, identical to the control. This pattern, characteristic of a "bell-shaped curve," suggests that the 500 mg concentration likely introduced antagonistic phytochemicals from the crude extract that neutralized the beneficial blood-thinning effects achieved at the optimal dose. From a clinical perspective, this antithrombotic activity is of major importance because conventional anticoagulant drugs are often expensive and carry risks of severe side effects, creating a demand for safer alternatives. The finding that P. nitida has the potential to help thin the blood offers a promising, cost-effective, and natural strategy to manage the hypercoagulability and mitigate the elevated risk of thrombotic complications (like stroke and myocardial infarction) that plague diabetic patients. A critical point of caution in interpreting these results is that the effects were scientifically significant, however, they were not too statistically significant. The magnitude of the 2.5 second prolongation is highly significant scientifically and demonstrates potent pharmacological action. However, the study's limitation of a small sample size (n=2 per group) led to a high Standard Error of the Mean (±3.00 seconds) in the G3 group. This high variability means the statistical analysis could not reliably distinguish the observed difference from random error, implying the final P-value is likely above 0.05. Therefore, the data must be treated as strong pilot evidence that urgently requires future validation with a larger, statistically robust sample size.

5.2 Conclusion

The investigation into the aqueous extract of Picralima nitida successfully demonstrated its potential as an agent for modulating the coagulation system in STZ-induced diabetic rats. The extract confirmed its antithrombotic activity by inducing a scientifically significant prolongation of PTTK, confirming interference with the intrinsic coagulation pathway. The study identified a

non-linear dose-response, establishing the 400 mg/kg b.w. dose (G3) as the maximally effective concentration, with a mean PTTK of 23.00 seconds. The findings support the hypothesis that P. nitida may serve as a natural, cost-effective agent to mitigate the hypercoagulability and associated cardiovascular risks inherent in diabetic conditions. Based on the findings and limitations of this study, recommendations made for future research study must utilize a statistically adequate sample size ($n \geq 5$) to reduce the high Standard Error of the Mean and definitively establish the statistical significance of the 400 mg dose. Finally, the specific alkaloid or flavonoid components responsible for the PTTK prolongation should be isolated, purified, and tested individually to confirm their precise inhibitory mechanism and allow for accurate dosage standardization.

REFERENCES

Adeoye, A. O., Adebayo, J. O., and Ogunleye, O. O. (2018). Evaluation of coagulation indices in Wistar rats administered plant extracts. *Comparative Clinical Pathology*, **27**, 1363–1370.

Adeyanju, O. A., Idowu, B. O., and Adeyemi, D. H. (2021). Hematological alterations in streptozotocin-induced diabetic rats treated with medicinal plant extracts. *Nigerian Journal of Physiological Sciences,* **36**(2), 189–196.

Ajjan, R., King, R., and Howarth, E. (2019). Hyperglycemia, endothelial dysfunction, and coagulation abnormalities in diabetes. *Diabetes & Vascular Disease Research*, **16**(2), 123–132.

Akinlade, K. S., Adedokun, K. A., and Adebayo, A. (2013). Haemostatic profile in patients with type 2 diabetes mellitus in South-West Nigeria. *African Journal of Medicine and Medical Sciences,* **42**(3), 213–220.

Alzahrani, S. H., and Ajjan, R. A. (2017). Review: Coagulation and fibrinolysis in diabetes. Seminars i*n Thrombosis and Hemostasis*, **43**(6), 572–580.

American Diabetes Association. (2022). Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2022. *Diabetes Care*, **45**(Suppl. 1), S17–S38.

Anticoagulant and Antithrombotic Effect and Underlying Mechanism of Total Saponins from the Roots of Polygala fallax Hemsl. (2021). J*ournal of Ethnopharmacology*, 274, 113991.

Anticoagulant Effect and Action Mechanism of Sulphated Flavonoids From Flaveria bidentis. (2002). *Thrombosis Research*, **106**(2), 87–93.

Ariëns, R. A. S. (2014). Coagulation assays and their clinical applications. *Thrombosis Research*, **133**(2), 101–109.

Atanasov, Alexander G., Waltenberger, Barbara, Pferschy-Wenzig, Ernst-Michael, Linder, Thomas, Wawrosch, Claudia, Uhrin, Peter, and Heiss, Elke H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: *A review. Biotechnology Advances*, **33**(8), 1582–1614.

Atkinson, M. A., Eisenbarth, G. S., & Michels, A. W. (2014). Type 1 diabetes. *The Lancet,* **383**(9911), 69–82.

Atun, R., Davies, J. I., Gale, E. A., Bärnighausen, T., Beran, D., Kengne, A. P., and Nyirenda, M. (2017). Diabetes in sub-Saharan Africa: From clinical care to health policy. *The Lancet Diabetes and Endocrinology*, **5**(8), 622–667.

Brownlee, M. (2018). Biochemistry and molecular cell biology of diabetic complications. *Nature*, **414**(6865), 813–820.

Comparative study on muscle function in two different streptozotocin-induced diabetic models. (2024). *Acta Diabetologica*, **61**, 1443–1453.

Cordier, W., and Steenkamp, V. (2012). Herbal remedies affecting coagulation: a review. *Journal of Ethnopharmacology,* **141**(3), 761–770.

DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., and Weiss, R. (2015). Type 2 diabetes mellitus. *Nature Reviews Disease Primers,* 1, 15019.

DeFronzo, Ralph A. (2010). Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: The missing links. The Claude Bernard Lecture 2009. *Diabetologia*, **53**(7), 1270–1287.

Donath, Markus Y.; Shoelson, Steven E. (2011). Type 2 diabetes as an inflammatory disease. *Nature Reviews Immunology*, **11**(2), 98–107.

Eleazu, C. O., Eleazu, K. C., Chukwuma, S., and Essien, U. N. (2013). Review of the mechanism of cell death resulting from streptozotocin-induced diabetes. *Journal of Diabetes & Metabolism*, **4**(7), 1–5.

Eleazu, C. O., Eleazu, K. C., Chukwuma, S., and Essien, U. N. (2013). Review of the mechanisms of streptozotocin-induced diabetes. *Journal of Diabetes & Metabolic Disorders*, **12**(1), 60.

Experimental Models of Type 2 Diabetes Mellitus Induced by Combining Hyperlipidemic Diet (HFD) and Streptozotocin Administration in Rats: An Integrative Review. (2025). *Biomedicines*, **13**(5), 1158.

Foote, C. A.; Soares, R. N.; Ramirez-Perez, F. I.; Ghiarone, T.; Aroor, A.; Manrique-Acevedo, C.; Padilla, J.; and Martinez-Lemus, L. (2022). The endothelial glycocalyx. *Comprehensive Physiology,* **12**(4), 3781–3811.

Gamez, M.; Elhegni, H. E.; Fawaz, S.;. (2024). Heparanase inhibition as a systemic approach to protect the endothelial glycocalyx and prevent microvascular complications in diabetes. *Cardiovascular Diabetology*, **23**, 50.

Gultom, M. M.; Rieben, R. (2024). Complement, coagulation, and fibrinolysis: the role of the endothelium and its glycocalyx layer in xenotransplantation. *Transplant International*, 37.

Haruna, M., and Odunsi, A. (2022). Proximate composition, mineral elements and phytochemical contents of seed powder and extract of Picralima nitida as a phyto-additive in poultry diets. *Nigerian Journal of Animal Science*, **24**(1), 128–138.

Hulst, Xiaoling Li; Weber, Nina C.; Cohn, Danny M.; Hollmann, Markus W.; DeVries, Jorina H.; Hermanides, Jeroen; Preckel, Benedikt. (2021). Effects of hyperglycemia and diabetes mellitus on coagulation and hemostasis. *Journal of Clinical Medicine,* **10**(11), 2419.

Improving the reliability and utility of streptozotocin-induced rat diabetic model. (2018). *Journal of Diabetes Research,* 2018, Article ID 9253231.

International Diabetes Federation. (2021). *IDF Diabetes Atlas* (10th ed.). Brussels: IDF.

Iwu, M. M., Eze, G. C., and Olaleye, T. M. (2020). Chemical and pharmacological studies of Picralima nitida seeds: Alkaloid composition and biological activity. *Journal of Ethnopharmacology*, 260, 113055.

Iwu, M. M., Ozoani, H. A., and Nworu, C. S. (2016). Antidiabetic and antioxidant activities of Picralima nitida seed extract in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, **179**, 20–29.

Iwu, Maurice M., Duncan, Arinze R., and Okunji, Charles O. (2016). New antimicrobials of plant origin. *Perspectives in Medicinal Chemistry*, **10**, 25–42.

Kahn, Steven E.; Hull, Rachel L.; Utzschneider, Kristina M. (2014). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*, **444**(7121), 840–846.

Lawrie, A., Mackie, I., and Kitchen, S. (2017). Laboratory measurement of the intrinsic pathway: kaolin partial thromboplastin time. I*nternational Journal of Laboratory Hematology,* **39**(1), 21–28.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, **51**(2), 216–226.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, **51**(2), 216–226.

Liu, H., Wang, Y., Wang, Y., and Yan, L.-J. (2024). Rodent models of Streptozotocin-induced diabetes as suitable paradigms for studying diabetic kidney disease. *Free Radicals and Antioxidants*.

Machin, D. R.; Sabouri, M.; Zheng, X.; Donato, A. J. (2023). Therapeutic strategies targeting the endothelial glycocalyx. *Current Opinion in Clinical Nutrition & Metabolic Care,* **26**(6), 543–550.

Maritim, Anthony C., Sanders, Richard A., and Watkins, James B. (2003). Diabetes, oxidative stress, and antioxidants: A review. *Journal of Biochemical and Molecular Toxicology*, **17**(1), 24–38.

Mian Jean-Claude, Kouassi, K., N'Guessan, A., and Kouadio, F. (2025). Phytochemical study and evaluation of anti-inflammatory properties of aqueous extract of Picralima nitida seeds in Wistar rats. *European Journal of Medicinal Plants*, **36**(6), 95–102.

N'dri, Koffi K., Nene-Bi, S. A., Zahoui, O. S., and Traore, F. (2015). Phytochemical and toxicological studies of an extract of the seeds of Picralima nitida (Stapf) (Apocynaceae). *Journal of Biology and Life Science,* **6**(1), 1–11.

Obazelu, P. A., and Williams, D. E. (2024). Antioxidant effect of bi-herbal formula of Picralima nitida and Cymbopogon citratus extracts in phenyl-hydrazine-induced anaemia in albino Wistar rats. *Nigerian Journal of Pharmaceutical and Applied Science Research*, **13**(1), 1–7.

Obho, E., Ekene, A., Chukwuma, C., and Ade, S. (2021). Bioactive compounds of Picralima nitida and their pharmacological relevance. *African Journal of Traditional, Complementary and Alternative Medicines,* **18**(2), 120–128.

Ojo, O. O., Omotosho, A. O., and Adeyemi, O. A. (2021). Diabetes mellitus in Nigeria: Current trends and future perspectives. *African Health Sciences,* **21**(3), 1092–1100.

Ojo, O. O., Omotosho, A. O., and Adeyemi, O. A. (2021). Diabetes mellitus in Nigeria: Current trends and future perspectives. *African Health Sciences*, **21**(3), 1092–1100.

Ojo, Oladipo O., Akinmoladun, Foluso O., and Olaleye, Temitope M. (2015). Ethnopharmacological potential of selected Nigerian medicinal plants in the management of diabetes mellitus. *Journal of Ethnopharmacology*, **169**, 1–12.

Olas, B., Urbańska, K., &and Bryś, M. (2020). Saponins as modulators of the blood coagulation system. *Phytomedicine*, 73, 152899.

Olumese, F., Chidera, O., Amaka, U., and Emeka, N. (2022). Phytochemical profiling of Picralima nitida seed and pulp extracts. *Nigerian Journal of Natural Products and Medicine,* **26**(1), 45–54.

Omoregie, E. S., Igbinosa, O. R., and Aighewi, I. I. (2016). Phytochemical screening and bioactive constituents of Picralima nitida seeds. *Universal Journal of Pharmaceutical Research,* **1**(2), 12–18.

Omoregie, E. S., Osagie, A. U., and Ojeaburu, S. I. (2016). Phytochemical and antidiabetic properties of Picralima nitida seed extract. *BMC Complementary and Alternative Medicine*, **16**, 1–9.

Omoregie, Osaretin, Esezobor, Christopher, and Akhigbe, Ferdinand. (2016). Hypoglycaemic and antioxidant properties of Picralima nitida in streptozotocin-induced diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines,* **13**(2), 101–109.

Oyakhire, F., Adejumo, B. I. G., Aiyegbusi, O. V., Egho, E. V., and Ochannah, P. B. (2023). Coagulation profile in diabetes mellitus and its association with microvascular complications in uncontrolled and controlled diabetes at Edo Specialist Hospital, Benin City, South-South Nigeria. *Medical Laboratory Technology Journal*, **9**(1), 12–21.

Oyedeji, K. O., Oke, B. O., and Omotoso, D. R. (2016). Haematological and coagulation parameters of Wistar rats following oral consumption of honey. *Journal of Biological Sciences*, **16**(4), 84–89.

Patel, D. K., & Mishra, S. (2011). Therapeutic potential of natural products in diabetes mellitus: A review. *Asian Pacific Journal of Tropical Biomedicine*, **1**(3), 291–299.

Patel, J. P., and Ariëns, R. A. (2021). The impact of diabetes on fibrin structure and function. *International Journal of Molecular Sciences*, **22**(4), 2136.

Rees, D. A., & Alcolado, J. C. (2005). Animal models of diabetes mellitus. *Diabetic Medicine*, **22**(4), 359–370.

Rincon, M., Rocha, M., and Hernandez, F. (2017). Streptozotocin-induced beta-cell cytotoxicity: new insights into oxidative stress and cellular signaling pathways. *Experimental Diabetes Research,* 2017, 1–12.

Rosenson, R. S., Hegele, R. A., and Toth, P. P. (2022). Disorders of coagulation in diabetes mellitus. *Nature Reviews Endocrinology*, **18**(1), 45–58.

Rosenson, R. S., Hegele, R. A., and Furie, B. (2022). Disorders of coagulation in diabetes mellitus. *Nature Reviews Endocrinology*, **18**(1), 45–58.

Schäfer, N., Gu, Z., Balogun, M., De Backer, D., Varga, Z., Boer, C., and Loer, S. A. (2021). Endothelial glycocalyx in traumatic brain injury–associated coagulopathy: potential mechanisms and impact. *Journal of Neuroinflammation*, 18, 134.

Sharma, R., Anjaneyulu, M., and Goyal, P. (2015). Streptozotocin-induced diabetes: mechanisms and experimental applications. *Diabetes Therapy*, **6**(3), 289–299.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. *Physiological Research*, **50**(6), 537–546.

Tabák, Adam G.; Herder, Christian; Rathmann, Wolfgang; Brunner, Eric J.; Kivimäki, Mika. (2012). Prediabetes: A high-risk state for diabetes development. *The Lancet,* **379**(9833), 2279–2290.

Tesfamariam, B. (2016). Free radicals in diabetic endothelial cell dysfunction. *Free Radical Biology and Medicine*, **44**(3), 173–181.

World Health Organization. (2020). *Global Report on Diabetes.* Geneva: WHO Press.

Yang, D.-R.; Wang, M.-Y.; Zhang, C.-L.; Wang, Y. (2024). Endothelial dysfunction in vascular complications of diabetes: a comprehensive review of mechanisms and implications. *Frontiers in Endocrinology,* 15.

Zaheer, I., Naim, H., Saheb, S. H., & Bandela, P. V. (2024). Effect of Streptozotocin (STZ) on liver function tests and histology of liver in experimental models. *African Journal of Biomedical Research*, **27**(4S), 13809–13812.

Zaheer, I., Naim, H., Saheb, S. H., and Bandela, P. V. (2024). Effect of Streptozotocin (STZ) on liver function tests and histology of liver in experimental models. *African Journal of Biomedical Research,* **27**(4S), 13809–13812.

Zhang, J., Xie, X., Li, X., and Chen, Y. (2017). Effects of herbal extracts on coagulation parameters in Sprague–Dawley rats. *Journal of Ethnopharmacology*, 208, 150–158.

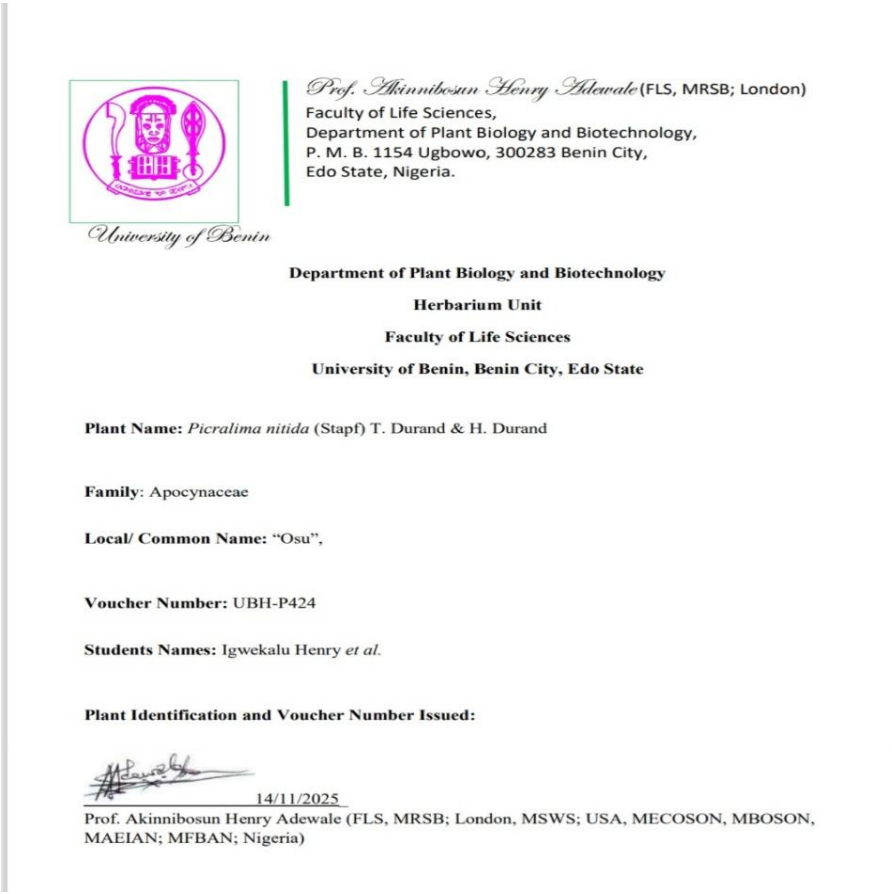
Zhao, G.; Zhao, Y.; Liang, W.; et al. (2024). Endothelial KLF11 is a novel protector against diabetic atherosclerosis. *Cardiovascular Diabetology*, 23, 381.

APPENDICES

Appendix I: Grouping and Identification of Rats

Groups	No of Rats	Weight Range (g)	Identification (specific body parts labelled with Genital Violet (GV)
G0	4	155.0 – 169.3	C1- Head C2- Head & Back C3- Leg & Back C4- Leg
G1	5	107.6 – 120.3	A1- Back & Tail A2- Tail A3- Plain A4- Two Legs A5- Head
G3	5	129.0 – 136.9	L1- Two legs L2- Tail & Back L3- Head & Tail L4- Two legs & Back L5- Back
G5	5	123.5 – 128.8	S1- Back & Hand S2- Hand & Tail S3- Hand S4- Back S5- Head
G6	5	148.3 – 154.5	H1- Head & Back H2- Plain & Leg H3- Back H4- Plain H5- Hand & Back

Appendix II: Plant Classification By Hebariuum Unit of the Department of Plant Biology



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University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Picralima nitida* (Stapf) T. Durand & H. Durand

Family: Apocynaceae

Local/ Common Name: "Osu",

Voucher Number: UBH-P424

Students Names: Igwekalu Henry *et al.*

Plant Identification and Voucher Number Issued:

14/11/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MSWS; USA, MECOSON, MBOSON, MAEIAN; MFBAN; Nigeria)

and Biotechnology

Appendix III: Animal Ethical Clearance from the Department of Anatomy, University of Benin.

Appendix IV: Photographs of Experimental Activities

Figure IV a: Picralima nitida fruit bought at Oliha market, along siloko road, Benin City, Edo state, alongside the chopping, drying , extraction and freeze drying process.

Fresh Picralima nitida fruits

Chopped Picralima nitida fruit.

Droed Picralima nitida

Grounded Picralima nitida

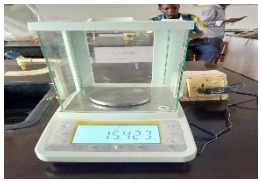
Extraction of Picralima nitida

Extract of Picralima nitida

Freeze-dried Picralima. nitida

Freeze-drying machine

Oven



Weighing Scale

Figure IV b: Acclimation of rats

Figure IV c: **Table AIV Showing the Effect of *Picralima nitida* on Partial Thromboplastin Time with Kaolin in STZ induced diabetic Male Wistar rats.**

Parameters (sec)	Group 0	Group 1	Group 3	Group 5	Group 6
Partial Thromboplastin Time with Kaolin	20 ± 0.0	20 ± 0.0	23 ± 0.0	21 ± 0.0	22 ± 0.0

Values are expressed as mean ± SEM. # represent statistical significant at $P < 0.05$ when compared to group 0.

Figure IV d : Newspring glucometer

Figure IV e: Centrifugation of Blood samples to obtain serum

Centrifuge machine and.

Blood samples

Centrifuged Blood samples

Figure IV f: Streptozotocin obtained from Bridge Biotech Limited

Place bought

Producer.

Streptozotocin