INVESTIGATING THE EFFECTS OF AQUEOUS EXTRACT OF *Picralima nitida* ON SERUM AMYLASE IN STREPTOZOTOCIN INDUCED DIABETIC MALE WISTAR RATS

BY

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CERTIFICATION

We the undersigned hereby certify that Eucharia Joseph GARBA carried out this work, in the department of Medical Biochemistry, University of Benin, Benin city and we approve same as adequate in scope and quality for the reward of Bachelors of Science degree (B.Sc.) in Medical Biochemistry.

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DEDICATION

I dedicate this work to the Almighty God who has been my source of strength, Grace and Wisdom through whose Grace and favor I have been able to run my course and scale through the hurdles of my academic pursuit.

ACKNOWLEDGMENT

My sincere gratitude goes to God Almighty for his divine intervention and strength granted to me throughout my academic journey.My gratitude also goes to Dr. E.S Oghabon; my supervisor, who gave me the understanding and assistance to carry out this work. My heartfelt thanks also goes to my family for all their constant encouragement, love and support. And to the two people who pushed me through this project work, never gave up on me and showed their support in various ways; Naomi Izibili and Oghenemarho Ugege, I love you both dearly. Last but not least, I want to thank myself . I want to thank myself for not giving up. I want to thank myself for standing strong even when I was at my lowest. I want to thank myself for pushing the storms through regardless. Thank you Garba Eucharia for being your mothers daughter.

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ABSTRACT

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, or elevated blood glucose levels, resulting from defects in insulin secretion, insulin action, or both. This study focused on the severe metabolic imbalance caused by Streptozotocin (STZ) induction in male Wistar rats . Diabetes mellitus was induced in male Wistar rats by a combination of a 2-week high-carbohydrates diet, followed by double doses (60 and 40 mg/kg respectively) of intraperitoneal injection of STZ. Diabetic rats were then treated for two weeks with either distilled water (control), Glibenclamide (5 mg/kg, positive control), or *picralima nitida* extract at two doses (200 mg/kg and 500 mg/kg). The findings revealed that all groups treated with *Picralima nitida* showed a decrease in α-amylase levels compared to the diabetic group that received no treatment. However, this reduction was not sufficient to bring the enzyme activity back to the levels observed in non-diabetic (control) rats. Among the doses tested, the low dose produced the most stable regulatory effect, showing results that were comparable, though slightly less effective, than glibenclamide. This indicates that *Picralima nitida* may not promote pancreatic repair or fully restore enzyme levels but instead functions as a modulator of enzyme activity. Such regulation could aid in controlling post-meal blood glucose spikes, suggesting the plant extract's potential as an α-amylase inhibitor in type 2 diabetes management. Overall, the evidence supports its role more as a regulatory agent than a definitive treatment.

CHAPTER ONE

1.1 INTRODUCTION

Natural products and plants historically have been used for several years for the discoveries of new drugs (Creed *et al*., 2021).

Picralima nitida, commonly known as 'Akuamma' is a west African tree whose seeds, fruits, rind, and other parts are traditionally used to treat ailments such as fever, malaria, diarrhea, hypertension, and diabetes, underlining its ethnomedicinal potential (Falodun *et al.,* 2006*;* Adegoke and Oloyede*,* 2013).

Studies have shown its extracts to be effective in inhibiting pancreatic amylase *(Etim et al., 2018).* Hence in modern days, huge attention has been directed towards recognition of plants with antidiabetic that may be used effectively for human consumption (Grover *et al.,* 2002).

Pancreatic α-amylase, involved in dietary starch breakdown (Webb *et al.,* 2017) play vital roles in glucose regulation where excess activity of α-amylase can lead to exaggerated blood glucose sugar.

Biological studies further support *Picralima nitida's* pharmacological promise; its fruit extract exhibits anti-inflammatory, antipyretic, antimicrobial, and antimalarial activities*,* while its hypoglycemic effects in normal and diabetic rabbits suggests potential for glucose regulation beyond insulin-dependent pathways. Plant derived compounds are increasingly investigated for enzyme modulation with fewer adverse effects than conventional drugs.

This study aims to assess the influence of aqueous extract of fruit on pancreatic amylase enzyme in Streptozotocin induced diabetic Male Wistar albino rats following a controlled feeding period, to provide enlightenment as a potential therapeutic agent against diabetes.

1.1 Aim of research

The aim of the study is to evaluate the effects of aqueous extract of fruit on pancreatic amylase in Streptozotocin induced diabetic male Wistar albino rats.

1.2 Scope of study

This study focuses on investigating the effects of aqueous extract of *Picralima nitida* fruit on the activity of the enzyme involved in glucose metabolism: pancreatic amylase. This research would examine if the extract exhibits inhibitory activity against these enzymes under controlled laboratory conditions.

1.3 Objective of study

To determine the impact of *Picralima nitida* fruit extract on pancreatic amylase activity in male Wistar albino rats.

To compare enzyme activity levels between treated and control groups.

To compare the effects of *Picralima nitida* fruit extract with that obtained from a standard hypoglycemic drug (glibenclamide).

CHAPTER TWO

LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is taken from the Greek word *diabetes*, meaning siphon - to pass through and the Latin word *mellitus* meaning sweet. A review of the history shows that the term "diabetes" was first used by Apollonius of Memphis around 250 to 300 BC. Ancient Greek, Indian, and Egyptian civilizations discovered the sweet nature of urine in this condition, and hence the propagation of the word Diabetes Mellitus came into being (Sapra and Bhandari, 2023).

Mering and Minkowski, in 1889, discovered the role of the pancreas in the pathogenesis of diabetes (Sapra and Bhandari, 2023).

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, or elevated blood glucose levels, resulting from defects in insulin secretion, insulin action, or both (Banday, Sameer, and Nissar, 2020; Patil, Mane, Datkhile, *et al.*, 2025). Uncontrolled hyperglycemia leads to long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Migdalis, 2024).

Notably, insulin plays an important role as an anabolic hormone, affecting the metabolism of carbohydrates, lipids, and proteins (Poznyak *et al.,* 2020).

Without proper treatment, uncontrolled diabetes can lead to various complications such as coma, confusion, and in rare cases, death from ketoacidosis or nonketotic hyperosmolar syndrome if not treated (Poznyak *et al.,* 2020).

Chronic hyperglycemia in synergy with the other metabolic aberrations in patients with diabetes mellitus can cause damage to various organ systems, leading to the development of disabling and life-threatening health complications, most prominent of which are microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular complications leading to a 2-fold to 4-fold increased risk of cardiovascular diseases (Yang T. *et al.,* 2024).

The prevalence of DM has been rising dramatically, with projections indicating a substantial increase in cases worldwide over the next two decades (Hossain *et al.,* 2024).

The main subtypes of DM are Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM), which classically result from defective insulin secretion (T1DM) and/or action (T2DM) (Sapra and Bhandari, 2023).

While T1DM and T2DM remain the primary classifications, the identification of more nuanced subtypes based on underlying pathophysiology is gaining traction (Patil *et al*., 2025).

2.1.1 EPIMEDIOLOGY OF DIABETES MELLITUS

Diabetes is a worldwide epidemic. With changing lifestyles and increasing obesity, the prevalence of DM has increased worldwide. The global prevalence of DM was 589 million in 2025 (International Diabetes Federation, 2025).

According to the International Diabetes Federation (IDF), in 2025, about 11.1% of the adult population had diabetes. Of these, 252 million were undiagnosed. With an increase in age, the prevalence of DM also increases. About 25% of the population above 65 years of age has diabetes (Centers for Disease Control and Prevention, 2024; Sinha *et al*., 2024).

In 2014, the WHO announced that 8.5% of adults aged 18 and above were affected by diabetes. In 2019, diabetes was responsible for 1.5 million deaths, with 48% of these occurring before the age of 70. Additionally, diabetes led to another 460,000 deaths due to kidney disease, and roughly 20% of cardiovascular-related deaths were attributed to elevated blood glucose levels (Antar *et al.,* 2023).

The onset of T2DM is usually later in life, though obesity in adolescents has led to an increase in T2DM in younger populations. T2DM has a prevalence of about 9% in the total population of the United States, but approximately 25% in those over 65 years. The International Diabetes Federation estimates that 1 in 11 adults between 20 and 79 years had DM globally in 2015.

From 2000–2019, there was a 3% rise in standardized mortality rates related to diabetes. In lower-middle-income countries, the mortality rate associated with diabetes increased by 13% (Antar *et al.,* 2023).

Type 2 diabetes accounts for 90–95% of all diabetes. This form encompasses individuals who generally have relative (rather than absolute) insulin deficiency and have peripheral insulin resistance (i.e., decreased biological response to insulin) (Diabetes care, 2024)

2.1.2 CLASSIFICATION OF DIABETES MELLITUS

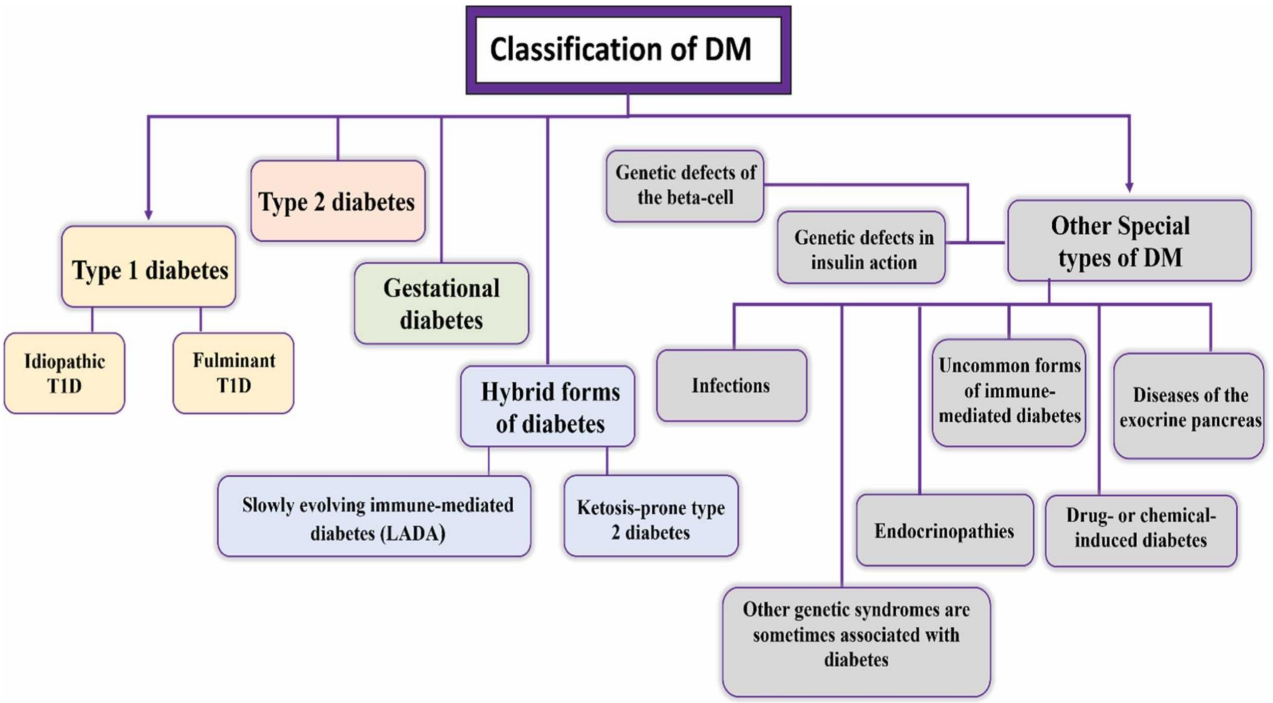

Fig 2.1.2 The newest classification **of** DM represents the different types and subtypes of diabetes, including Type 1 Diabetes, Type 2 Diabetes, Gestational Diabetes, Hybrid Forms of Diabetes (LADA and Ketosis-prone Type 2 Diabetes), and Other Special Types of Diabetes (Antar *et al.,* 2023).

2.1.2.1 TYPE 1 DIABETES MELLITUS

Type 1 diabetes (T1D) can be detected well before abnormal insulin secretion starts, with a steady decline starting at least two years before diagnosis (ElSayed *et al.*, 2023).

Type 1 diabetes (T1D) is a condition characterized by the immune-mediated destruction of insulin-producing pancreatic β-cells, leading to absolute insulin deficiency (Lucier and Mathias, 2021).

The metabolic, genetic, and immunogenetic characteristics of T1D are heterogeneous, with age-related differences necessitating a personalized approach for each individual. Underlying genetic risk is present in many individuals with the disease. Hence, the American Diabetes Association (ADA) recommends that first- and second-degree relatives of individuals with T1D be screened and offered T1D autoantibody testing (Holt *et al.,* 2021).

Diabetic ketoacidosis is more prevalent among young patients with new-onset T1D (Ehrmann *et al*., 2020). Disease-modifying therapy has now been approved in the early preclinical stages of T1D to delay the onset of clinical diabetes (Ramos *et al.,* 2023).

2.1.2.1.1 IDIOPATHIC TYPE 1 DIABETES MELLITUS

This type of T1D shows no evidence of autoimmune destruction of pancreatic beta cells. People with idiopathic diabetes may experience episodic ketoacidosis as well as insulin insufficiency (Antar *et al.,* 2023).

2.1.2.1.2 FULMINANT TYPE 1 DIABETES MELLITUS

Unlike classical autoimmune type 1 diabetes (AT1D), individuals with FT1D have a much shorter duration of hyperglycemic symptoms, present with a greater severity of ketoacidosis and hyperglycemia, yet have misleadingly normal glycated hemoglobin (HbA1c) at presentation (Luo *et al.,* 2020).

It causes an incredibly quick and practically complete β-cell death that leaves almost no residual insulin output (Antar *et al.,* 2023).

This condition is mainly attributed to some environmental and hereditary causes. Through an increased immune response without discernible formation of autoantibodies attacking pancreatic *β*-cells, an antiviral immune response may cause the loss of pancreatic *β*-cells (Antar *et al.,* 2023).

2.1.2.2 TYPE 2 DIABETES MELLITUS

This is a chronic condition where the body is unable to produce insulin or doesn't utilize it properly leading to high blood sugar levels.

Type 2 diabetes frequently goes undiagnosed for many years, because hyperglycemia develops gradually and, at earlier stages, is often not severe enough for the individual to notice the classic diabetes symptoms caused by hyperglycemia, such as dehydration or unintentional weight loss (Diabetes care, 2024)

2.1.2.3 GESTATIONAL DIABETES MELLITUS (GDM)

Gestational Diabetes mellitus (GDM) is a state of maternal maladaptation to the physiological insulin resistance of the second half of pregnancy (Martis *et al.,* 2025).

GDM is hyperglycemia first identified during pregnancy (Patil *et al*., 2025). It is considered a medical complication of pregnancy that increases the risk of adverse outcomes for both mother and offspring (Dion and Rudan, 2023).

Another factor that promotes abnormally increased insulin resistance is maternal obesity early in pregnancy due to higher free fatty acid levels, which inhibits maternal glucose uptake and stimulates hepatic gluconeogenesis (Sweeting *et al.,* 2022).

Not only is the prevalence of type 1 diabetes and type 2 diabetes increasing in individuals of reproductive age but there is also a dramatic increase in the reported rates of gestational diabetes mellitus (GDM) (Diabetes care, 2025). Gestational Diabetes mellitus is a state of maternal maladaptation to the physiological insulin resistance of the second half of pregnancy (Martis *et al.,* 2025).

Women with a history of GDM face a substantially increased long-term risk (7- to 10-fold higher) of developing T2DM later in life (Dion and Rudan, 2023). Offspring are also pre-programmed for cardiometabolic risks, including obesity and dysglycemia, creating an intergenerational cycle of risk (Dion and Rudan, 2023). Increased maternal leptin and placental hormones like hPL contribute to maternal insulin resistance and fetal macrosomia (Martis *et al.*, 2025).

2.1.3 HYBRID FORMS OF DIABETES

2.1.3.1 SLOWLY EVOLVING IMMUNE-MEDIATED DIABETES (LADA)

Slowly evolving immune-mediated diabetes, also known as latent autoimmune diabetes in adults(LADA resembles type 2 diabetes clinically but is characterized by the presence of pancreatic autoantibodies associated with autoimmune diabetes (Antar *et al.,* 2023).

The criteria used to diagnose LADA typically involve positive glutamic acid decarboxylase (GAD) autoantibodies, age older than 35 years at the time of diagnosis, and no immediate need for insulin therapy in the first 6–12 months after diagnosis (Antar *et al.,* 2023).

2.1.3.2 KETOSIS-PRONE TYPE 2 DIABETES

Ketosis-prone type 2 diabetes is a unique clinical condition primarily seen in young African Americans and populations in sub-Saharan Africa. It is characterized by episodes of ketosis and severe insulin deficiency at the initial presentation, resembling type 1 diabetes or diabetic

ketoacidosis. However, individuals with this condition eventually enter remission and do not require insulin treatment (Antar *et al.,* 2023).

2.1.4 OTHER SPECIAL TYPES OF DIABETES MELLITUS

2.14.1 GENETIC DEFECTS OF THE BETA CELL

Maturity-onset diabetes of the young (MODY): This type of diabetes is associated with abnormal monogenetic in β-cell function. It typically appears at a young age, usually before 25 years old, and is characterized by reduced insulin secretion with little to no abnormalities in insulin action.

There are 14 subtypes of MODY, amongst which the most common ones are MODY-3 and MODY 2 accounting for 15%–25% and 30%–50% respectively. MODY-5 caused by a mutation in hepatic nuclear factor 1B (HNF-1B) is responsible for less than 5% of all cases of MODY (Mateus *et al.,* 2020).

2.1.4.2 GENETIC DEFECTS IN INSULIN ACTION

Certain gene mutations of insulin receptors can lead to abnormalities in insulin action. These mutations associated with insulin can cause a variety of metabolic abnormalities, ranging from elevated insulin levels and mild high blood sugar to severe diabetes (Antar *et al.,* 2024).

In some cases, individuals with these mutations may display additional symptoms such as acanthosis nigricans (skin darkening), virilization(development of male characteristics), and enlarged cystic ovaries in women (Antar *et al.,* 2024).

2.1.4.3 DISEASES OF THE EXOCRINE PANCREAS

Diabetes can arise from several conditions that lead to widespread damage to the pancreas. Such conditions include infection, pancreatitis, pancreatic carcinoma, trauma, and pancreatectomy(surgical removal of the pancreas). However, significant damage to the pancreas is necessary for diabetes to develop, while sometimes, small affected portions of the pancreas due to adenocarcinomas can also be linked to diabetes.

2.1.4.4. ENDOCRINOPATHIES

Diabetes can be secondary to the overproduction of counter-regulatory hormones, such as in Acromegaly(excess Growth Hormone), Cushing's Syndrome (excess cortisol), and Pheochromocytoma (excess catecholamines) (Martis *et al*., 2023).

2.1.4.5 DRUGS OR CHEMICAL INDUCED DIABETES

Certain drugs and toxins have the potential to interfere with insulin secretion or action, either on their own or by triggering diabetes in individuals who already have insulin resistance (Antar *et al.,* 2023). The use of certain medications, such as glucocorticoids and some antipsychotics, can severely impair glucose metabolism, leading to secondary diabetes, particularly in susceptible patients (Martis *et al*., 2023).

2.1.5 UNCOMMON FORMS OF IMMUNE MEDIATED DIABETES

2..1.5.1 STIFF-MAN SYNDROME

This is an autoimmune neurological disorder often associated with high-titer antibodies like Glutamic acid decarboxylase (GAD), the same autoantigen found in T1DM, highlighting an overlap in autoimmune pathology (Bose and Jacob, 2025). Patients with Stiff-man syndrome may develop T1DM (Pugliese *et al*., 2020).

2.1.5.2 ANTI-INSULIN RECEPTOR ANTIBODY RELATED DIABETES

Antibodies against the insulin receptor can interfere with insulin binding, leading to diabetes. In certain instances, these antibodies can function as insulin agonists, leading to hypoglycemia instead of hyperglycemia. Anti-insulin receptor antibody-related diabetes is a rare, severe form of insulin resistance often referred to as Type B Insulin Resistance Syndrome (IR-B) (Pugliese, 2024).

It is characterized by the presence of autoantibodies directed against the insulin receptor (IR) on target cells (Dhandapani *et al.*, 2022).

2.2 OXIDATIVE STRESS AND DIABETES

Oxidative stress is now considered an indispensable mechanism linking hyperglycemia to cellular damage in DM (Crasto *et al.*, 2021).

Increased ROS production damages blood vessels, promotes inflammation, and impairs endothelial function. These factors contribute to the development of diabetic vascular complications, including diabetic retinopathy (a leading cause of blindness), nephropathy, other sensorial damage, and cardiovascular diseases (Darenskaya *et al.,* 2021).

Furthermore, oxidative stress-induced damage affects various organs and tissues, exacerbating diabetic complications. Kidneys, nerves, and eyes are particularly vulnerable. Oxidative stress disrupts normal renal function, leading to diabetic nephropathy. It also

damages nerves, contributing to the development of diabetic neuropathy, which can manifest as pain, numbness, and impaired sensations. Additionally, oxidative stress plays a role in the development of diabetic retinopathy, leading to vision impairment or even blindness (Yousef *et al.,* 2023; Sasso *et al.,* 2022).

2.3 OXIDATIVE STRESS AND B-CELLS DYSFUNCTION

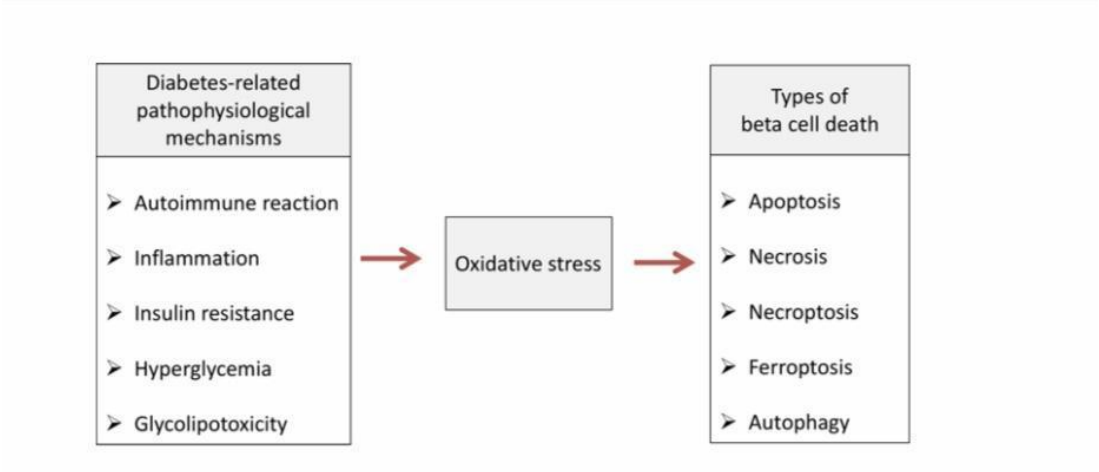

Fig 2.3 Oxidative stress-mediated pathophysiological mechanisms cause various types of beta cell death in diabetic conditions. Pathophysiological processes such as autoimmune reaction, inflammation, insulin resistance, hyperglycemia/glucotoxicity and lipotoxicity lead to pancreatic beta cell death in diabetic condition by inducing oxidative stress which is involved in apoptosis, necrosis, necroptosis, ferroptosis and autophagy (Dinic *et al.,* 2021)

2.4 ANTIOXIDANTS AND DIABETES

The relationship between antioxidants and Diabetes Mellitus (DM) is a key area of current research, revolving around the central role of oxidative stress in the pathogenesis and progression of diabetic complications (Swarup *et al.*, 2023). Chronic hyperglycemia is known to fuel the excessive production of Reactive Oxygen Species (ROS), which overwhelms the body's natural antioxidant defense systems (Swarup *et al*., 2023; Banday *et al.,* 2020).

Elevated glucose levels increase substrate entry into the mitochondrial electron transport chain, causing a rise in the electrochemical gradient and leading to the leakage of electrons, which form superoxide (Swarup *et al.*, 2023).

2.4.1 COMPLICATION OF DIABETES MELLITUS

2.4.1.1 MICROVASCULAR COMPLICATION OF DIABETES MELLITUS

DIABETIC KIDNEY DISEASE (DKD)

Elevation in glucose levels leads to both metabolic and hemodynamic changes, including glomerular hyperfiltration, podocyte injury, and progressive albuminuria, while hypertension accelerates glomerular damage (de Boer *et al.,* 2022). Genetic predisposition, along with lifestyle factors such as obesity and smoking, further increases the risk. Dyslipidemia and oxidative stress contribute to endothelial dysfunction and tubulointerstitial injury (Xu *et al.,* 2023), and inflammation (Oda *et al.,* 2023), and activation of fibrotic pathways play important roles in disease progression (Mohandes *et al.,* 2023; Mlyarnska *et al.,* 2024).

DIABETIC RETINOPATHY

Diabetic retinopathy is the most common cause of blindness worldwide. In DR, vision loss is usually attributed to diabetic macular edema (DME) that impairs central vision, or proliferative diabetic retinopathy (PDR), that might lead to the formation of new blood vessels and fibrous tissue, resulting in fractional retinal detachment and preretinal or vitreous haemorrhage. Risk factors include the duration of diabetes, poor glycemic control, hypertension, and nephropathy (Patil, 2025).

DIABETIC NEUROPATHY

DN, also known as diabetic kidney disease, is a common and severe complication of diabetes that leads to chronic kidney disease. If left unnoticed and untreated, the condition may result in the development of Charcot neuroarthropathy, foot ulceration and finally foot amputation with high impact on quality of life and overall life expectancy (Faselis *et al.*, 2020).

SEXUAL DYSFUNCTION

Sexual dysfunction in type 2 diabetes mellitus patients is an often overlooked complication, despite the high impact of this condition on quality of life. The pathogenesis of erectile dysfunction (ED) in diabetic patients is very complex and it is a mixture of vasculopathic, neuropathic and hormonal changes that are attributed to diabetes mellitus. It is a manifestation of microangiopathy, autonomic neuropathy and macroangiopathy and as a

result, erectile dysfunction could be possibly exploited as an early biomarker for diabetic complications enabling early intervention and better outcomes (Faselis *et al.*, 2020).

2.4.1.2 MACROVASCULAR COMPLICATIONS OF DIABETES MELLITUS.

Patients who have diabetes mellitus tend to have coronary artery, cerebrovascular, myocardial infarction and peripheral vascular disease more often, at an earlier age, and more extensively than the nondiabetic population. Hypertension, elevated blood lipid concentrations, and cigarette smoking are other risk factors for developing macrovascular complications.

2.5 DIABETOGENES

The term diabetogenes refers to the specific chemical compounds, drugs, or infectious agents that are capable of inducing diabetes mellitus in humans or, more commonly, in experimental animal models (Lenzen, 2024). These agents primarily achieve their effect by selectively damaging or destroying the insulin-producing β-cells in the pancreas, leading to a state of absolute or functional insulin deficiency and subsequent hyperglycemia (Banday *et al*., 2020).

2.5.1 COMPARING ALLOXAN WITH STREPTOZOTOCIN AS DIABETOGENIC AGENTS

Streptozotocin has notable advantages over alloxan as chemical agents or induction of experimental diabetes, thus, is often preferred to the latter (alloxan). For instance, streptozotocin has longer half-life (15 min against 1.5 min of alloxan) (Macdonald *et al*., 2017). This makes it more stable in solution before and after injection into animals.

Streptozotocin induced hyperglycemia is relatively more stable and for a longer duration (as much as three months compared to alloxan-induced hyperglycemia that can only be sustained for less than a month). Moreover, the mechanism of streptozotocin diabetogenicity is less associated with cellular toxicity, hence, lesser animal mortality. Alloxan on the contrary, induces diabetes by a mechanism characterized by incidences of ketosis, reactive oxygen species toxicity (ROS), and high mortality rate which is particularly a major setback in experimental diabetes studies (Szkudelski, 2001).

One reason for this is that streptozotocin is more selective to islet beta cells than alloxan which causes severe damage to other cell types which express GLUT2 (systemic toxicity) (Macdonald *et al*., 2017). More so, streptozotocin-induced diabetes is associated with well

characterized diabetic complications unlike alloxan-induced diabetes (Lenzen, 2008). In addition, compared to alloxan, streptozotocin diabetogenicity is not severely interfered with by blood glucose level. Overall, streptozotocin diabetogenicity is more effective and with lesser variation with animal species (Macdonald *et al*., 2017).

2.5.2 STREPTOZOTOCIN (STZ)

Streptozotocin is an antimicrobial agent and has also been used as a chemotherapeutic alkylating agent. Streptozotocin is a naturally occurring methylnitrosourea antineoplastic antibiotic compound. Streptozotocin (STZ) was originally identified in the late 1950s as an antibiotic and was discovered in a strain of the soil microbe *Streptomyces achromogenes* In the mid-1960s, STZ was found to be selectively toxic to the beta cells of the pancreatic islets and thus it is used in animal models of diabetes and as a medical treatment for cancers of the beta cells. STZ is an odorless ivory-colored crystalline powder or pale yellow crystals, with the solubility of 5070 mg l1 in water at 25°C. Streptozotocin is soluble in alcohol and ketones and slightly soluble in polar organic solvents and insoluble in nonpolar organic solvents.

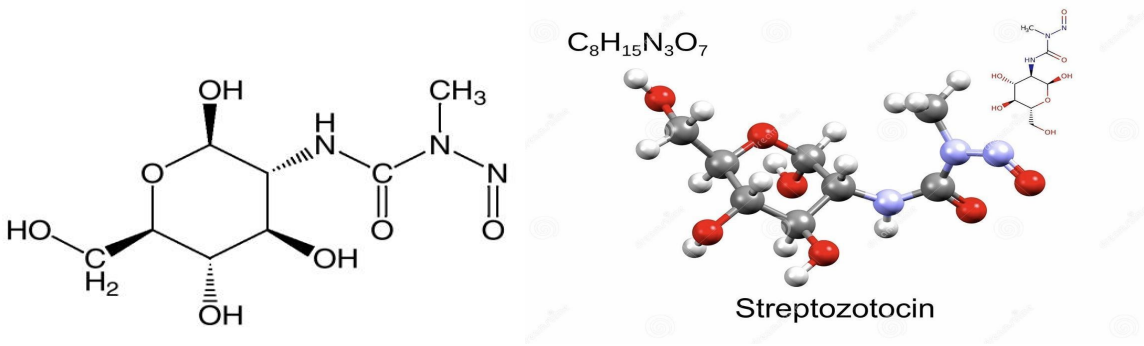

Fig 2.5.2 Chemical formula, skeletal formula, and 3D ball-and-stick model of chemotherapeutic drug streptozotocin (Sharand Chand, 2020).

2.5.3 STRUCTURAL FEATURES OF STREPTOZOTOCIN

Streptozotocin (2-deoxy-2-[3-methyl-3-nitrosourea] 1-D-glucopyranose) exists in two anomers,α and β forms that can be separated by chromatographic methods .It is a pale yellow to off-white crystalline powder. The molecular weight of streptozocin is265 g/mol, chemical formula is C8H15N3O7. The molecular structure of STZ is familiar to2-deoxy-D-glucose substituted at C2 with an N-methyl-N-nitrosourea group. This is the cytotoxic portion of STZ

in beta-cell damage. Streptozotocin is a nitrosourea compound of glucosamine with a methyl group at one end and a glucose molecule at another end.

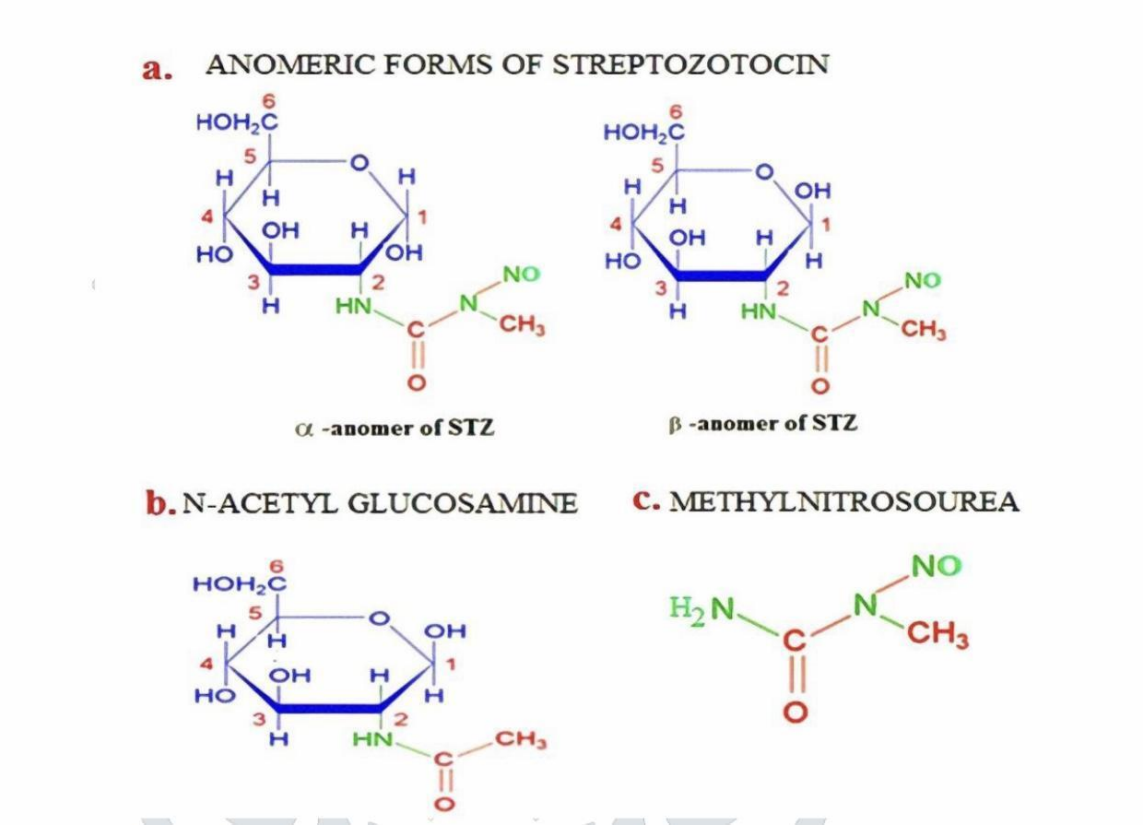

Figure 2.5.3 Chemical Structure of STZ (a. α and β Anomeric forms of STZ b.Structural analog of STZ – N-acetyl glucosamine C. cytotoxic moiety of STZ- N-methyl-N-Nitrosourea) (JETIR, 2023).

2.5.4 STREPTOZOTOCIN: MECHANISM OF ACTION

STZ continues to be understood as a glucosamine-nitrosourea compound that acts as a toxic glucose analogue (Banday *et al.,* 2020). Its selectivity for the β-cells is governed by the high expression of the GLUT2 glucose transporter on the cell membrane, which facilitates its entry (Titisari *et al*., 2025). Once inside, the methylnitrosourea moiety is released, functioning as a potent alkylating agent that causes extensive DNA strand breaks and fragmentation, which is the initial cytotoxic event (Banday *et al.,* 2020).

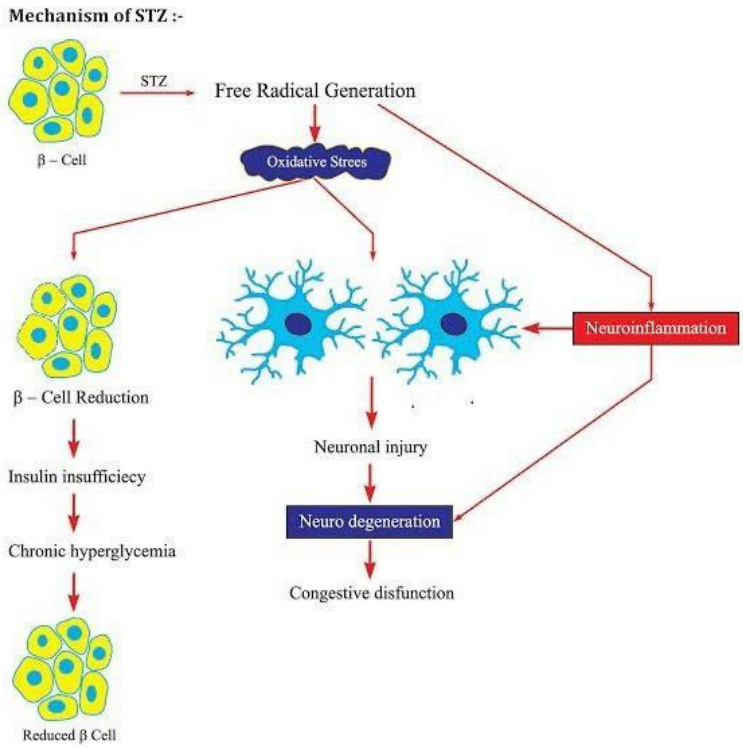

Fig 2.5.4 Mechanism of action of streptozotocin (Raj kumar, 2017).

2.5.5 BETA CELL SELECTIVITY OF STREPTOZOTOCIN

Streptozotocin is a nitrosourea analogue in which the N-methyl-N-nitrosourea (MNU) moiety is linked to the carbon-2 of a hexose. The toxic action of streptozotocin and chemically related alkylating compounds requires their uptake into the cells. The specific ability of Streptozotocin (STZ) to target and destroy the insulin-producing β-cells is fundamental to its use as a diabetogenic agent. This selectivity is primarily mediated by the expression of the GLUT2 glucose transporter on the β-cell membrane (Banday *et al.*, 2020; Titisari *et al.*, 2025).

The high expression of the GLUT2 transporter on β-cells, in contrast to its low expression on other insulin-sensitive tissues (like muscle or fat cells), confers the critical selectivity necessary for STZ's diabetogenic effect (Titisari *et al.*, 2025). This selective uptake allows the toxic moiety of STZ to accumulate within the β-cell, reaching cytotoxic concentrations not attained elsewhere (Banday *et al.*,, 2020).

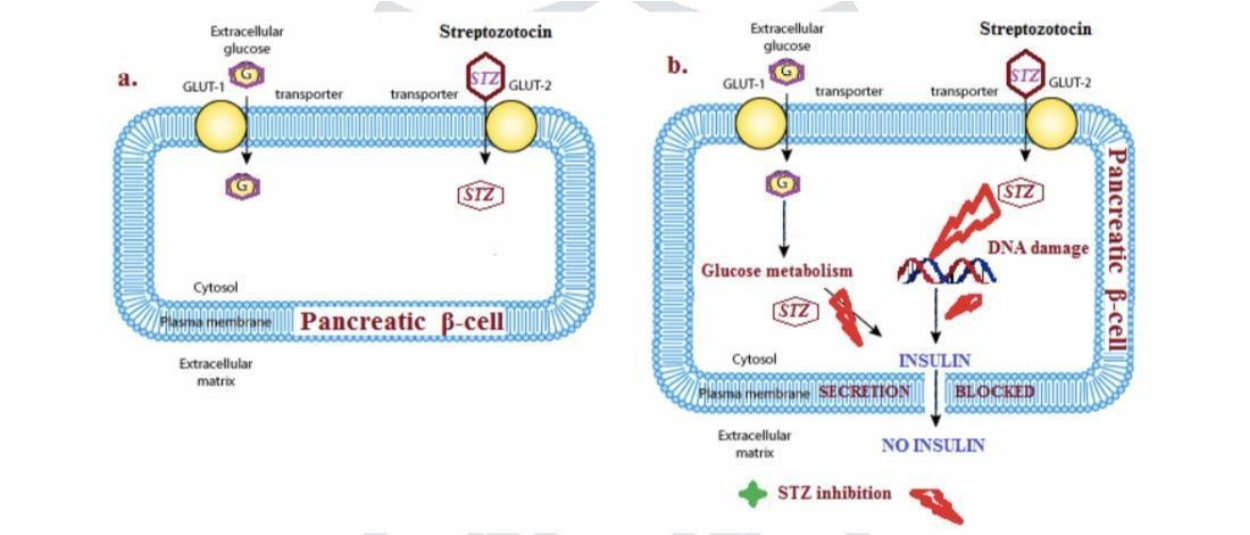

Fig 2.5.5 Selective uptake of Streptozotocin by Beta cells of the pancreas (JETIR, 2023).

2.5.6 BETA CELL TOXICITY OF STREPTOZOTOCIN

STZ's structure, containing a methylnitrosourea moiety attached to a glucose molecule, grants it recognition by the beta-cell's plasma membrane (Banday, Sameer, and Nissar, 2020. The beta-cell is selectively targeted because it expresses high levels of the (GLUT 2) glucose transporter (Titisari *et al*., 2025). This transporter facilitates the rapid and concentrated influx of STZ into the cytoplasm, achieving a lethal concentration that is not reached in other cells (Banday *et al.,* 2020). Once inside the beta-cell, STZ initiates two major, interconnected pathways of destruction. First is DNA Alkylation, where it targets the DNA nucleus to cause extensive strand breakage, thus leading to cellular injury.

The massive DNA damage activates the nuclear repair enzyme Poly(ADP-ribose) polymerase (PARP) (Banday et al., 2020). The activation of PARP consumes enormous amounts of the cellular energy molecule NAD+ (Nicotinamide Adenine Dinucleotide) in an attempt to repair the DNA (Ghasemi et al., 2023). This leads to a severe metabolic collapse due to ATP and NAD+ depletion. Furthermore, STZ metabolism contributes to the generation of Reactive Oxygen Species (ROS), including Nitric Oxide (NO), which contribute to oxidative stress and precipitate beta-cell death via apoptosis and necrosis (Banday *et al*., 2020).

2.6 *Picralima nitida* MEDICINAL OVERVIEW

2.6.1 BOTANICAL DESCRIPTION AND ETHNOMEDICINAL USES

Picralima nitida (Staph) (Apocynaceae) otherwise known as Akuama or 'Abeere' among the Yoruba speaking of South–Western Nigeria, is a medicinal plant with diverse uses *(*Kazeem *et al.,* 2013*).*

This plant is the first species of the genus to be described and is widely distributed in the vast majority of the forest of equatorial Africa, particularly in Cameroon, Nigeria, Congo, Ghana, or Uganda, where its different parts are used for therapeutic purposes *(*Akabassi *et al.,* 2020*,* Akabassi *et al.,* 2021).

Picralima nitida is an understorey tree which reaches up to 4-35 m in height, crown dense, trunk 5-60 m diameter; cylindrical, the wood is pale yellow, hard, elastic, fine-grained and taking a high polish. The flowers are white (about 3cm long) and they have ovoid fruits which become yellow when mature. The leaves are broad (3-10 cm) and oblong (6-20 cm long) with tough tiny lateral nerves of about 14 to 24 pairs.

The seeds of its ovoid fruit, whose characteristic yellowish color, as well as the bark, or its roots, are used empirically in traditional medicine, chewed, eaten or built in form of decoction to treat malaria, or typhoid fever, particularly in Africa (Dibong *et al.,* 2011, Iyamah and Idu, 2015, Jiofack *et al.,* 2009).

They are also used in several villages in Benin in the treatment of diabetes, sexual weakness, hemorrhoid, headaches and stomach aches, cough, and also as an analgesic (Akabassi *et al.,* 2021). Preparations from different parts of the plant are employed as crude drug or crude herbal extract as remedy for various kinds of human diseases.

The practitioners address a wide range of human health issues by using raw materials and extracts from various *P. nitida* components, which could be linked to the various secondary metabolites including alkaloids, triterpenes flavonoids,lycosides, polyphenols, akuammicine, saponins, and tannins (Adeola *et al.,* 2024).

Fig 2.6.1 *Picralima nitida* fruit (Dzotam and Kuete, 2023).

2.6.2 PHYTOCHEMICAL PROFILE OF *Picralima nitida* FRUIT EXTRACT

Phytochemical screening of *Picralima nitida* has revealed the presence of alkaloids, tannins, saponins, steroids, flavonoids, terpenoids, steroids, and glycosides in the plant (Olumese *et al*., 2023).

Alkaloids are the predominant bioactive compounds that have, so far, been isolated from the seeds of *Picralima nitida* (Alcover *et al*., 2020).

Alkaloids are the major class of phytochemicals isolated from *Picralima nitida*. The first set of alkaloids isolated from *Picralima nitida* are the indole alkaloids; akuammine, pseudoakuammine, akuammidine, akuammicine, akuammigine, pseudoakuammigine, akuammiline and akuammenine. Alkaloids are the predominant bioactive compounds that have, so far, been isolated from the seeds of *Picralima nitida* (Alcover *et al*., 2020).

These phytochemicals are primarily believed to boost food and medicinal plants' antioxidant capacities, considerably assisting in the fight against various pathological conditions like cancer, diabetes, aging, cardiovascular disease, and other degenerative diseases (Yu et al., 2021).

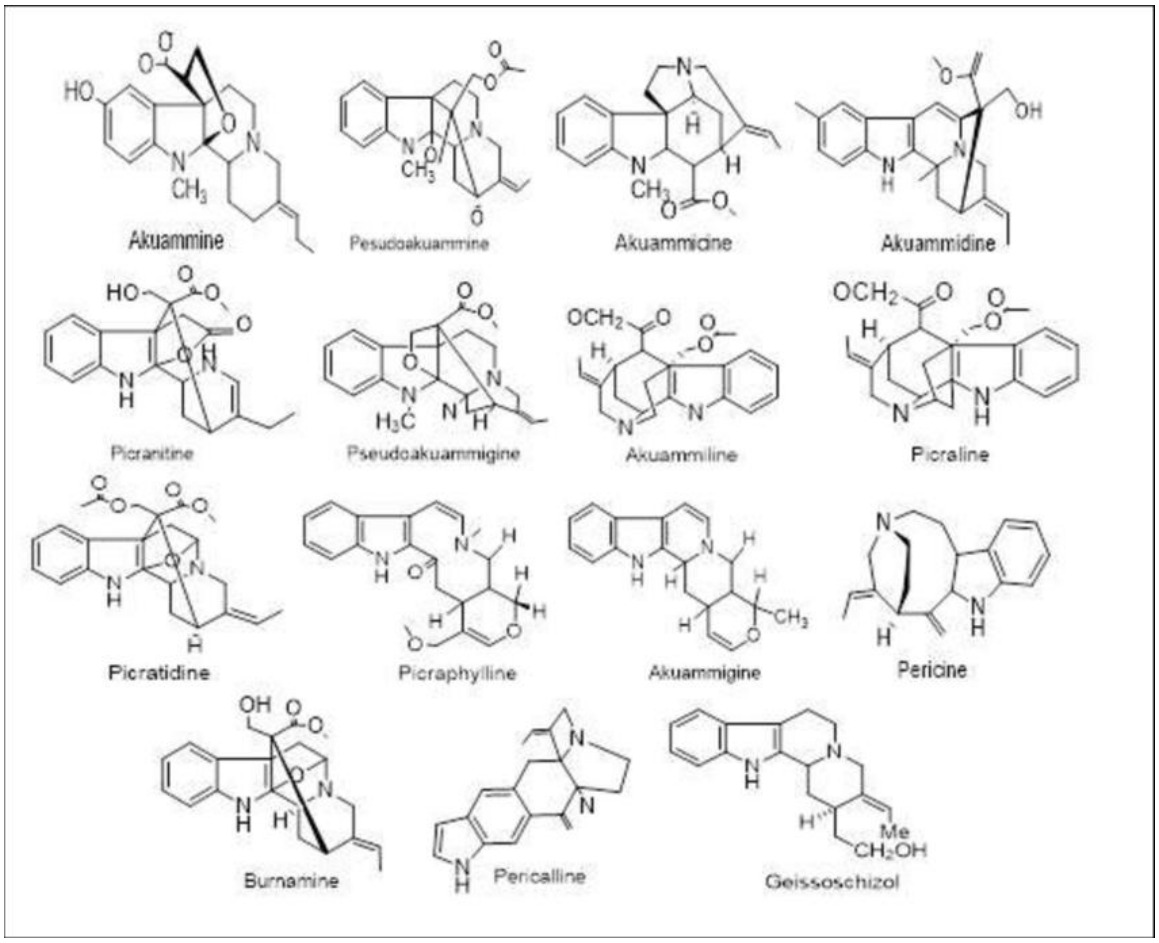

Fig 2.6.2 chemical structures of alkaloids isolated from *Picralima nitida* (Koto-Te-Nyiws, 2023).

2.6.3 PHARMACOLOGICAL PROPERTIES OF *Picralima nitida*

Based on the claimed ethnomedicinal uses, scientists have investigated a number of pharmacological parameters such as antimalarial, anti-inflammatory, analgesic, antidiabetic, antimicrobial, antioxidant, antiulcer, cytotoxic and toxicological profile of extracts as well as compounds isolated from *Picralima nitida* (Olumese *et al*., 2023).

Analgesic and anti-inflammatory properties: Studies on alkaloids isolated from *Picralima nitida* seeds such as akuammine, pseudo-akuammigine, akuammicine, and akuammiline, have shown that they target opioid receptors (Zhu *et al.,* 2021) thus producing pain relieving effects. Research has also demonstrated that extracts from the stem bark of *Picralima nitida* possess significant anti-inflammatory activity, effectively reducing inflammation in animal models (Oladosu *et al., 2021).*

Antidiabetic and hypoglycemic properties: Extracts of *Picralima nitida* have been shown to reduce plasma glucose and insulin resistance in high fat, high-fructose-fed rats (Okoro *et al.,* 2020).

Antimalarial properties: The plant's root bark extract has been shown to have a concentration-dependent antioxidant activity in mice infected with *plasmodium berghei* (Erharuyi *et al., 2023).*

2.7 PANCREATIC AMYLASE: OVERVIEW, STRUCTURE AND REGULATION

Pancreatic amylase is a powerful enzyme that plays a vital role in the digestion of complex carbohydrates. Disruptions in pancreatic amylase secretion or activity can lead to carbohydrate malabsorption and related health issues (Fend, 2023).

Structurally, it is a single chain polypeptide with a molecular weight of approximately 55 kDa and is composed of three domains: the catalytic domain (domain A), a carbohydrate-binding domain (domain B), and a C-terminal β-sheet domain (domain C).

Recent studies show that in T2DM, the functional integrity of this axis is compromised (Abdulkareem *et al.*, 2024). This derangement leads to a functional impairment of the exocrine cells due to the endocrine dysfunction (Darenskaya *et al*., 2021). The prevailing observation is that patients with T2DM frequently exhibit significantly lower serum amylase levels compared to healthy controls (Daruwala *et al.*, 2023).

Multiple studies confirm that lower serum amylase levels are associated with T2DM (Daruwala *et al.*, 2023). This reduction is often more pronounced in patients with poorer glycemic control (higher HbA1c levels) and longer duration of the disease (Abdulkareem *et al.*, 2024). This negative correlation suggests that as the metabolic dysfunction progresses, the exocrine pancreas is increasingly affected (Daruwala *et al.*, 2023).

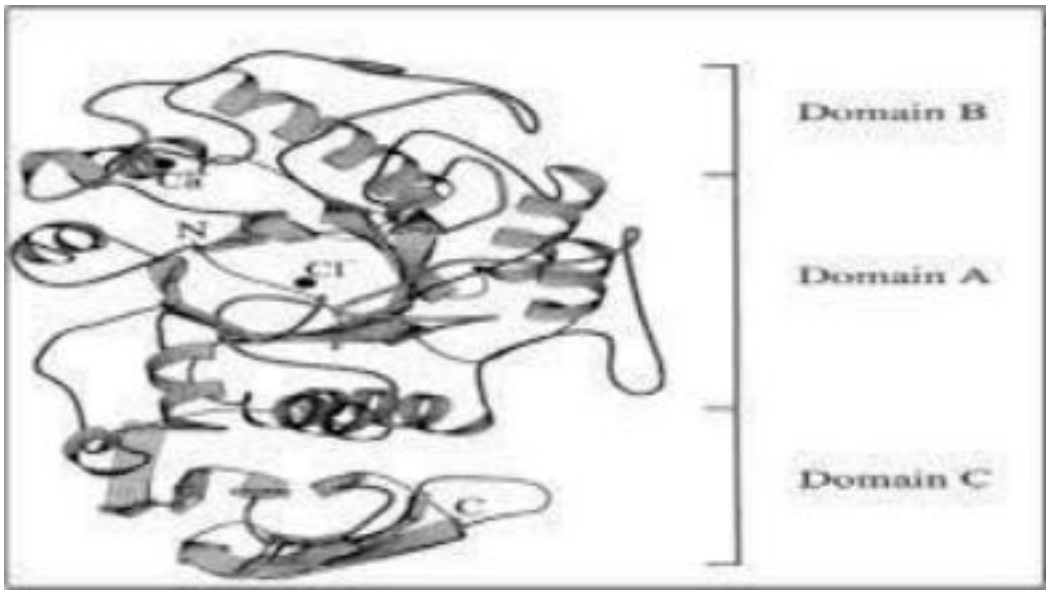

Fig 2.7 3D structure of human pancreatic amylase (Abdulkareem *et al.,* 2024)

2.7.1 ROLES OF PANCREATIC AMYLASE IN CARBOHYDRATE METABOLISM

Pancreatic amylase plays a critical role in the enzymatic breakdown of dietary polysaccharides into disaccharides and oligosaccharides in the small intestine. Pancreatic amylase is essential for carbohydrate metabolism, specifically focusing on the digestion of complex starches in the small intestine (Dion and Rudan, 2023).

Pancreatic amylase is secreted by the acinar cells into the duodenum, where it hydrolyzes the α-1,4 glycosidic bonds in starch and glycogen (Swarup *et al.*, 2023). This process breaks complex carbohydrates into disaccharides (like maltose) and α-dextrins, which are then further converted to absorbable glucose by brush border enzymes (Dhandapani *et al.,* 2022).

Dysregulation or inhibition of pancreatic amylase activity has therapeutic implications in the management of metabolic disorders such as obesity and type 2 diabetes mellitus

2.7.2 NATURAL INHIBITORS AND MECHANISM OF ACTION

The inhibition of pancreatic amylase is a recognized strategy for controlling hyperglycemia by slowing carbohydrate digestion and glucose absorption. Natural inhibitors are commonly found in plant extracts, particularly those rich in polyphenolic compounds, alkaloids, terpenoids, and flavonoids . These inhibitors can act through various mechanisms:

i. Competitive inhibition, where the inhibitor mimics the substrate and binds to the active site;
ii. Non-competitive inhibition, involving binding to a site distinct from the active site;

iii. Uncompetitive inhibition, which occurs only after substrate binding

2.7.3 THE ROLE AND DYSREGULATION OF PANCREATIC AMYLASE IN DIABETES MELLITUS

Pancreatic alpha-amylase is a crucial digestive enzyme synthesized and secreted by the exocrine pancreas, responsible for hydrolyzing starch into simpler sugars like maltose and maltotriose (Shrestha *et al*., 2023). Its function is tightly linked to the endocrine pancreas through the intricate insulo-acinar axis, making it highly susceptible to the pathological changes characteristic of diabetes mellitus (DM).

2.7.3.1 BIOSYNTHESIS AND REGULATED SECRETION OF PANCREATIC AMYLASE

The production and release of pancreatic amylase is a high-fidelity process carried out by the pancreatic acinar cells, which boast one of the highest rates of protein synthesis in the mammalian body (Gorelick *et al.,* n.d). This process follows the classical regulated secretory pathways.

A. BIOSYNTHESIS AND PROCESSING

1. **Synthesis in the Rough Endoplasmic Reticulum (RER):** Amylase synthesis begins when ribosomal subunits attach to messenger RNA (mRNA) for the amylase gene AMY2A or AMY2B (Abdulkareem, 2024).A hydrophobic "signal" sequence on the nascent polypeptide targets the ribosome complex to the outer surface of the RER, where the protein is synthesized into the rough endoplasmic reticulum lumen (Gorelick *et al.,* n.d).
2. **Post-Translational Modification:** Within the RER, the newly synthesized amylase polypeptide undergoes critical modifications, including disulfide bridge formation and proper folding to achieve its tertiary and quaternary structures. Amylase is a calcium-dependent metalloenzyme, and its functional integrity relies on the presence of calcium and specific anions, particularly chloride (Shrestha *et al*., 2023). Misfolded proteins are subject to the ER quality control system; chronic failure of this system leads to ER stress, which can impair overall acinar cell function (Gorelick *et al.,* n.d).

3. **Transport to the Golgi Complex:** Processed proteins are transported from the RER to the Golgi complex. Here, further post-translational modifications, such as glycosylation, occur, and the enzymes are concentrated (Gorelick *et al.,* n.d).
4. **Sorting and Packaging:** The Golgi complex is responsible for sorting digestive enzymes and packaging them into specialized secretory vesicles known as zymogen granules. These granules serve as the storage site for amylase and other digestive proenzymes (Gorelick *et al.,* n.d).

B. Regulated Secretion (Exocytosis)

1. **Stimulation:** Cholecystokinin(CCK) is released from the duodenal mucosa in response to the presence of fat and protein digestion products, while Acetylcholine(ACh)is released from vagal nerve endings.
2. **Intracellular Signaling:** Both Cholecystokinin and Acetylcholine bind to specific receptors on the acinar cell, leading to the generation of intracellular signals. A key mediator of this process is the rise in cytosolic free calcium concentration (Ca2+), which is released from Endoplasmic reticulum (ER) stores (Gorelick *et al.,* n.d).
3. **Exocytosis:** The increase in cytosolic Ca2+ drives the movement of zymogen granules to the apical (luminal) plasma membrane. This process involves a complex machinery of proteins, including GTP-binding proteins, which mediate the fusion of the granule membrane with the plasma membrane, releasing the stored amylase into the pancreatic ductal system for transport to the duodenum (Gorelick *et al.,* n.d).

2.7.3.2 DYSREGULATION OF PANCREATIC AMYLASE SECRETION IN DIABETES

Diabetes mellitus, both Type 1 (T1DM) and Type 2 (T2DM), often presents with varying degrees of exocrine pancreatic dysfunction, frequently leading to pancreatic exocrine insufficiency (PEI), defined by the inadequate secretion of enzymes like amylase

The Insulo-Acinar Axis and Disruption

The regulation of amylase is highly dependent on the "insulo-acinar axis"—a paracrine relationship where high concentrations of insulin, secreted from the islets of Langerhans, reach the surrounding acinar cells via the peri-insular portal vasculature before entering the systemic circulation (Abdulkareem, 2024).

1. **Insulin's Trophic Effect:** Insulin acts as a trophic factor on the acinar cells, binding to insulin receptors and stimulating various mechanisms, including the regulation of amylase gene transcription, enhancement of acinar protein synthesis, and potentiation of amylase secretion (Abdulkareem, 2024).
2. **Disruption in DM:** In Diabetes mellitus DM, the deficiency (in T1DM) or resistance (in T2DM) to insulin action profoundly disrupts this trophic signaling, directly impacting the acinar cell's ability to synthesize and secrete amylase (Abdulkareem, 2024).

2.7.3.4 DYSREGULATION MECHANISMS BY DIABETES TYPES

Type 1 Diabetes Mellitus

T1DM involves autoimmune destruction of beta-cells, leading to absolute insulin deficiency.

- **Insulinopenia and Atrophy:** The loss of insulin's trophic effect is the primary mechanism. Studies have shown a significant reduction in pancreas organ size (atrophy) inT1DM patients, often involving greater atrophy in the body and tail where beta-cells are more concentrated (Bruggeman and Schatz, 2023). This atrophy directly reduces the acinar cell mass available for amylase production.
- **Autoimmunity:** Leading hypotheses suggest that T1DMis a disorder of both the endocrine and exocrine pancreas from the start. Autoantibodies targeting islet cells may also trigger inflammatory or autoimmune destruction of exocrine cells (Bruggeman and Schatz, 2023).

Type 2 Diabetes Mellitus

T2DM involves a complex mix of insulin resistance and defective insulin secretion.

- **Insulin Resistance and Hyperglycemia:** Chronic hyperglycemia and insulin resistance cause damage to the pancreatic acinar cells and the intricate insulo-acinar axis (Patel *et al*., 2023).
- **Anti-Insulin Hormone Activity:** Some studies suggest that the increased activity of anti-insulin hormones (like glucagon, which is often dysregulated in T1DM may contribute to acinar atrophy and reduced enzyme secretion (Bruggeman and Schatz, 2023, Patel *et al*., 2023).
- **Observed Variability:** The findings on serum amylase levels in T2DM are more variable than in T1DM While many studies show a significant decrease in mean serum amylase and lipase activity compared to healthy controls, especially in long-standing disease or cases with very low serum insulin (Patel *et al*., 2023).

2.7.3.5 THE INFLUENCE OF DIABETES ON PANCREATIC AMYLASE

The overall influence of diabetes is a cascade that progresses from regulatory and expression changes to functional insufficiency.

A. Influence on Amylase Gene Expression and Synthesis

Experimental models and clinical data confirm that pancreatic amylase expression is insulin-dependent. Studies dating back to 1981 demonstrated that amylase mRNA is reduced in the pancreas of diabetic subjects (such as diabetic mice) and that this reduction can be recovered by insulin treatment, clearly indicating that insulin is a trophic and regulatory signal for amylase gene transcription and synthesis. The lack of insulin signaling in T1DM, thus directly suppresses the machinery needed to produce the enzyme.

B. Functional Consequences (Pancreatic Exocrine Insufficiency)

The dysregulation of amylase synthesis and secretion leads directly to the functional deficit known as Pancreatic Exocrine Insufficiency (PEI).

- **Maldigestion:** Insufficient secretion of amylase into the small intestine results in the maldigestion of complex carbohydrates (starch). The clinical threshold for

symptomatic Pancreatic Exocrine Insufficiency (malabsorption) is often reached when pancreatic enzyme activity falls below 10% of normal levels

- **Nutritional Deficits:** Chronic maldigestion can lead to deficiency of micronutrients (like fat-soluble vitamins A, D, E and K) macronutrients, potentially exacerbating weight loss and malnutrition, particularly in T1DM
- **Clinical Marker:** Low serum amylase is now considered a potential marker for impaired pancreatic function associated with metabolic syndrome and diabetes [1, 10]. Studies have shown that the reduction in serum amylase levels correlates negatively with the duration of the disease and positively with the severity of hyperglycemia HbA, suggesting that amylase activity reflects the chronicity and progression of pancreatic damage in diabetes mellitus (Patel *et al*. 2023).

2.8 CLINICAL RELEVANCE

The high prevalence Pancreatic Exocrine Insufficiency in diabetes mellitus up to 50% in some studies, with rates generally higher in T1DM. It underscores the importance of the endocrine-exocrine crosstalk (Bruggeman and Schatz, 2023). The clinical symptoms of Pancreatic Exocrine Insufficiency (flatulence, abdominal discomfort, diarrhea) are often vague and can be misdiagnosed as side effects of common diabetic medications (e.g.,Glibenclamide) (Koryps *et al*., 2018). Therefore, the assessment of pancreatic enzymes, such as fecal elastase-1 (a diagnostic tool for Pancreatic Exocrine Insufficiency and serum amylase) is increasingly recommended for comprehensive evaluation and management of long-standing or poorly controlled diabetes patients ((Koryps *et al*., 2018, Shetty *et al*., 2018).

2.9 GLIBENCLAMIDE: OVERVIEW

Glibenclamide, also known as glyburide, is an essential oral anti-diabetic agent belonging to the second-generation class of sulfonylureas. It is widely used as an adjunct to diet and exercise to improve glycemic control in adults diagnosed with Type 2 Diabetes Mellitus (T2DM) (Ahmed *et al*., 2023)

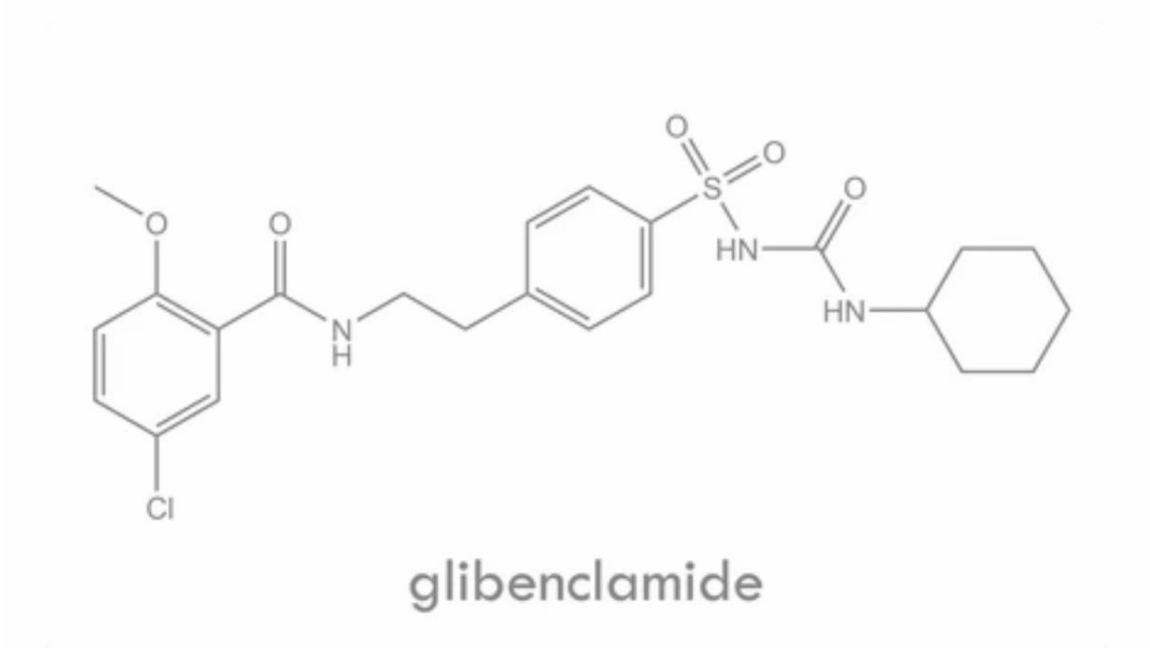

Fig 2.9 Chemical structure of glibenclamide (MedChemExpress).

2.9.1 MECHANISM OF ACTION

Glibenclamide exerts its primary blood glucose-lowering effect by stimulating the release of insulin from the pancreatic beta cells, a process dependent on the functionality of these cells (DrugBank Online, 2025).

The mechanism involves binding to the sulfonylurea receptor 1 (SUR1), which is the regulatory subunit of the ATP-sensitive potassium channels K (ATP) found on the pancreatic beta-cell membrane (Ahmed et al., 2023; DrugBank Online, 2025. Glibenclamide binds to and inhibits the potassium (K_(ATP) channels, causing them to close (DrugBank Online, 2025). The closure of these potassium channels prevents potassium ions from exiting the cell, leading to depolarization of the cell membrane). This depolarization subsequently opens voltage-gated calcium channels. The resulting influx of calcium ions into the beta cell increases the intracellular calcium concentration, which triggers the release of insulin-containing granules (DrugBank Online, 2025).

Studies have also suggested that glibenclamide can inhibit the enzyme Carnityl Acyl Transferase I (CAT-I) in the mitochondria, which could prevent the transport of long-chain fatty acids for beta-oxidation, offering another mechanism to prevent hyperglycemia (DrugBank Online, 2025).

2.9.2 PHARMOKINETICS

Glibenclamide is administered orally and is rapidly absorbed from the gastrointestinal tract

- **Absorption and Peak Concentration:** It is well-absorbed, with peak plasma concentrations generally occurring within 2 to 4 hours (Pharmacology of Glibenclamide, 2025).
- **Protein Binding:** Sulfonylurea drugs are extensively bound to serum proteins, primarily albumin (FDA Verification Portal, 2025).
- **Metabolism:** Glibenclamide undergoes extensive metabolism in the liver, principally through the cytochrome P450 isoenzymes CYP2C9 and, to a lesser extent, CYP3A4 (Effects and serum levels of glibenclamide, 2003; EFDA, 2023). It is metabolized into several derivatives, including the 4-trans-hydroxy (M1) and 3-cis-hydroxy (M2b) derivatives, which are pharmacologically active and contribute to the drug's sustained hypoglycemic effect (FDA Verification Portal, 2025).
- **Elimination:** The metabolites are eliminated in both urine and bile/feces, with approximately 50% excreted through each route (Pharmacology of Glibenclamide, 2025). The terminal elimination half-life is typically about 10 hours, but reported half-lives can vary between approximately 4 to 8 hours and 10 hours (FDA Verification Portal, 2025; Pharmacology of Glibenclamide, 2025).
- **Pharmacogenomics:** Genetic variations, such as polymorphisms in the *CYP2C9* gene, have been shown to affect the appropriate therapeutic response to glibenclamide. Similarly, polymorphisms in the *KCNJ11*gene, which encodes the K(ATP) channel subunit Kir6.2, are associated with an increased risk of secondary failure to the drug and hypoglycemic effects (Ghasemi *et al.,* 2022).

2.9.3 ADVERSE EFFECTS AND SAFETY PROFILE

Hypoglycemia (low blood sugar) is the most common and clinically significant adverse effect associated with glibenclamide therapy. This risk is greater compared to other sulfonylureas and can occasionally be severe, prolonged, and life-threatening (DrugBank Online, 2025).

Commonly reported side effects include:

- Headache, dizziness, and fatigue (Glibenclamide: Indications, Uses, Dosage, 2023).
- Gastrointestinal disturbances such as nausea, heartburn, abdominal discomfort, and diarrhea (Glibenclamide: Indications, Uses, Dosage, 2023; DrugBank Online, 2025).

- Weight gain (Glibenclamide: Indications, Uses, Dosage, 2023).

Less common or rare adverse effects include skin reactions (rashes, photosensitivity), allergic reactions, and liver function abnormalities (Glibenclamide: Indications, Uses, Dosage, 2023).

- **Cardiovascular Risk:** Concerns exist regarding the use of glibenclamide in diabetic patients with cardiovascular risk. Observational studies have suggested an association between glibenclamide use and an increased risk of cardiovascular mortality compared to agents like metformin or gliclazide, particularly in patients with diagnosed coronary disease (EFDA, 2023).

2.9.4 CLINICAL DOSING AND EFFICACY

Glibenclamide is typically taken orally, once daily with breakfast or the first main meal to maximize effectiveness and minimize hypoglycemia risk (Pharmacology of Glibenclamide, 2025). The usual starting dose for adults is 2.5 to 5 mg daily, with a usual maintenance dose ranging from 1.25 to 20mg daily, often given as a single dose (Pharmacology of Glibenclamide, 2025).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Equipment and Apparatus

Digital weighing scale (NEWSPRING Digital Scale, China; Model NS- 790)

Muslin cloth

Cotton wool

pH Meter (PHS-25 pH meter, China; Model PHS-25)

Chloroform

Refrigerator ((HAIER THERMOCOOL, China; Model HRF- 3500X)

Permanent markers

Dissecting kit

Methylated Spirit

Freeze Dryer (BIOBASE Freeze Dryer, China; Model BK- FD10S)

Oven (WINCOM Thermostat Oven, Model OV-A25SF)

Dry Herb Industrial Grinder

Distilled water

Centrifuge

Plain, EDTA and lithium heparin sample containers

Syringes (5 mL)

Insulin syringe (1mL)

16–18 gauge stainless-steel gavage needle

Glucometer (NEWSPRING Glucose Meter, China; Model KF-B12)

Nose mask

Lancets

Hand gloves

3.2 CHEMICALS AND REAGENT

Streptozotocin

Chloroform

Glibenclamide

Citrate buffer

Phosphate buffer

DNSA (Dinitrosalicyclic acid)

3.2.1 COLLECTION OF PLANT MATERIALS (*Picralima nitida)*

The *Picralima nitida* fruit was obtained from Oliha market, along Siloko Road, Benin City, Edo state, Nigeria. Each fruit weighed between 0.2kg-0.3kg. The plant material was identified in the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Nigeria. The Voucher Number given was UBH-P424 after the identification process was completed.

3.2.2 PREPARATION OF PLANT EXTRACT

The *Picralima nitida* fruits were washed thoroughly with clean water to remove dirt. The outer part of the fruit or the rind was manually peeled off and the fruit further cut into smaller chunks including the seeds. The chopped up fruits were then subjected to oven-dry at 45°C for a period of four(4) weeks to ensure a moisture free residue. The dried fruit weighed around 2,202.19g and further pulverized (crushed to powder). The powdered material was soaked in distilled water at a ratio of 1:10 w/v (100 g of powder in 1000 mL of water) for 72 hours (3 days). The mixture was stirred at intervals to ensure appropriate aqueous extraction and to encourage extraction efficiency. The mixture containing the powdered *Picralima nitida* and distilled water was filtered using a Muslin cloth. The filtrate was then evaporated to dryness using a freeze dryer. A total yield of 1,271.98g (57.78%) of the extract was obtained after freeze drying. Phytochemical analysis of the freeze dried aqueous extract revealed the presence of significant bioactive compounds. The extract was found to contain 39.24 mg GAE/g extract of total phenols and ~24.39 mg QE/g extract of flavonoids ~24.4 mg QE/g, as reported by (Ilenowa *et al.* 2024). The bioactive compounds are known for their antioxidant properties and contribute to the therapeutic potential of the extract.

3.3 EXPERIMENTAL ANIMALS

A total of twenty-four (24) adult male Wistar rats, with average weight of 107.6g-169.3g were obtained from the Department of Anatomy, University of Benin, Nigeria. The animals were kept in a well aerated room at the Department of Anatomy Anima house, University of Benin. The animals were housed in clean, well-ventilated plastic cages with soft wood shavings for bedding and were allowed the recommended diurnal 12 hours light and dark cycles. The animals were acclimatized for 4 weeks before commencement of study. The environmental temperature was between 25-29^{o} C. The rats were provided water ad libitum and standard feed (Chicken Grower Feed) as a basal diet. Treatment of the animals confirmed to the guidelines for the care and use of laboratory animals (NAS, 2011).

3.3.1 EXPERIMENTAL DESIGN

After acclimatization, the rats were weighed and divided into (4) groups of 5 rats and (1) group of 4 rats. They include: Group 0 (155g- 169.3g; Group 1 (107.6g – 120.3g); Group 2 (143.2g – 148.2g); Group 3 (138.7g – 142.2g); and Group 4 (148.3g – 154.5g). . Each rat was assigned a unique identification number based on the part of the body where the colour was applied. The rats were fed with the standard feed (Chicken grower mesh), clean tap water, the fruit extracts to be experimented upon at different doses depending on the weight of the rats and glibenclamide after induction of Streptozotocin. During this period, the animals were observed for toxicity and mortality.

3.3.2 EXPERIMENTAL GROUPING

Thirty-four(24) rats were divided into four (4) groups of five (5) rats and (1) group of (4) rats according to their varying body weight.

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

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

Group 2: Diabetic rats treated with Glibenclamide (glanil)(5mg/kg)

Group 4: Diabetic rats treated with Picralima nitida extract (200 mg/kg).

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

3.4 INDUCTION OF DIABETES

The animals were fasted overnight, with which their weight blood glucose checked prior to induction. Each animal was induced with a single dose of freshly prepared streptozotocin at a

dose of 60mg/kg body weight intra-peritoneally. After induction, the rats were allowed to drink 0.4% glucose solution for 24 hours to prevent initial hypoglycemia. Blood glucose levels were measured 72 hours (3 days) post-induction using a glucometer. Rats with fasting blood glucose levels above 200mg/dL were considered diabetic. Re-induction was done a week after some rats were found to neither exhibiting diabetic symptoms nor having increased blood glucose after streptozotocin induction. After induction, the rats were allowed to drink 0.4% glucose solution for 24 hours to prevent hypoglycemia. Blood glucose levels were measured 72 hours post-induction using a glucometer. Rats with fasting blood glucose levels above 110 mg/dL were considered diabetic.

3.4.1 PREPARATION OF STREPTOZOTOCIN

The streptozotocin was prepared by dissolving 0.30g of streptozotocin in 10 mL of 0.1 M citrate buffer (pH 4.5). The buffer solution was mixed thoroughly until the streptozotocin completely dissolved. Preparation was done under low light conditions to prevent degradation, after which the resulting solution was stored in a refrigerator until use. A second batch of streptozotocin was freshly prepared by dissolving 0.20g of streptozotocin 0.05M citrate buffer (pH 4.5).

3.4.2 DOSAGE CALCULATION OF STREPTOZOTOCIN

i) For a standard dose of 60mg/kg; 60mg of streptozotocin is required for 1kg body weight of rat

ii) For a rat weighing 154g, the exact dose to be given is ;

$$60\ \text{mg} \times 154\ \text{g}/1000\ \text{g}$$
$$= 9.24\ \text{mg}$$

So, the volume of streptozotocin solution required for a rat weighing 154g was calculated as follows, with the concentration of 0.3g (300mg) of Streptozotocin in 10mL

300mg = 10mL

Volume = 9.24mg×10mL/300mg

= 0.31mL

3.4.2.1 RE-INDUCTION OF STREPTOZOTOCIN

The dosage of Streptozotocin used for induction was 60 mg/kg body weight. The dose was calculated as follows:

i) For a standard dose of 40mg/kg; 40mg of streptozotocin is required for 1kg body weight of rat

ii) For a rat weighing 110g, the exact dose to be given is ;

40mg ×110g/1000 g

= 4.40mg

So," the volume of streptozotocin solution required for a rat weighing 110g was calculated as follows, with the concentration of 0.2g (200mg) of Streptozotocin in 10mL

200mg = 10mL

Volume = 4.40mg×10mL/200mg

= 0.25mL

3.4.3 PREPARATION OF CITRATE BUFFER

The citrate buffer was freshly prepared by 0.1L(100mL) of 0.1M citrate buffer, pH 4.50, by dissolving 20.80g/L citric acid monohydrate and 0.29g trisodium citrate dihydrate in ≈80 mL of water. Then, the pH.was checked and adjusted to 4.50 and then volume was brought to 0.1L(100mL).

3.4.3.1 PH CALCULATION

The pH was calculated using the nderson–Hasselbalch equation;

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

20.80g citric acid monohydrate (A) and 0.29g trisodium citrate dihydrate (B) in ≈800 mL of water. Then, the pH.was checked and adjusted to 4.50 and then volume was brought to to 1L(1000mL).

0.1M citrate buffer (pH 4.5) were prepared by mixing appropriate amounts of citric acid and sodium citrate in distilled water. The buffer was mixed thoroughly and ensured to be free from light and moisture exposure.

A second batch of citrate buffer was prepared for reinduction alongside streptozotocin. The calculation for pH goes as follows;

The citrate buffer was freshly prepared by 0.1L(100mL) of 0.05 M citrate buffer, pH 4.50, by dissolving 1.04g citric acid monohydrate and 0.0147g trisodium citrate dihydrate in ≈80 mL of water. Then, the pH was checked and adjusted to 4.50 and then volume was brought to to 0.1L(100mL).

The pH was calculated using the Henderson–Hasselbalch equation:

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

1.04g citric acid monohydrate (A) and 0.0147g trisodium citrate dihydrate (B) in ≈80 mL of water. Then, the pH.was checked and adjusted to 4.50 and then volume was brought to 0.1L(100mL).

0.05M citrate buffer (pH 4.5) were prepared by mixing appropriate amounts of citric acid and sodium citrate in distilled water. The buffer was mixed thoroughly and ensured to be free from light and moisture exposure.

3.5 ADMINISTRATION OF EXTRACT

The aqueous extract of *Picralima nitida* fruit was introduced was used to introduce into the rats by oral gavage. Four (4) groups of rats were confirmed diabetic, while two (2) groups received the extract via oral gavage in addition to feed and water in doses of 200mg/kg and

500mg/kg respectively. The extract was administered orally once daily using a calibrated syringe and the stainless-steel oral gavage needle for a treatment period of (10) days.

3.5.1 DOSAGE CALCULATION OF *Picralma nitida*

The required concentrations of the extract were prepared based on the individual body weight of the rats.The dosage was calculated as follows :

i) For a standard dose of 200mg/kg;
200mg of extract is required for 1kg body weight of rat

ii) For a rat weighing 136.6 g, the exact dose to be given is ;

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

$$= 27.32\ \text{mg}$$

Hence, the volume of aqueous extract solution required for a rat weighing 136.6 g was calculated as follows, with the concentration of 1g/10mL,

1g = 10mL

1000mg = 10mL

$$\text{Volume} = \frac{27.32\ \text{mg} \times 10\text{mL}}{1000\ \text{mg}}$$

Volume = 0.27mL

1g (1000 mg) of the extract was dissolved in 10mL of distilled water to make the stock solution.

3.6 ADMINISTRATION OF GLIBENCLAMIDE (GLANIL))

Glibenclamide (glanil) was used as the standard hypoglycemic drug in comparison with the *Picralima nitida* fruit extract administered to one of diabetic groups. The drug was obtained from RX Pharmacy, Ekosodin, Benin City, Edo state Nigeria.The required dose was prepared freshly by dissolving the appropriate amount of the drug tablet (5mg) in distilled water. The

drug was administered once daily by oral gavage using a calibrated syringe and stainless-steel gavage needle.

3.6.1 DOSAGE CALCULATION FOR GLIBENCLAMIDE

The dosage of glibenclamide for the rats in Group was calculated individually based on their body weight. For example, for a rat weighing 170.1 g, with a standard dosage of 5 mg/kg body weight:

i) 5 mg of glibenclamide is required for 1 kg (1000 g) of body weight.

ii) For a rat weighing 170.1g, the exact dose to be given is ;

$$\frac{5\text{mg} \times 170.1\text{g}}{1000\text{g}} = 0.85\text{mg}$$

Hence, the volume of glibenclamide solution required for a rat weighing 170.1g was calculated as follows, with the concentration of 1g (1000mg) /10mL:

1000mg = 10mL

$$\text{Volume} = \frac{0.85\text{mg} \times 10\text{mL}}{1000} = 0.009\text{mL}$$

Thus, 0.009 mL of the glibenclamide solution was administered to a rat weighing 170.1 g.

3.7 COLLECTION OF BLOOD SAMPLES

Blood samples for glucose test: Animals were allowed to fast overnight for at least 12 hours. Blood samples were collected from the tail with a sterile lancet, and a drop of blood was collected into test strips to measure glucose levels using a glucometer.

Blood samples for the Assay: After an overnight fast, blood samples were collected from the heart after sacrificing the rats. The samples were collected into plain bottles for biochemical analyses. The samples were allowed to clot at room temperature and subsequently

centrifuged at 3000 rpm for 30 minutes. The resulting clear serum supernatant was carefully transferred into labeled Eppendorf tubes.

3.8 WEIGHT AND BLOOD GLUCOSE EVALUATION

The rats received their respective doses of the extract daily for seven (10) days. Throughout this period, body weight and blood glucose levels were monitored at the beginning and end of the study.

The results presented below include the initial and final body weights of each rat, the changes in weight, as well as the initial and final glucose levels and the corresponding differences in glucose values.

Table 1: Weight and blood glucose evaluation

Group	Label	ID	Baseline weight (g)	Baseline FBG (mg/dl)	Weight (g) Before Induction	FGB(Mg/dl) Before Induction	FGB(Mg/dl) 4 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	169.1	76	365
		S2	145.6	68	160.8	83	127
		S3	144.0	56	182.1	86	86
		S4	148.2	88	178.2	94	270
		S5	144.9	70	154.7	95	61
G2	Glibencalimde drug (oral)	M1	123.5	76	136.1	104	232

		M2 M3 M4 M5	124.5 126.6 127.5 128.7	76 90 76 68	158.8 139.4 145.6 157.3	81 88 99 83	144 88 133 83
G3	P.nitidia extract (200mg,oral)	D1 D2 D3 D4 D5	138.7 140.5 140.7 141.8 142.2	72 70 58 85 72	141.6 152.6 156.2 161.0 161.1	74 104 72 77 79	50 135 43 279 130
G4	p.nitida extract (500mg, oral)	H1 H2 H3 H4 H5	151.8 154.3 152.1 154.5 158.3	77 72 65 50 85	175.6 166.0 166.2 182.4 149.4	99 97 76 67 94	194 250 263 90 238

3.9 DETERMINATION OF SERUM ALPHA AMYLASE ACTIVITY

The assay mixture containing 200µl of 0.02M sodium phosphate buffer, 50µl serum, were incubated for 10mins at room temp, followed by addition of 200µl of starch in all test tubes. The reaction was terminated with the addition of 400µl DNS reagents and placed in boiling

water bath for 5mins, cooled and diluted with 1ml of distilled water and absorbance was measured at 540nm. Standard calibration graph of maltose (100ug/mL)was used to calculate the amount of maltose produced by alpha amylase activity.

Table 2: Assay protocol for alpha-amylase

NO. OF TUBES	**1**	**2**	**3**	**4**	**5**	**6**
Starch (100ug/mL)	-	0.2	0.4	0.6	0.8	1
Phosphate buffer (pH 6.9)	1	0.8	0.6	0.4	0.2	2
Alpha amylase (mL)	0.25	0.25	0.25	0.25	0.25	0.25
Incubated for 10 min at room temperature						
DNS (mL)	0.5	0.5	0.5	0.5	0.5	0.5
Boiled for 5 min						
Abs at 600nm		0.206	0.384	0.561	0.738	0.984
Amount (ug)		20	40	60	80	100

3.9.1 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

4.0 RESULTS

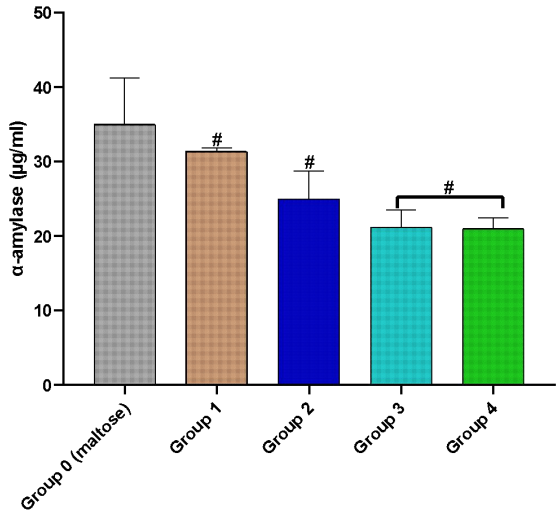

Figure 4.0: Effect of *Picralima nitida* on α-amylase concentrations on Streptozotocin induced diabetic Male Wistar rats. Values are expressed as mean ± SEM. # represent statistical significance at P < 0.05 when compared to group 0. From the study, it was shown that all the groups (1-4) significantly decreased α-amylase concentrations when compared to group 0 (maltose). This decrease was mostly observed in 3 and

I. The highest mean was observed in the control group 0 (34.94 ± 6.25), while the lowest was observed in both Group 3 *Picralima* nitida low dose (21.19 ± 2.29) and Group 4 *Picralima nitida high dose* (20.98 ± 1.46).

CHAPTER FIVE

5.0 DISCUSSION

The current study investigated the effect of *Picralima nitida*extract on serum pancreatic amylase activity in streptozotocin (STZ)-induced diabetic male Wistar rats. Serum amylase is known to serve as an indicator of pancreatic exocrine function, with its activity often disrupted in diabetic states. When Streptozotocin is induced, it causes destruction of pancreatic β-cells which in turn leads to decreased insulin production. Since insulin plays a role in stimulating pancreatic enzyme secretion, this dysfunction can result in decreased amylase activity. The objective of analyzing serum alpha-amylase was to assess the physiological impact of *Picralima nitida* on the pancreas of streptozotocin-induced diabetic rats. The results successfully established the diabetic baseline in contrast to the healthy state:

- **Healthy Baseline (Control):** The control group exhibited the highest mean amylase activity (34.94 ± 6.25) representing the normal physiological level in a healthy organism.
- **Disease Baseline (Diabetic Untreated):** The Streptozotocin induced diabetic group showed a numerically lower mean activity (31.34 ± 0.47). This depression is characteristic of chronic or advanced diabetes where the streptozotocin has caused significant destruction of pancreatic tissue (both beta-cells and acinar cells), leading to pancreatic burnout and reduced enzyme synthesis and secretion.

The administration of the standard drug (Glibenclamide) and the plant extract *Picralima nitida* resulted in a dramatic shift in enzyme activity, which shows a strong pharmacological intervention on pancreatic physiology.

- **Glibenclamide Effect:** The standard anti-diabetic drug significantly reduced the enzyme activity compared to the Diabetic Untreated baseline (24.94 ± 3.75). This suggests the drug is effective at reducing systemic stress or stabilizing the pancreas, thereby lowering the amount of amylase released into the serum.

- **Regulatory Action of *Picralima nitida*:** Both doses of *Picralima nitida* further reduced the serum alpha-amylase activity to the lowest levels observed in the study.

The profound decrease in amylase activity by *Picralima nitida* is interpreted as a beneficial, anti-inflammatory, or enzyme-regulating effect, rather than a sign of further toxicity for the following reasons:

1. **Anti-Inflammatory Action:** *Picralima nitida* possesses alkaloid compounds that lessen the inflammatory response linked with the diabetic state.. Pancreatic inflammation (pancreatitis) causes enzyme leakage and high serum amylase. By suppressing the elevated systemic or local inflammation associated with chronic diabetes, *Picralima nitida* reduces the abnormal efflux of the enzyme into the bloodstream. Reduced inflammation helps in reducing
2. stress and leakage of enzymes from the pancreatic tissue, resulting in lower serum levels.
3. **No Sign of Acute Toxicity:** Acute pancreatic toxicity would typically cause an initial increase in amylase as cells die and release their contents. The observed suppression indicates a long-term, stable, and controlled change in physiological regulation.
4. **Lack of Recovery:** *Picralima nitida* did not show any sign of recovery effect, as recovery would mean raising the depressed STZ induced diabetic group levels (31.34) back towards the healthy level (34.94).

5.1 CONCLUSION

Based on the numerical data for serum alpha-amylase activity, the following conclusions are drawn:

1. There was depressed enzyme activity in the Diabetic Untreated group compared to the Healthy Control, consistent with pancreatic damage.
2. *Picralima nitida* at both low and high dosages exerts a strong, dose-independent suppressive effect on serum alpha-amylase activity, driving the levels significantly below both the diabetic baseline and the Glibenclamide-treated group, although maximum therapeutic saturation for this particular enzymatic pathway is achieved at the medium dose, indicating no need for a higher concentration.

3. The profound reduction in serum amylase levels is interpreted as a strong indicator of the anti-inflammatory and pancreatic-stabilizing potential of *Picralima nitida* in the diabetic condition.
4. The results suggest that *Picralima nitida* could be a highly effective agent for reducing pancreatic stress in diabetes, a property that is crucial for managing the chronic progression of the dise

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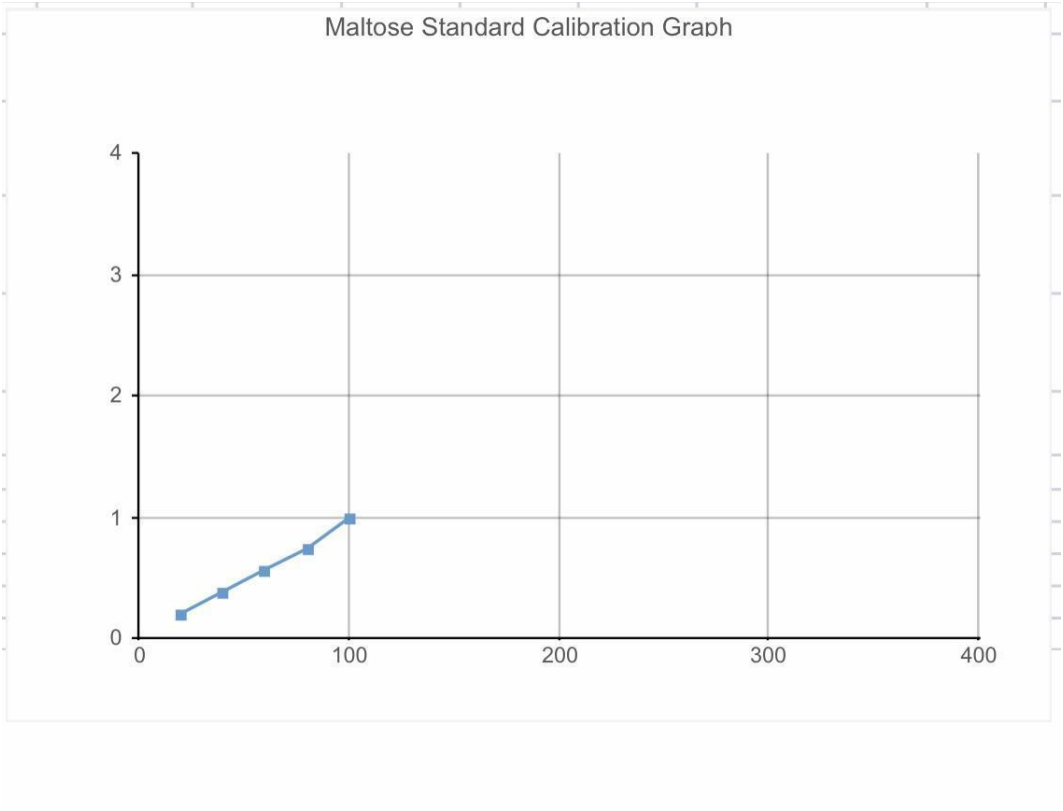
APPENDICES

Appendix A Table 1: Raw serum amylase values (U/L) for all Experimental Groups

SAMPLE ID	Maltose Abs	Const	Const	**Maltose (ug/mL)**
A1	0.189	0.006	0.0096	**19.5208**
A2	0.298	0.006	0.0096	**30.875**
A4	0.217	0.006	0.0096	**22.4375**
C1	0.227	0.006	0.0096	**28.6875**
C2	0.397	0.006	0.0096	**41.1875**
D2	0.227	0.006	0.0096	**28.6875**
D4	0.205	0.006	0.0096	**21.1875**
D5	0.307	0.006	0.0096	**31.8125**
M1	0.183	0.006	0.0096	**18.8958**
M4	0.227	0.006	0.0096	**23.4792**

APPENDIX B: MALTOSE STANDARD CALIBRATION GRAPH



APPENDIX C: MEAN ± SEM OF SERUM AMYLASE ACTIVITY IN EXPERIMENTAL RATS COMPARED TO CONTROL (GROUP 0)

Parameters (ng/mL)	Group 0 (maltose)	Group 1	Group 2	Group 3	Group 4
α-amylase (µg/ml)	34.94 ± 6.3	31.34 ± 0.5$^{\#}$	24.94 ± 3.8$^{\#}$	21.19 ± 2.3$^{\#}$	20.98 ± 1.5$^{\#}$

APPENDIX D: ETHICAL CLEARANCE